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Testing Copper-Speciation Predictions in Freshwaters over a Wide Range of Metal−Organic Matter Ratios

Imad A.M. Ahmed †*
John Hamilton−Taylor †
Stephen Lofts ‡
Johannes C. L. Meeussen §
Chun Lin †
Hao Zhang †
William Davison†

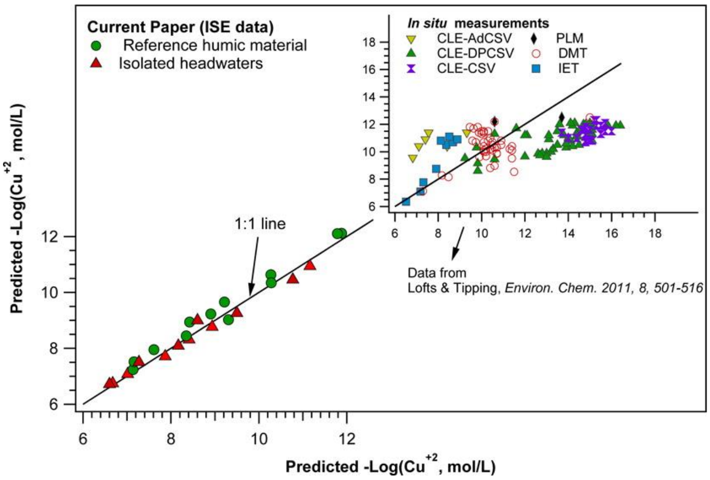
†The University of Lancaster, Lancaster Environment Centre, Lancaster, LA1 4YQ, United Kingdom
‡Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, United Kingdom
§Soil Physics, Ecohydrology and Groundwater Management Group, Wageningen University, Wageningen, Netherlands

* Corresponding author: Tel: +44 (0) 1524 594534; fax: +44 (0)1524 593985
e-mail: i.ahmed@lancaster.ac.uk

Abstract

The harsh chemical conditions involved in the isolation of fulvic acids (FA) and humic acids (HA) have been identified as a possible contributing factor to the significant mismatch between in situ measurements and model predictions of trace metal speciation in freshwaters, resulting from the use of isolated FA and HA in model calibration. A set of experimental assays were developed to enable Cu binding to DOM to be measured over the full range of [Cu]/[DOC] ratios (~1–460 μmol g–1) observed in surface freshwaters. They were applied to the widely used and traditionally isolated Suwannee River HA and FA and to DOM isolated from headwater streams by a mild procedure using minimal chemical treatment. Good agreement was observed between measured free ion activities and those predicted using both WHAM/Model VII and NICA–Donnan speciation models for both traditionally and mildly isolated DOM. Agreement to within a factor of 2 for WHAM/Model VII contrasts with 100-fold differences previously reported between in situ Cu2+ measurements and model predictions for a wide range of conditions. The results demonstrate that (a) existing speciation models are capable of accurately predicting Cu-humic binding in natural waters at environmentally realistic [Cu]/[DOC] ratios, under equilibrium conditions, and (b) that the isolation procedures traditionally used for HA and FA do not appreciably affect their binding characteristics.

Abstract graphic



Introduction

The chemical speciation of metals is fundamental to understanding their reactivity, mobility, bioavailability, and toxicity in aquatic, sediment, and soil systems. Model predictions of the chemical speciation of cationic trace metals are increasingly being used in a wide range of applications in freshwater systems. Examples include their use in Environmental Risk Assessments(1, 2) and in studies of ecotoxicity,(3, 4) environmental fate,(5) bioaccumulation,(6) and metal solubility.(7) One of the greatest challenges of modeling metal speciation in freshwaters has been reliable prediction of metal binding to natural dissolved organic matter (DOM). The principal software packages currently in use for this purpose include vMINTEQ,(8) ECOSAT(9) and ORCHESTRA, which all incorporate the NICA-Donnan model,(10, 11) and versions of WHAM, incorporating humic substances binding Model VI or VII.(12-14) Both WHAM and NICA-Donnan are equilibrium-based models, calibrated using published data on metal and proton binding to fulvic and humic acids, obtained from freshwaters and soils by various standard isolation schemes. Since they are calibrated more or less with the same binding data, the two models give broadly similar predictions of metal-humic binding, although there are differences in detail. Despite many advances being made in recent years, significant uncertainties remain in our ability to make reliable predictions and measurements of metal speciation under natural conditions.(15) For example, agreement between in situ measurements in solution and model (WHAM) predictions of free-ion concentrations is currently only within an order of magnitude for Al, Co, Zn, and Cd, and 2 orders of magnitude for Cu and Pb.(16) Hamilton–Taylor et al.(15) identified a number of factors that may contribute to the observed discrepancies. On the modeling side, there is uncertainty as to whether isolated humic fractions are truly representative of freshwater DOM, since it has been suggested that the extremes of pH used during the isolation of fulvic and humic acids may introduce artifacts that could affect their binding properties.(17) Another factor is that other unspecified ligands (e.g., low molecular weight organic ligands, microbial exudates,(18, 19) anthropogenic compounds,(20-22) and sulfides(23)) may have a significant effect on the measured speciation. There is also the question of whether an equilibrium approach is always appropriate to field conditions. For example, the rates of equilibration with strong humic binding sites may be sufficiently slow that in natural waters, where there is a dynamic supply of dissolved metal, the free ion activity may not be controlled by these sites. There are also uncertainties that relate to the model itself. Single (mean) values of binding parameters are routinely used so that the effects of natural variability,(24, 25) particularly in the nature of the humic material, are generally not assessed. Model-input concentrations of metals and humic substances (HS) are normally based on measurements of total filterable (e.g., < 0.45 μm) metal and dissolved organic carbon (DOC), with some assumptions being made about the humic fraction of the measured DOC.(26, 27) The use of filterable concentrations can lead to errors in predicted free ion concentrations due to the presence of inorganic colloids (e.g., Fe oxides).(28, 29) While comparisons between in situ measurements and model predictions are important for validation purposes and for indicating the total scale of the combined errors associated with measurements and predictions, they are not well-suited to determining the significance of the individual potential sources of error, described above.

