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Laboratory measurements and modelling 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 0.005-0.7 M in NaCl and mixed Na-Mg-Ca chloride solutions, with trace metal concentrations of ~5×10^{-7} M, a PHA concentration of 10 mg/l, and at constant pH values of ~7.8 (Co and Cu) and ~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 (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.

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 modelling 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 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 river-borne 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 ~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

5   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 test Humic Ion-Binding Model V. Following 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

10   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

15   Aqueous Model, WHAM (Tipping, 1994). This model takes into account the interactions of metals with (i) inorganic ligands (OH^-, HCO_3^-, CO_3^{2-}, SO_4^{2-}, 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

20   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

25   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 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

30   proton-dissociating sites. The model permits the binding of the first hydrolysis product (e.g. $CuOH^+$ in the case of 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:

$$RH^Z + M^{z+} = RM^{Z+z-1} + H^+ \tag{1}$$

where R is the humic molecule, Z is the charge on the humic molecule (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:

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

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

$$w = P\log_{10}I \tag{3}$$

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 Eq. 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 pK_{MHB} vary linearly with pK_{MHA}, so that it is possible to characterise metal binding with a single parameter, -$\log_{10}K_{MHA}$ or pK_{MHA} (Tipping, 1994). The strength of metal-humic binding therefore increases with decreasing values of 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 pK_{MHA} for 31 metals, estimated from literature data or by linear free-energy correlations. The (non-specific) 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 about 0.1 M. 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 M (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 sub-model 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)

5   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 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.

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Metal-humic binding is expressed as %-metal bound and as ν_M, the total amount of metal specifically bound to the humic molecule (mol g^{-1}). The term ν_M is comparable to $\left[RM^{Z+z-1} \right]$ in Equation 2 divided by the concentration of humic substances (g l^{-1}), but it relates to the total amount bound by all the heterogeneous monodentate and bidentate

15   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 10-90% humic-bound because of problems associated with quantifying whichever is the smaller, the humic-bound fraction or the non-bound fraction (Lead et al., 1998). Consequently, it was

20   necessary to obtain a spread of binding values within this range in order to provide a reliable test of WHAM. In the case of Cu, this required a series of binding experiments at a relatively low pH (~4.5) (see Section 3). It follows from the law of mass action (see Equation 2) that the percentage bound between 10 and 90% will be highly sensitive to pH and the pK_{MHA}, so that humic binding within this range also provides a

25   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 M NaCl, indicate that the range 10-90% 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 pK_{MHA} of 0.6 (pK_{MHA} 1.25 to 1.85) for Cu and 1.1 (pK_{MHA} 2.2 to 3.3) for Co. The variable pH

30   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

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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 humic acid fraction (PHA) was extracted as described by Reid et al. (1990). Briefly, the peat was wet sieved (4 mm mesh) and then treated with

10 nitrogen-saturated NaOH (0.1 M 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 days in the dark to allow complete precipitation of humic acid and then the suspension was recentrifuged and the supernatant discarded. The resulting PHA sample was subjected to successive treatments in nitrogen-saturated

15 1 M KOH, HCl (pH 2) and a 0.3 M HF- 0.1 M HCl mixture in order 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. Prior to the experiments, 1 g/l stock solutions of PHA were prepared in Milli-Q water and stored in the dark at 4°C.

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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

25 l HDPE screw top bottles, using Spectra/Por cellulose ester dialysis tubing (2000 MWCO) that had been thoroughly prewashed in Milli-Q water. Preliminary experiments were carried out in order to determine equilibration times (~24 h) for Cu and Co through the membranes, under the solution conditions used in the main experiments.

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Two simple analogue solutions of estuarine systems were prepared using AnalaR reagents, one containing NaCl only and the other containing NaCl, $MgCl_2$ and $CaCl_2$ 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 M, 0.35 M and 0.7 M. The solutions were spiked with Perkin Elmer acidic metal standards to give final concentrations of 4.7×10^{-7} M Cu and 5.1×10^{-7} M Co. Analar NaOH (0.1 M) and HNO_3 (10% by vol.) were added as required to adjust the

5   pH of the solutions. Experiments were undertaken at a single narrow pH range (~7.7-8.0) in the case of Co and at two narrow pH ranges (~4.4-4.7, 7.7-8.1) in the case of Cu. The high pH solutions were equilibrated with atmospheric CO_2 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

10   relevant trace-metal free, ionic medium, then sealed and inserted into each experimental solution. Experiments were allowed to reach dialysis equilibrium over 4 days 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

15   conditions (see Table 1).