The main aims of the present work were to develop and apply a simple protocol to investigate one of the key identified uncertainties, namely whether model parametrization, using isolated fulvic and humic acids, provides a true measure of humic binding under natural conditions. The effects of variable model inputs and inorganic colloids were also investigated. In order to achieve the aims, a number of key methodological requirements are identified. The analytical technique must be reliable and well-tested, and should provide a well-defined speciation measure (preferably the free-ion concentration). While ion-selective electrodes (ISE) meet this requirement and have been used before for model testing, they have the disadvantage of limited sensitivity, typically requiring metal addition prior to measurement.(30) However, the critical issue in model testing is the [metal]/[DOC] concentration ratio rather than the absolute metal concentration. Because of the heterogeneous nature of humic binding sites,(17) it is important that the measurements cover a range of [metal]/[DOC] ratios, including those found in natural waters. Our approach combines ISE measurements with preconcentration of DOM, to allow metal binding measurements at realistic [metal]/[DOC] ratios. Given the aims of the work, it is essential that the preconcentration method avoid the harsh chemical conditions experienced during the isolation of fulvic and humic acids. Reverse osmosis(22) and low-temperature rotary evaporation(31) have both been used in recent years for isolating and preconcentrating DOM without affecting its reactivity or integrity (e.g., molecular size distribution).(32, 33) Finally, the field sites were selected to ensure, as far as possible, that humic substances were the only significant organic ligands present.

Materials and Methods

Sampling and Isolation of DOM

Freshwater samples were collected from Rough Sike (54.688° N 2.380° W) and Brown Gill (54.776° N 2.389° W), two headwater streams in the North Pennines, England. Rough Sike (RS) is a remote, high altitude (560 m) stream within the Moor House National Nature Reserve, whose catchment is dominated by blanket peat of total depth 1–4 m.(34) Vegetation is dominated by heather (Calluna vulgaris), cotton grass (Eriophorum spp.), and Sphagnum moss. The Brown Gill (BG) site is at an altitude of 430 m. The lower part of its catchment comprises mainly uncultivated grasslands on acid loams and clays, and is used for rough grazing. The upper part is formed of blanket peat. There are also disused lead-mine workings within the catchment. A total of 100 L of water samples was collected from August to September 2010 over five visits (20 L each time) from midstream points at both sites. Prior to sampling, HDPE plastic containers (10 L) were soaked with 4% Decon-90 overnight and 10% HCl followed by a thorough rinsing with deionized water. Containers were also prerinsed three times on site with streamwater.

The DOM isolation method was based on that of Thacker et al. (31) but was modified so that the ion-exchange step to remove Ca and Mg was undertaken before evaporative concentration, in order to preclude CaCO3 precipitation and hence possible loss of DOM. Sample processing was begun on the day of sampling and involved sequential filtration through Whatman GF/C (<1.2 μm) and GF/F (<0.7 μm) glass-fiber filter papers. The filtration system comprised a polycarbonate in-line filter holder (Geotech Environmental Equipment, Inc.), connected to a peristaltic pump with Marprene tubing. The system was washed using 0.1 M HCl and flushed (20 L) with deionized water before use. The first 250 mL of the collected filtrate from each location were discarded and the remaining sample passed through a 250 cm3 column of Na-saturated Amberlite IR-120 (Na^+) resin at a flow rate of 50 cm^3 min^{-1}. The column was preconditioned by flushing with 1 L of NaNO3 (0.1 M) at a flow rate of 30 cm^3 min^{-1} followed by 5 L deionized water. This preconditioning step was repeated three times and subsamples from column eluates were collected to test the DOC bleed from the resin column. Repeated tests indicated a DOC bleed below the detection limit of the TOC analyzer (∼0.05 mg L^{-1}). The column eluate from a prescribed sample was then concentrated in vacuo using a low-temperature rotary evaporator (Büchi Rotavapor R-220). Samples were added incrementally to an amber evaporation flask in a 318 K water bath, while maintaining a vacuum of 10 mbar. The combined concentrate (∼3 L) from the five 20 L samples was subjected to a further concentration step to ∼800 mL and then filtered through a 0.22 μm surfactant-free MCE MF-Millipore membrane. All sample storage during and after processing was undertaken at 4 °C in the dark. The volumes of the final isolates were determined to enable calculation of DOC recoveries. Detailed chemical analysis was performed on final isolates from the RS and BG sites. The concentrations of trace metals and major cations were analyzed using ICP-MS (Thermo Xseries-II) and ICP-OES (Thermo iCAP 6000), respectively. The concentrations of nitrate, chloride, sulfate, and phosphate were analyzed by ion chromatography (Dionex DX100), and ammonium analyzed colorimetrically. Alkalinity was determined by Gran titration. DOC concentration was determined using a Shimadzu TOC-VCPN analyzer. The concentrations of the chelating agents EDTA (ethylenedinitrilotetraacetate) and NTA (nitrilotriacetate) in isolated DOM were determined by a gas chromatographic method using the international standard ISO 16588:2002.(35)

Metal-Binding Assays and Cu Free-Ion Measurement

We used a laboratory-assay approach, in which free-metal ion (FMI) activities were measured in standardized solutions, at a wide range of [metal]/[DOC] ratios, pH values and competing metal concentrations. This approach enabled simultaneous execution of multiple experiments with long equilibration times, facilitating the efficient collection of binding data over a diverse range of conditions. Copper was used as the target metal because of the known reliability of its FMI measurement by ion-selective electrode (30, 36) and because the humic-binding models are better calibrated with copper than with any other metal.(10, 13). Six laboratory assays were run in triplicate with the target conditions shown in Table 1. In addition to the two natural DOM isolates, Suwannee River fulvic acid (SRFA 2S101F) and humic acid (SRHA 2S101H), obtained from the International Humic Substances Society (IHSS), were used to make up two parallel series of reference assays. The background ionic strength was 100 ± 0.7 mM in all cases, to ensure optimum electrode performance and for consistency with the concentrated assay solutions. The measured chemical compositions of the DOM isolates and concentrated stock solutions of SRFA and SRHA were input into WHAM VII (see Speciation Modeling) to calculate the volume of KNO3 or/and Ca(NO3)2 solutions required to produce the required ionic strength, taking account of the various dilutions required for the assays. Calcium, as Ca(NO3)2, was added only to assays #5 and #6 at a [K]/[Ca] molar ratio of 1:1 in SRFA and SRHA assays and at a ratio of 3:1 in isolated DOM assays. All reagents were of analytical grade or higher and were used without further purification. Fisher Scientific analytical standards (1000 mg L^{-1}) of Cu, Ni and Zn were used for assay spikes and ISE calibration. All plastic-ware were soaked in 10% HNO3 (v/v) for at least 48 h and rinsed thoroughly in deionized water before use. Deionized ultrapure water with a resistivity of 18.2 MΩ.cm (Milli-Q, Millipore) was used throughout the study. Assays 1–5 were maintained at pH 6.0 ± 0.1 by the additions of 1 mM 2-(N-morpholino)ethanesulphonic acid buffer (MES). Binding of Cu by MES was examined and found to be negligible under the experimental conditions of the assays. Small additions of 0.1 M HNO3 or 0.1 M KOH (Fixanal, Fulka) were required to bring the pH of assay #6 to 4.0 ± 0.05. The samples were kept on an orbital shaker and left to equilibrate for 2 weeks in the dark before undertaking Cu-ISE measurements. The final trace-metal compositions of each of the triplicated assay solutions were measured by ICP–MS and their pH determined using an Orion Ross Sure-Flow combination pH electrode, calibrated following procedures recommended by Davison.(37).

Free Cu2+ activities were measured with an Orion Ionplus Cupric solid-state combination ISE (model 9629BNWP, Thermo Fisher Scientific) with a built-in Sure-Flow reference junction. After each measurement, the sensing element was rinsed with deionized water and then immersed sequentially in 0.025 M H2SO4 for 5 min and a 10% Chelex-100 suspension (pH 9) for another 5 min, before a final thorough water rinse. This procedure consistently produced initial electrode potentials below −390 mV. Electrode drift was minimized by gentle polishing (30 s) of the shiny surface with a 0.3 μm wet alumina polishing strip (Orion 948201) before each calibration. The effect of photoinduced reaction at the ISE probe surface and in DOM-containing samples was minimized by placing the titration cell or sample container in an opaque Perspex box. The electrode response and the calibration curve were determined using 100 mL Cu(NO3)2 solution volumes over a concentration range of 1.0–1000 μM at an ionic strength of 100 M (KNO3). For low [CuTot], better results and faster stabilization of the Cu-ISE were obtained in ≥100 mL solution volumes (see Figure S1 of the Supporting Information) and hence all assays were carried out using 100 mL solutions. To avoid the precipitation of Cu(OH)2(s), all of the calibration standards were maintained at pH ≈ 4.5 using small additions of 0.1 M HNO3 (Suprapure, Merck). To minimize losses on container walls, calibration solutions were prepared in acid–cleaned containers and left overnight. On the following day, the solutions were decanted and containers rinsed briefly in Milli-Q water then new calibration solutions were prepared in the same containers. The total Cu concentration in each calibration solution was determined by ICP–MS. Normally, the Cu-ISE electrode is capable of detecting Cu2+ activity to pCu (−log10 Cu, mol L–1) ~7.4 (~40 nM), but Cu detection can be extended well beyond this limit by buffering Cu concentration using 1,2-diaminoethane (EN).(38) Extended calibration of Cu-ISE was carried out by pH-titration of solutions (100 mL) containing 0.1 mM Cu(NO3)2 and 2.5 mM 1,2-diaminoethane monohydrate (Merck) at an ionic strength of 0.1 M adjusted using KNO3. To avoid problems caused by memory effects of the Cu-ISE electrode, acidimetric titration (from pH ~ 9.8 to 6.0) was undertaken using an automatic piston buret (Schott, Titronic Universal 20 mL). Changes in the ionic strength during the titration were explicitly taken into account for each experimental data point. The ISE potential and pH readings were taken on an Orion 720Aplus meter with an input impedance of >1012 Ω. Calibration solutions were flushed with N2(g) for 5 min, then pre-moistened N2(g) was slowly passed through the headspace over the solutions in order to exclude O2(g) and CO2(g) during calibration. The pH was recorded using a code written in Visual Basic every 10 s until its drift was <0.005 min–1. After pH stabilization, the Cu-ISE cell potential was measured at 30 s intervals until its drift was <0.2 mV min–1. The maximum stabilization time was ~45 min at low Cu2+ activity (pCu > 8) and <30 min at higher Cu2+ activities. The minimum time for a single Cu-ISE measurement was set at 15 min. All ISE measurements were carried out in a constant temperature (290 ± 3 K) class 100 clean room. To further control solution temperature, samples were placed in a small water bath on a digital controlled precision hot plate. This approach minimized variations in temperature between calibrations and in samples to ≤0.5 K. Solutions were stirred at a constant speed (500 rpm). The same measurement protocol was used for assay samples following an equilibration period between DOM and the added metal of at least 2 weeks. The Visual Basic code produced a real-time response time curve of the Cu-ISE, facilitating a visual decision on whether a particular potential reading represented a true equilibrium state (see Figure S2 of the Supporting Information). Stable readings were obtained within 45–60 min in all assay samples, except those of assay 4 where a stable reading was established after ~4 h (see Figure S2 of the Supporting Information) due to the low [Cu]/[DOC] ratio.

The total Cu concentration in each calibration solution, determined by ICP–MS, was generally within ±2% (spikes plus carry-over in the DOM isolates) of the concentrations calculated from the added standards. The free Cu2+ ion activities in the calibration solutions were obtained from the measured total concentrations, using WHAM VII, and then used to create the extended calibration graph shown in Figure S3 of the Supporting Information. Critical stability constants of EN complexes were taken from Smith and Martell (39, 40) and added to the WHAM VII database. The free-ion estimates were checked against predictions obtained with PHREEQC,(41) MINEQL+,(42) and ORCHESTRA (43) and in all cases were found to be within ±1%, owing to slight differences in stability constants between different thermodynamic databases. The Cu-ISE calibration data show excellent linearity (R2 = 0.999, nsample = 26) and reproducibility in the range pCu 3–18 (Figure S3 of the Supporting Information), together with a slope (28.7 ± 0.08 mV/log M) close to the theoretical Nernstian response (29.1 mV/log M) at 293 K. The main interference in the assays is most likely to arise from the chloride ion. Bryan (44) reported interference at 100 mM chloride, but none at 10 mM. The chloride concentrations in the assay solutions (Tables S1–S4 of the Supporting Information) were <10 mM, except in assays 4–6 for Brown Gill when slightly higher concentrations were present (13 mM Cl).