After 4 days, 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

20   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_3. 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.

25   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

30   metal analysis was carried out by 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, pers. comm.).

4. RESULTS AND WHAM PREDICTIONS

In the NaCl medium at a pH of ~7.8, Co-humic binding decreased markedly with

5 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), while 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

10 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}

15 within the range 10-90% 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 (Figs. 1a-f).

With the assumption of a fixed relationship between pK_{MHA} and pK_{MHB}, the former is

20 the only parameter in Model V that directly affects metal binding. The default values of all the other Model V parameters, including the electrostatic parameter P in Equation 3, were obtained independently by fitting the large number of published data sets on proton binding by humic and fulvic acids (e.g. see Tipping and Hurley, 1992). Therefore the default predictions shown in Fig. 1 can only justifiably be improved on

25 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 (Figs. 1e and 1f). Cu-humic binding at high pH

30 (Figs. 1c and 1d) 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 (10-90% 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 ~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 (Figs. 1a and 1e). 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 negligible 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 $\nu_{Cu}/a_{Cu^{2+}}$ 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 $\nu_{Cu}/a_{Cu^{2+}}$ 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 $\nu_{Cu}/a_{Cu^{2+}}$ ratio was reflected in a measurable change within the range 10-90 % bound.

The factors causing the observed dependence of metal-humic binding on I in NaCl

5   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 Equations 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,

10   1993), and it is for this reason that, in Model V, monovalent metals are assumed to bind only nonspecifically 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.

15   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 ~0.15 M. Therefore in terms of both absolute concentrations and their ionic strength trends, carbonate species cannot account for the observed binding trends. Chloride

20   complexation is clearly significant in both the Co and low pH, Cu experiments, varying from <1 to ~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 ~3 with increasing I from 0.005 to 0.7 M. Therefore, at first appearance, chloride complexation and simple activity coefficient effects both appear to be important in explaining the

25   metal-humic binding trends shown in Figs. 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.

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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

5   Equation 2 that the only factors then affecting the amount of specifically bound metal are the exponential term 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

10   charge has the effect of counteracting to some degree the effect of increased shielding on metal ion binding. To obtain fixed values of $a_{M^{2+}}$ and pH, the total trace metal concentrations at I=0.7 M were varied until WHAM gave the same $a_{M^{2+}}$ values as those predicted at I=0.005 M with the original total trace metal concentrations (4.7 x 10^{-7} M Cu and 5.1 x 10^{-7} M Co). Table 6 shows the final values of variables used in the

15   calculations. The predicted differences in $\nu_M / a_{M^{2+}}$ between the 0.005 and 0.7 M solutions of each series (Table 6), at fixed values of $a_{M^{2+}}$ and pH, are similar to those predicted under the experimental conditions (Tables 3-5) . It follows that the electrostatic (coulombic) effect is the dominant factor underlying the observed variations in metal-humic binding in NaCl solutions.

20

Although the electrostatic sub-model 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.,

25   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.

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5.2. Metal-Humic Binding in the presence of Ca and Mg

The results shown in Fig. 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-modelling study of Mantoura et al. (1978) that predicted a

5   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_o) at a single set of conditions (pH 8.0 and $I = 0.02$ M), and used these constants to make predictions of metal speciation under estuarine conditions using an

10   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

15   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 (Equation 1). However, a measure of the relative binding strengths was obtained by

20   comparing the ratios of the equilibrium constants for Ca, Mg, Cu and Co (Table 7). Trace metal binding occurs principally at bidendate 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 pK values) (Tipping, 1994). The comparison therefore used the pK_{MHA} value for each metal multiplied by 2,

25   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 250000:8:1 in the Mantoura study. The latter ratios were obtained using the median values of the ranges shown in Table 7. If the lowest K_o for Cu was used instead, the Mantoura ratios would be 16000:8:1.