Speciation Modeling

Chemical speciation was calculated primarily using the equilibrium models: WHAM/Model VII(16) and NICA–Donnan.(45) WHAM VII is a recent development in the WHAM series of models that includes the same inorganic speciation code as previous versions but a simplified parameter set for metal-HS binding. Compared with Model VI, it provides improved descriptions of metal binding at high pH and an expanded database for cation binding to HS. Model VII also includes, for the first time, a Monte Carlo routine for estimating the effects of uncertainty in metal binding parameters.(24) In this analysis, the principal metal-binding constant (log KMA) of each metal is varied in a series of model runs by random sampling from a normal distribution around the mean default value. The normal distributions are based on the different values of log K_{MA}, obtained from model calibration with independent published binding data sets. Log K_{MA} uncertainty calculations for all dissolved metal ions were undertaken for each assay, using a sample size of 1999 parameter sets, while the HA and FA proton parameters remained unchanged. The ORCHESTRA (43) equilibrium framework was used for NICA–Donnan speciation calculations. Metal-inorganic complexes were calculated using MINTEQ database version 4.0.(46) The generic NICA-Donnan model parameters for humic acid and fulvic acid were used as provided by Milne et al.(47) Activity coefficients were calculated using the Davies equation with both models.

The compositions of the assay solutions shown in Tables S1–S4 of the Supporting Information were used to define the model inputs. In the case of pH and the trace metal concentrations, including Al and Fe, the measured values in each of the triplicated assay solutions were used in separate model runs. All solutions were assumed to be fully oxidized and in equilibrium with atmospheric CO2, at a partial pressure of 3.5×10^{-4} atm. The predicted Cu^{2+} activity was found to be insensitive to alternative assumptions about CO2. The fraction of chemically active DOM in natural waters generally requires adjustment to predict metal binding accurately. A range of assumptions about the chemically active DOM fraction have been used, e.g., 65% FA and 35% inert,(30, 48) 69% FA and 31% inert,(49) 41% FA and 59% inert (25), and 40% FA, 40% HA, and 20% inert.(50) We tested three assumptions of active DOM (total DOM assumed to be 2-times DOC by weight) fractions: 65% FA and 35% inert; 55% FA, 10% HA, and 35% inert; and 100% FA. The saturation status of the assay solutions was examined with respect to $Al(OH)_3$ and $Fe(OH)_3$ as part of the model runs. In WHAM Model VI, the $Fe(OH)_3$ solubility expression as a function of pH is based on calculated activities of FeIII in natural waters as described in detail by Lofts et al.(7) On the basis of the same work of Lofts et al.,(7) the activities and solubility expression of FeIII were recalculated using Model VII.(24)

Results and Discussion

DOM Isolate and Assay-Solution Compositions

Comparisons between the major-ion compositions of the DOM isolates and the mean filtered streamwater compositions (Table S5 of the Supporting Information) show that more than 96% of the Ca was removed from the source waters by the ion-exchange step. The measured recovery (97–98%) of DOM for the two natural waters compares favorably with recoveries (70–107%)(31, 51) obtained using the same technique, with the order of the ion-exchange and evaporation steps reversed, and are similar to that (~95%) obtained with reverse osmosis.(22) The assay-solution compositions (Tables S1–S4 of the Supporting Information), including the ionic strengths calculated by WHAM VII from the input compositions, were generally close to the target values (Table 1). In the case of BG and SRHA, lower concentrations of DOC were used in assays 2–6 because of limited availability of DOM. The measured pH values and total Cu concentrations were invariably close to the target values, and showed little variation between the triplicated assay solutions. The standard deviations of these two variables are shown for each assay in Tables S1–S4 of the Supporting Information. Table 2 compares the range of the concentration ratios of total dissolved Cu to DOC in unpolluted and polluted streams with those in the assay solutions. It is evident that the assays encompass the typical environmental range, thus fulfilling one of the key requirements of the experimental study. The concentrations of NTA and EDTA in the isolated DOM were below detection (<20 nM), hence excluding the possibility of significant Cu-binding by these anthropogenic ligands.

Cu-Binding Assays

The experiments resulted in similar patterns of Cu speciation across the six assays for all the IHSS standards (Figure 1) and DOM isolates (Figure 2). Both figures show that the measured Cu^{2+} activities decreased with decreasing [Cu]/[DOC] ratios (assays 1 to 4), in the absence of competing metals (assay #3 cf. assay #5), and with increasing pH (assay #5 cf. assay #6). The associated error bars are based on the triplicated experiments and represent the combined effects of experimental and analytical variability (small differences in experimental conditions and errors). The figures also show a series of predictions of Cu^{2+} activities, based on the WHAM/Model VII and NICA–Donnan models and using various assumptions. In the case of the DOM isolates (Figure 2), the columns correspond to the activities obtained using the most common assumption employed in applications of WHAM to natural waters, concerning the nature of DOM (65% FA, 35% inactive). In all cases, the predictions shown as columns, used the measured concentrations of Fe and Al as model inputs of Fe(III) and Al. The associated error bars represent the variability (±1SD) obtained from the uncertainty analysis in the WHAM predictions. The Cu^{2+} activity predicted by both models generally agreed well with the experimental measurements. Uncertainty in the WHAM VII predictions was always greater than the variability associated with the assay experiments, and the difference between predictions and measurements was almost invariably within the uncertainty of the predictions. Because WHAM VII has only recently been published, the resulting predictions were compared with those of its widely used predecessor, WHAM VI. The two versions of the model generally gave very similar results in the case of fulvic acid, with a maximum difference in pCu of ~0.4 (see Figure S4 of the Supporting Information). Differences were more evident in the case of humic acid, with the largest (~0.8 pCu) occurring with assay #5, involving competition at pH 6. When sufficient fractions of the discrete sites of the WHAM VII model take part in the adsorption process, the differences between NICA-

Donnan and WHAM VII predictions are likely to be small. However, at low Cu^{2+} activities, the number of discrete sites that takes part in the adsorption becomes small which explain the observed differences between NICA-Donnan and WHAM VII predictions in assay #4 (Figures 1 and 2). Results clearly demonstrate that model predictions do not deviate from experimental observations at high DOM concentrations. While some degree of aggregation cannot be discounted, this suggests that any resulting effects on Cu-DOM binding are insignificant.