30   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^{-1}) and from the default total binding site concentration (4.94 x 10^{-3} mol g^{-1}) 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 4.35-5.25 x 10^{-3} mol g^{-1}. In the Mantoura study, the Cu concentration was 1 x 10^{-8} M but a number of trace metals were included in the model prediction, giving a total trace metal concentration of 5.2 x 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 experimental 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. While 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 since 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 (Figs. 2a and 2b) assuming equilibrium with CO_2 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 3×10^{-7} M, which is 1-2 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 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 0.5-5 % 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 (Figs. 2e and 2f). 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 M ionic strength in mixed salt solution (Fig. 1d), the seawater simulation was rerun with the pK_{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 modelling 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 x 10^{-7} M). Natural variability was considered by running simulations for one trace metal at a time with alternative pK_{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 pK_{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 ~70% in seawater when the humic substances were assumed to be fulvic acid. For humic acid, Cu binding remained above 85% at all pK_{MHA} values. The predicted effect of humic variability was even greater for Co, especially with respect to the extreme pK_{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 Figs. 3a-d). The question then arises as to how representative the Co pK_{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 pK_{MHA} value (~2.5) for Co obtained in the present study for a peat humic acid and the previously reported correlation between pK_{MHA} values and the analagous values for lactic acid (Tipping and Hurley, 1992) both indicate that the default pK_{MHA} value of 2.7 for humic acid is the most representative. However, the above simulations do highlight the need for further high quality measurments 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 high metal:humic ratios used in the simulations. A small competition effect is predicted in the case of Co binding (Figs. 3a and 3b), but effectively none at all in the

case of Cu (Figs. 3c and 3d), 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 (1×10^{-8} M and 1×10^{-7} M), over a range of humic concentrations between 0.2 and 1 mg l^{-1}. One of these simulations (1×10^{-8} M Cu and 0.2 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 ~0.5 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. $v_{Ca} + v_{Mg}$) and Ca, Mg and Cu (i.e. $v_{Ca} + v_{Mg} + v_{Cu}$). The trends in $v_{Ca} + v_{Mg} + v_{Cu}$ 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 competition effects were smaller than might have been expected. Since metal binding occurs preferentially at bidendate 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 v values and the default concentration (4.94×10^{-3} mol g^{-1}) of binding sites for humic acid in the WHAM database (Tipping, 1994). The v values shown in Figure 4b correspond to a maximum site occupancy of 35-48%.

Both the experimental and modelling 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 (~7.5-8.1) used in the modelling.

Assumption 1 means that K_o is independent of I, implying that there is no electrostatic

5   effect on the humic molecule. Thus the conditional binding constants measured at

I=0.02 M 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 M, pH=8.0, 1×10^{-6}

10   M humic binding sites, and 1×10^{-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 Equation 3) was then set to zero, so

that the exponential term in Equation 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

15   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

20   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

2.20×10^{-3} mol g^{-1}, when compared with the results for seawater using the default

25   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 M produced a far greater lowering of

30   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 M and a pH of 8.0 than in full-strength seawater at pH 8.3. This result is counter-intuitive 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 M, 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 modelling 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 (0.85-1.38 x 10^{-3} 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 non-acid freshwaters. Another important factor in maintaining binding, even in soft waters, is that the combined concentrations of Ca and Mg (e.g. 325 µM in Esthwaite Water - see Table 8) are far in excess of the total number of humic binding sites (e.g. 5 µM at 1 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 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 like Co. Both the experimental and modelling 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 mgl^{-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 is 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 Figs. 3a and 3c). 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 (~0.8-1.4 x 10^{-3} 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 ~0.1 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

5 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

10 interpretation is supported by the predictions for Co at a pK_{MHA} value of 1.9 in Fig. 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 Figs. 3a and 3c). It follows that this effect will be highly sensitive to the exact pK_{MHA} values for trace metals over a critical range (e.g. ~1.9-2.7

15 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 multi-component equilibrium systems that are sometimes counter-intuitive.

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In this study, data interpretation and model predictions have been based on Model V as the humic sub-model 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

25 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

30 humic sub-model, since Equation 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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Table 1. The measured pH (mean and standard deviation) in the binding experiments, with the numbers of experimental replicates shown in parentheses.

	Experimental series[*]										
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)

[*]Experiments in NaCl solutions indicated by /Na, those in mixed NaCl, $MgCl_2$, $CaCl_2$ solutions by /NaMgCa

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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

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

I (/M)	ν_{Co} (mol g^{-1})/10^{-6}	$\frac{\nu_{Co}}{a_{Co^{2+}}}$ (l g^{-1})	a_{Co}^{2+} (nM)	γ_{Co}^{2+}	[Co^{2+}] (nM)	[CoCl$^+$] (nM)	[CoCl$^+$] (as % of total Co)	[Co^{2+}]
0.005	53	25000	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

[*]ν_{Co} - concentration of Co specifically bound to humics; [ ] denotes solution concentrations; a_{Co}^{2+} - free ion activity; and γ_{Co}^{2+} - activity coefficient of free ion.

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

I (/M)	ν_{Cu} (mol g⁻¹)/10⁻⁶	$\dfrac{\nu_{Cu}}{a_{Cu^{2+}}}$ (l g⁻¹)	a_{Cu}^{2+} (nM)	γ_{Cu}^{2+}	$[Cu^{2+}]$ (nM)	$[CuCl^{+}]$ (nM)	$[CuCl]$ (as % of total Cu)	$[Cu^{2+}]$
0.005	57	1.9×10^{8}	0.0003	0.74	0.0004	0.000003	6.0×10^{-7}	7.2×10^{-5}
0.15	46	1.0×10^{7}	0.0046	0.31	0.015	0.0017	3.5×10^{-4}	3.1×10^{-3}
0.35	46	3.8×10^{6}	0.012	0.25	0.051	0.01	2.2×10^{-3}	1.1×10^{-2}
0.7	48	1.7×10^{6}	0.028	0.23	0.12	0.048	1.0×10^{-2}	2.6×10^{-2}

[*] n_{Cu} - concentration of Cu specifically bound to humics; [ ] denotes solution concentrations; a_{Cu}^{2+} - free ion activity; and γ_{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 (/M)	ν_{Cu} (mol g⁻¹)/10⁻⁶	$\dfrac{\nu_{Cu}}{a_{Cu^{2+}}}$ (l g⁻¹)	a_{Cu}^{2+} (nM)	γ_{Cu}^{2+}	$[Cu^{2+}]$ (nM)	$[CuCl^{+}]$ (nM)	$[CuCl]$ (as % of total Cu)	$[Cu^{2+}]$
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

[*] n_{Co} - concentration of Co specifically bound to humics; [ ] denotes solution concentrations; a_{Co}^{2+} - free ion activity; and γ_{Co}^{2+} - activity coefficient of free ion.

Table 6. A comparison between the metal humic binding, expressed as n_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 (M)	pH	Total metal (nM)	a_M^{2+} (nM)	Predicted $\dfrac{\nu_M}{a_{M^{2+}}}$ (l g⁻¹) fixed a_M^{2+} and pH	as in Tables 3-5
Co	0.005	7.68	510	3.2	1.6×10^{4}	2.5×10^{4}
Co	0.7	7.68	21	3.2	19	19
Cu, high pH	0.005	7.73	470	0.0003	1.6×10^{8}	1.9×10^{8}
Cu, high pH	0.7	7.73	6.0	0.0003	2.0×10^{6}	1.7×10^{6}
Cu, low pH	0.005	4.46	470	65.9	580	910
Cu, low pH	0.7	4.46	590	65.9	290	290

n_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]	4.7×10^{-7} M	[Cu]	1.0×10^{-8} M
		[total metals]	5.2×10^{-8} M
[binding sites]	5×10^{-5} M	[binding sites]	1×10^{-6} M
pK_{CaHA}	3.2	$\log_{10} K_o$ for Ca	3.6-4.1[*]
pK_{MgHA}	3.3	$\log_{10} K_o$ for Mg	3.4-4.1[*]
pK_{CuHA}	1.5	$\log_{10} K_o$ for Cu	8.0-10.4[*]
pK_{CoHA}	2.7	$\log_{10} K_o$ for Co	4.5-4.9[*]

[*]Typical values, omitting outliers.