Predictions are also shown in Figures 1 and 2 with the DOC and Fe(III) concentrations separately set to zero. The former case emphasizes the scale of organic complexation and indicates that the prediction of inorganic speciation is very similar with the two models. The predictions at zero Fe(III) highlight a significant difference between the models. With WHAM VII, the absence of Fe(III) invariably resulted in a significantly lower Cu^{2+} activity (by 0.2–1.0 pCu), consistent with the competition between Cu^{2+} and Fe^{3+} reported by Tipping et al.(52) Although constants for Fe binding to HS are uncertain,(53) without the inclusion of Fe(III), agreement between experimental measurements and WHAM VII predictions would be considerably poorer. NICA-Donnan predicted a similar Fe–Cu competition effect for SRHA (i.e., humic acid), but little or no competition when the humic material was fulvic acid (SRFA, RS and BG). The models were also run with the competing metal concentrations variously set to zero. These simulations indicated that the competition effect seen in assay #5 (cf. assay #3) was due mainly to Ca. The competition effects of Zn and Ni were both relatively small, even though they were each present at ~5-times the Cu total concentration. A WHAM simulation considering a range of [K]/[Ca] ratios showed no apparent effect of Ca competition on Cu-DOM binding (see Figure S5 of the Supporting Information).

Both the WHAM VII and ORCHESTRA models predicted that Fe(III) and Al hydroxides were under-saturated in all assay solutions, despite the fact that equilibrium dialysis measurements on the two DOM isolates indicated the presence of submicrometer colloidal particles of Fe(III) “oxides”. Under-saturation can be explained by the fact that the isolates were significantly diluted in the assay solutions and the hydrogen-ion concentration was invariably increased by over an order of magnitude in the assays relative to the isolates. Thus, the extent of solubilization was increased compared to its original state in the isolates. The long (2 weeks) incubation time used in the assays would have increased the likelihood of complete dissolution. A “worst-case” speciation calculation with both models, assuming no dissolution, indicated that the extent of Cu adsorption by Fe oxide would be negligible, compared with that complexed by DOM and so adsorption to any remaining solid phase would not affect the comparisons between measured and modeled concentrations.

Figure 3 shows that predicted and measured Cu^{2+} activities correlate well for all the assay data. The WHAM VII predictions lie very close to the 1:1 line throughout the range, whereas with NICA-Donnan predicted activities are slightly and systematically low at high pCu. Because of this trend, root-mean-square errors (RMSE) based on the differences between the measured and predicted activities were higher for NICA-Donnan (Table 3). For both models, RMSE values were similar for IHSS standards and natural DOM isolates. The RMSE (0.21 pCu) obtained for the isolates with WHAM VII is equivalent to a prediction accuracy of a factor of ~2. It is unlikely that any greater accuracy can be expected, given the scale of the model uncertainty due to the natural variability in binding strengths of aquatic humic substances (see Figure 2). This finding suggests that errors associated with the isolation of humic substances for model calibration are small compared with the 2 orders of magnitude differences for Cu^{2+}, observed between in situ measurements and WHAM VII across a much wider range of water types.(16) Recent studies have systematically examined some of the other uncertainties in making speciation predictions, such as the significance of other ligands (22) and the active DOM fraction.(55)

Mueller et al.(55) found that two measures of the spectroscopic quality of DOM correlated with individually optimized values of the active DOM fraction (assumed to be entirely FA) across eight Canadian Shield lakes, suggesting the possibility of a simple method for predetermining the active fraction for use as a model input. An indication of the sensitivity of the models to different assumptions about the active fractions is given in Figure S6 of the Supporting Information and Table 3, based on the alternative model inputs used in the present study. The effect of the various assumptions on the RMSE values (Table 3) indicates that small but significant improvements to model predictions could be made, especially if a reliable method existed for predetermining the active DOM fraction of individual waters.

Environmental Context

The assay results of this work demonstrate that existing speciation models can successfully predict copper binding by naturally occurring organic matter, measured over an environmentally relevant range of [Cu]/[DOC] ratios (1–460 μmol g^{-1}), using a well-proven analytical technique under carefully controlled conditions. A similar conclusion was reached in an earlier study where measurements were made in natural waters with high Cu contents (30) but importantly our measurements, using isolates, have extended the range of [Cu]/[DOC] to lower values, typical of unpolluted waters. It follows that the methodology routinely used to isolate fulvic and humic acids, on which the models rely for calibration purposes, does not significantly alter the binding characteristics of the materials, at least for Cu, and most probably for other metals binding strongly to carboxylic and phenolic groups. However, as this work has focused on only two waters, both dominated by humic substances, similar studies of a wider range of waters including those with anthropogenic sources of DOC, are warranted. Given the previously reported discrepancies between in situ measurements and modeling, there is a need for further studies focused on specific uncertainties in speciation modeling, so that the significance of each can be determined and, where possible, the modeling strategies refined. The ionic strength of freshwaters is substantially less than that of the concentrated isolates measured here. This mismatch in ionic strength is a potential source of errors, but it is accounted for by the speciation models. Thus, ionic strength is unlikely to be a major factor explaining differences between in situ measurements and model predictions.

The two systematic differences between WHAM VII and NICA-Donnan identified in this study, involving Cu–Fe(III) competition for fulvic acid and Cu^{2+} predictions at low activities ($<10^{-9}$ M), also warrant a further study. In this context, it is important to remember that at low metal activities both the NICA-Donnan and WHAM VII would be outside their calibration range meaning that different prediction results are likely. The chosen pH range (4–6) in this study is opportune as it provides reliable results due to the negligible Cu–carbonate complexation. However, this pH range limits our conclusions in respect of how well the models predict speciation in natural waters. Nevertheless, this work presents an experimental system suitable for extending the analysis to waters at higher pH.

Discrepancies between free ion measurements and predictions may be related to measurement inaccuracies, as well as to modeling errors and uncertainties. In their assessment of the published data, Lofts and Tipping (24) noted that competitive ligand exchange (CLE) voltammetry generally measured Cu free-ion concentrations far below (down to 10^{-16} M) the associated WHAM VII predictions (generally $>10^{-12}$ M), and that the differences were consistent with previous criticisms of the CLE methodology, when applied to strong binding metals. Concentrations of Cu and DOC for the various river and lake waters measured in the original CLE studies (56-60) were used to calculate the [Cu]/[DOC] ratios, which were in the range 2–30 μmol g^{-1}. As the assay ratios used in this work embrace this range, it is significant that the free Cu^{2+} concentrations measured in the assays were consistent with the Model VII predictions over the range 10^{-6}–10^{-12} M, in contrast to the situation with the CLE studies.

Supporting Information

Detailed compositions of assay solutions containing IHSS fulvic and humic acids, DOM isolated from Rough Sike and Brown Gill sites are provided. Additionally, figures on the response time curves and the potentiometric response curve of Cu–ISE, comparison of model predictions of Cu–DOM binding between WHAM VII, WHAM VI, and NICA–Donnan model, and the sensitivity of the WHAM VII and NICA–Donnan models to alternative assumptions about the active DOM fraction are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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References

1. USEPA Aquatic Life Ambient Freshwater Quality Criteria–Copper (2007 Revision); U.S. Environmental Protection Agency, Office of Water: Washington, D.C., 2007.
2. ICMM; EuroMetaux.; Defra; EURAS MERAG: Metals Environmental Risk Assessment Guidance; The International Council on Mining and Metals (ICMM): London, UK, 2007.
3. Stockdale, A.; Tipping, E.; Lofts, S.; Ormerod, S. J.; Clements, W. H.; Blust, R. Toxicity of proton-metal mixtures in the field: linking stream macroinvertebrate species diversity to chemical speciation and bioavailability Aquat. Toxicol. 2010, 100, 112– 119.
4. Lofts, S.; Spurgeon, D. J.; Svendsen, C.; Tipping, E. Deriving soil critical limits for Cu, Zn, Cd, and Pb: A method based on free ion concentrations Environ. Sci. Technol. 2004, 38, 3623– 3631.
5. Tipping, E.; Rothwell, J. J.; Shotbolt, L.; Lawlor, A. J. Dynamic modelling of atmospherically-deposited Ni, Cu, Zn, Cd and Pb in Pennine catchments (Northern England) Environ. Pollut. 2010, 158, 1521– 1529.
6. Tipping, E.; Vincent, C. D.; Lawlor, A. J.; Lofts, S. Metal accumulation by stream bryophytes, related to chemical speciation Environ. Pollut. 2008, 156, 936– 943.
7. Lofts, S.; Tipping, E.; Hamilton-Taylor, J. The chemical speciation of Fe(III) in freshwaters Aquat. Geochem. 2008, 14, 337– 358.
8. Gustafsson, J. P.Windows version of MINTEQA2.
9. Keijzer, M. G.; Van Riemsdijk, W.a Computer Program for the Equilibrium Calculation of Speciation and Transport in Soil-Water Systems (ECOSAT 4.7); Wageningen Univiversity: Wageningen, The Netherlands, 2002.
10. Benedetti, M. F.; Milne, C. J.; Kinniburgh, D. G.; Van Riemsdijk, W. H.; Koopal, L. K. Metal ion binding to humic substances: Application of the non-ideal competitive adsorption model Environ. Sci. Technol. 1995, 29, 446– 457.

11. Kinniburgh, D. G.; Van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena, M. J. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency Colloids Surf. A. 1999, 151, 147– 166.
12. Tipping, E. WHAM—A chemical-equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site electrostatic model of ion-binding by humic substances Comput. Geosci. 1994, 20, 973– 1023.
13. Tipping, E. Humic ion-binding model VI: An improved description of the interactions of protons and metal ions with humic substances Aquat. Geochem. 1998, 4, 3– 48,
14. Lofts, S.; Tipping, E. WHAM6. Windermere Humic Aqueous Model. Equilibrium Chemical Speciation for Natural Waters; Natural Environment Research Council: Lancaster, 2001.
15. Hamilton-Taylor, J.; Ahmed, I. A. M.; Davison, W.; Zhang, H. How well can we predict and measure metal speciation in freshwaters? Environ. Chem. 2011, 8, 461– 465,
16. Tipping, E.; Lofts, S.; Sonke, J. E. Humic ion-binding model VII: A revised parameterisation of cation-binding by humic substances Environ. Chem. 2011, 8, 225– 235,
17. Tipping, E. Cation Binding by Humic Substances; Cambridge Environmental Chemistry Series; Cambridge University Press: Cambridge, UK, 2002.,
18. Xue, H.; Sigg, L. Comparison of the complexation of Cu and Cd by humic or fulvic acids and by ligands observed in lake waters Aquat. Geochem. 1999, 5, 313– 335,
19. Wu, F.; Tanoue, E. Isolation and partial characterization of dissolved copper-complexing ligands in streamwaters Environ. Sci. Technol. 2001, 35, 3646– 3652
20. Xue, H.; Sigg, L.; Kari, F. G. Speciation of EDTA in natural waters: Exchange kinetics of Fe-EDTA in river water Environ. Sci. Technol. 1995, 29, 59– 68
21. Nowack, B.; Xue, H.; Sigg, L. Influence of natural and anthropogenic ligands on metal transport during infiltration of river water to groundwater Environ. Sci. Technol. 1997, 31, 866– 872
22. Baken, S.; Degryse, F.; Verheyen, L.; Merckx, R.; Smolders, E. Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands Environ. Sci. Technol. 2011, 45, 2584– 2590
23. Tang, D.; Warnken, K. W.; Santschi, P. H. Distribution and partitioning of trace metals (Cd, Cu, Ni, Pb, Zn) in Galveston Bay waters Mar. Chem. 2002, 78, 29– 45,
24. Lofts, S.; Tipping, E. Assessing WHAM/Model VII against field measurements of free metal ion concentrations: model performance and the role of uncertainty in parameters and inputs Environ. Chem. 2011, 8, 501– 516,
25. Groenenberg, J. E.; Koopmans, G. F.; Comans, R. N. J. Uncertainty analysis of the nonideal competitive adsorption–Donnan model: Effects of dissolved organic matter variability on predicted metal speciation in soil solution Environ. Sci. Technol. 2010, 44, 1340– 1346