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

Component	Esthwaite	Seathwaite (mM)	Seawater (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
NO_3	30	36	0
SO_4	115	42	0.0293
HCO_3	385	0	0.0024
pH	7.8	5.1	8.3

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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^{-1} humic acid and 1×10^{-8} M total Cu, using different values of the electrostatic constant (P) and pK_{MHA} (see text for full explanation). The Esthwaite Water predictions are also given for two other humic acid concentrations.

Water type	P	pK_{MHA}	$n_{Ca} + n_{Mg}$ (mol g^{-1}) $/10^{-3}$	$\dfrac{[\text{humic - Cu}]}{[\text{total Cu}]}(\%)$
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=2mgl^{-1}	default	default	1.211	99.9
Esthwaite, pH=7.8, humics=10mgl^{-1}	default	default	1.208	100.0

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Figure 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 ~7.8, (b) Co in Na-Mg-Ca chloride solution at pH

~7.8, (c) Cu in NaCl solution at pH ~7.8, (d) Cu in Na-Mg-Ca chloride solution at pH ~7.8, (e) Cu in

NaCl solution at pH ~4.6, (f) Cu in Na-Mg-Ca chloride solution at pH ~4.6. Error bars represent the

standard deviations of experimental replicates.

Figure 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."

Figure 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.