26. Unsworth, E. R.; Warnken, K. W.; Zhang, H.; Davison, W.; Black, F.; Buffle, J.; Cao, J.; Cleven, R.; Galceran, J.; Gunkel, P.; Kalis, E.; Kistler, D.; Van Leeuwen, H. P.; Martin, M.; Noel, S.; Nur, Y.; Odzak, N.; Puy, J.; Van Riemsdijk, W.; Sigg, L.; Temminghoff, E.; Tercier-Waeber, M. L.; Toepperwien, S.; Town, R. M.; Weng, L. P.; Xue, H. B. Model predictions of metal speciation in freshwaters compared to measurements by in situ techniques Environ. Sci. Technol. 2006, 40, 1942– 1949
27. Warnken, K. W.; Lawlor, A. J.; Lofts, S.; Tipping, E.; Davison, W.; Zhang, H. In situ speciation measurements of trace metals in headwater streams Environ. Sci. Technol. 2009, 43, 7230– 7236
28. Vasyukova, E.; Pokrovsky, O. S.; Viers, J.; Dupré, B. New operational method of testing colloid complexation with metals in natural waters Appl. Geochem. 2012, 27, 1226– 1237,
29. Lyvén, B.; Hassellöv, M.; Turner, D. R.; Haraldsson, C.; Andersson, K. Competition between iron- and carbon-based colloidal carriers for trace metals in a freshwater assessed using flow field-flow fractionation coupled to ICPMS Geochim. Cosmochim. Acta 2003, 67, 3791– 3802,
30. Bryan, S.; Tipping, E.; Hamilton-Taylor, J. Comparison of measured and modelled copper binding by natural organic matter in freshwaters Comp. Biochem. Phys. C 2002, 133, 37–49,
31. Thacker, S. A.; Tipping, E.; Baker, A.; Gondar, D. Development and application of functional assays for freshwater dissolved organic matter Water Res. 2005, 39, 4559–4573, ,
32. Kitis, M.; Kilduff, J. E.; Karanfil, T. Isolation of dissolved organic matter (dom) from surface waters using reverse osmosis and its impact on the reactivity of dom to formation and speciation of disinfection by-products Water Res. 2001, 35, 2225– 2234, ,
33. Peuravuori, J.; Pihlaja, K. Preliminary Study of Lake Dissolved Organic Matter in Light of Nanoscale Supramolecular Assembly Environ. Sci. Technol. 2004, 38, 5958– 5967
34. Tipping, E.; Billett, M. F.; Bryant, C. L.; Buckingham, S.; Thacker, S. A. Sources and ages of dissolved organic matter in peatland streams: Evidence from chemistry mixture modelling and radiocarbon data Biogeochemistry 2010, 100, 121– 137,
35. ISO 16588:2002 Water quality—Determination of six complexing agents – gas chromatographic method; International Organization for Standardization: Geneva, Switzerland, 2002.Google Scholar
36. Christl, I.; Milne, C. J.; Kinniburgh, D. G.; Kretzschmar, R. Relating ion binding by fulvic and humic acids to chemical composition and molecular size. 2. Metal binding Environ. Sci. Technol. 2001, 35, 2512– 2517
37. Davison, W. A practical guide to pH measurement in freshwaters TRAC-Trends Anal. Chem. 1990, 9, 80– 83,

38. Avdeef, A.; Zabronsky, J.; Stuting, H. H. Calibration of copper ion selective electrode response to pCu 19 Anal. Chem. 1983, 55, 298– 304

39. Martell, A. E.; Smith, R. M. Critical Stability Constants: Inorganic Complexes; Plenum Press: New York, 1976; Vol. 4.Google Scholar

40. Martell, A. E.; Smith, R. M. Critical Stability Constants: Amines; Plenum Press: New York, 1975; Vol. 2.Google Scholar

41. Parkhurst, D. L.; PHREEQC, A.Computer Program for Speciation, Reaction-Path, Advective Transport, and Inverse Geochemical Calculations; US Department of the Interior: Lakewood, CO, 1995.Google Scholar

42. Schecher, W. D.; McAvoy, D. C. MINEQL+: A software environment for chemical-equilibrium modeling Comput. Environ. Urban Systems 1992, 16, 65– 76,

43. Meeussen, J. C. L. ORCHESTRA: An object-oriented framework for implementing chemical equilibrium models Environ. Sci. Technol. 2003, 37, 1175– 1182

44. Bryan, S. E. Testing models of chemical speciation in freshwaters. PhD Thesis, Lancaster University: Lancaster, UK, 2001.Google Scholar

45. Kinniburgh, D. G.; Van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena, M. J. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency Colloids Surf., A 1999, 151, 147– 166,

46. US-EPA MINTEQA2/PRODEFA2, A Geochemical Assessment Model for Environmental Systems: User Manual Supplement for Version 4.0; National Exposure Research Laboratory, Ecosystems Research Division: Athens, GA, 1999.Google Scholar

47. Milne, C. J.; Kinniburgh, D. G.; Van Riemsdijk, W. H.; Tipping, E. Generic NICA-Donnan model parameters for metal-ion binding by humic substances Environ. Sci. Technol. 2003, 37, 958– 971