Figure 4. WHAM predictions for seawater at total Cu concentrations of 1×10^{-8} M (circles) and 1×10^{-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
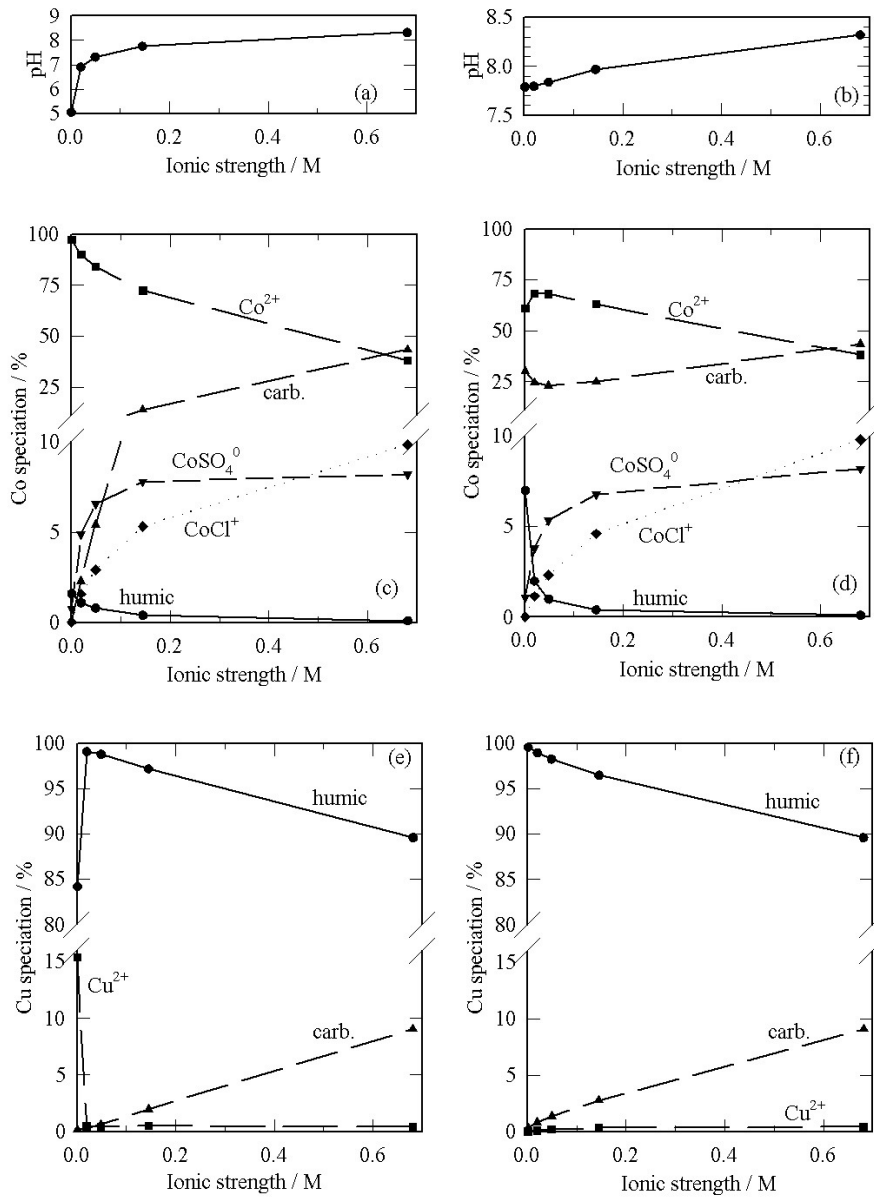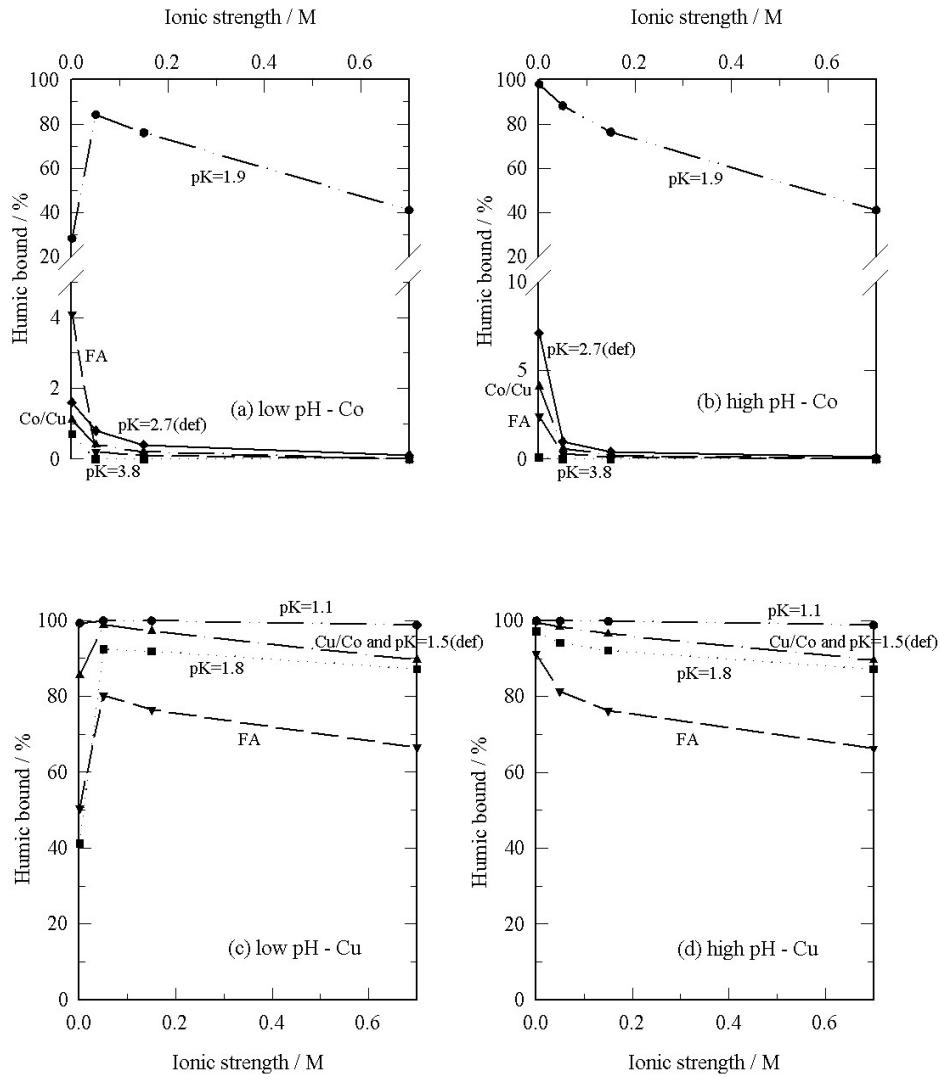
symbols).

Fig. 1

Fig. 2

Fig. 3

(a) low pH - Co

(b) high pH - Co

(c) low pH - Cu

(d) high pH - Cu

Fig. 4