48. Tipping, E.; Rieuwerts, J.; Pan, G.; Ashmore, M. R.; Lofts, S.; Hill, M. T. R.; Farago, M. E.; Thornton, I. The solid–solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales Environ. Pollut. 2003, 125, 213– 225, ,

49. Vulkan, R.; Zhao, F. J.; Barbosa-Jefferson, V.; Preston, S.; Paton, G. I.; Tipping, E.; McGrath, S. P. Copper speciation and impacts on bacterial biosensors in the pore water of copper-contaminated soils Environ. Sci. Technol. 2000, 34, 5115– 5121

50. Zhao, L. Y. L.; Schulin, R.; Weng, L.; Nowack, B. Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns Geochim. Cosmochim. Acta 2007, 71, 3407– 3418,

51. Gondar, D.; Thacker, S. A.; Tipping, E.; Baker, A. Functional variability of dissolved organic matter from the surface water of a productive lake Water Res. 2008, 42, 81– 90, ,

52. Tipping, E.; Rey-Castro, C.; Bryan, S. E.; Hamilton-Taylor, J. Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation Geochim. Cosmochim. Acta 2002, 66, 3211– 3224,
53. Hiemstra, T.; Van Riemsdijk, W. H. Biogeochemical speciation of Fe in ocean water Marine Chemistry 2006, 102, 181– 197,
54. Sigg, L.; Black, F.; Buffle, J.; Cao, J.; Cleven, R.; Davison, W.; Galceran, J.; Gunkel, P.; Kalis, E.; Kistler, D.; Martin, M.; Noël, S.; Nur, Y.; Odzak, N.; Puy, J.; Van Riemsdijk, W.; Temminghoff, E.; Tercier-Waeber, M.-L.; Toepperwien, S.; Town, R. M.; Unsworth, E.; Warnken, K. W.; Weng, L.; Xue, H.; Zhang, H. Comparison of Analytical Techniques for dynamic trace metal speciation in natural freshwaters Environ. Sci. Technol. 2006, 40, 1934– 1941
55. Mueller, K. K.; Lofts, S.; Fortin, C.; Campbell, P. G. C. Trace metal speciation predictions in natural aquatic systems: Incorporation of dissolved organic matter (DOM) spectroscopic quality Environ. Chem. 2012, 9, 356– 368,
56. Meylan, S.; Odzak, N.; Behra, R.; Sigg, L. Speciation of copper and zinc in natural freshwater: Comparison of voltammetric measurements, diffusive gradients in thin films (DGT) and chemical equilibrium models Anal. Chim. Acta 2004, 510, 91– 100,
57. Plöger, A.; Fischer, E.; Nirmaier, H.-P.; Laglera, L. M.; Monticelli, D.; Van den Berg, C. M. G. Lead and copper speciation in remote mountain lakes Limnol. Oceanogr. 2005, 50, 995– 1010,
58. Guthrie, J. W.; Hassan, N. M.; Salam, M. S. A.; Fasfous, I. I.; Murimboh, C. A.; Murimboh, J.; Chakrabarti, C. L.; Grégoire, D. C. Complexation of Ni, Cu, Zn, and Cd by DOC in some metal-impacted freshwater lakes: A comparison of approaches using electrochemical determination of free-metal-ion and labile complexes and a computer speciation model, WHAM V and VI Anal. Chim. Acta 2005, 528, 205– 218,
59. Xue, H.; Oestreich, A.; Kistler, D.; Sigg, L. Free cupric ion concentrations and Cu complexation in selected Swiss lakes and rivers Aquat. Sci.–Res. Across Boundaries 1996, 58, 69– 87,
60. Xue, H.; Sigg, L. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake Limnol. Oceanogr. 1993, 38, 1200– 1213

List of tables

Table 1. Target Conditions of the Laboratory Binding Assays

assay no.	ionic strength/medium	pH	DOC mg dm^{-3}	added metals (μM)	[Cu]/[DOC] μmol g^{-1}
1	100 mM KNO_3	6.0	5	Cu(2.5)	500
2	100 mM KNO_3	6.0	40	Cu(2.5)	62.5
3	100 mM KNO_3	6.0	100	Cu(1)	10
4	100 mM KNO_3	6.0	100	Cu(0.1)	1
5	100 mM (K/Ca[a])NO_3	6.0	100	Cu(1), Zn(5), Ni(5)	10
6	100 mM (K/Ca[a])NO_3	4.0	100	Cu(1), Zn(5), Ni(5)	10

[a] Ca added as Ca(NO3)2 with a molar ratio of [K]/[Ca] of 1:1 in IHSS SRFA and SRHA assays (see Tables S1 and S2 of the Supporting Information) and a ratio of 3:1 in the natural water assays (see Tables S3 and S4 of the Supporting Information.)

Table 2. Total Dissolved Cu (filtered <0.45 or <0.2 μm) and DOC Concentrations, And Their Ratios, In Polluted and Unpolluted Streams, And in the Assay Solutions

stream characteristics	Cu_{Total} (nM)	DOC (mg L^{-1})	[Cu]/[DOC] (μmol g^{-1})	references
polluted	30	1.9	16	54
unpolluted, draining peat moorland	22	15	1.5	54
34 streams, polluted/unpolluted mix	2.4–217	0.6–13.2	1.0–310[a]	27
Brown Gill assays	55–2428	5 −50	1– 460	this study
Rough Sike assays	103–2219	5 −100	1 – 430	this study

[a] Based on measured ratios for individual streams.

Table 3. Root Mean Square Error (RMSE) Values Obtained from the Differences between Measured and Predicted Values for Various Model Inputs of Fulvic Acid (FA) and Humic Acid (HA) Fractions

model	SRFA	SRHA	Rough Sike	Brown Gill
WHAM (FA = 0.65)			0.273	0.159
WHAM (FA = 0.55, HA = 0.10)			0.261	0.139
WHAM (FA = 1.0)	0.318	0.373[a]	0.498	0.312
NICA-Donnan (FA = 0.65)			0.629	0.399
NICA-Donnan (FA = 0.55, HA = 0.10)			0.789	0.509
NICA-Donnan (FA = 1.0)	0.475	0.594[a]	1.03	0.206

[a] HA = 1.0 used as the input.

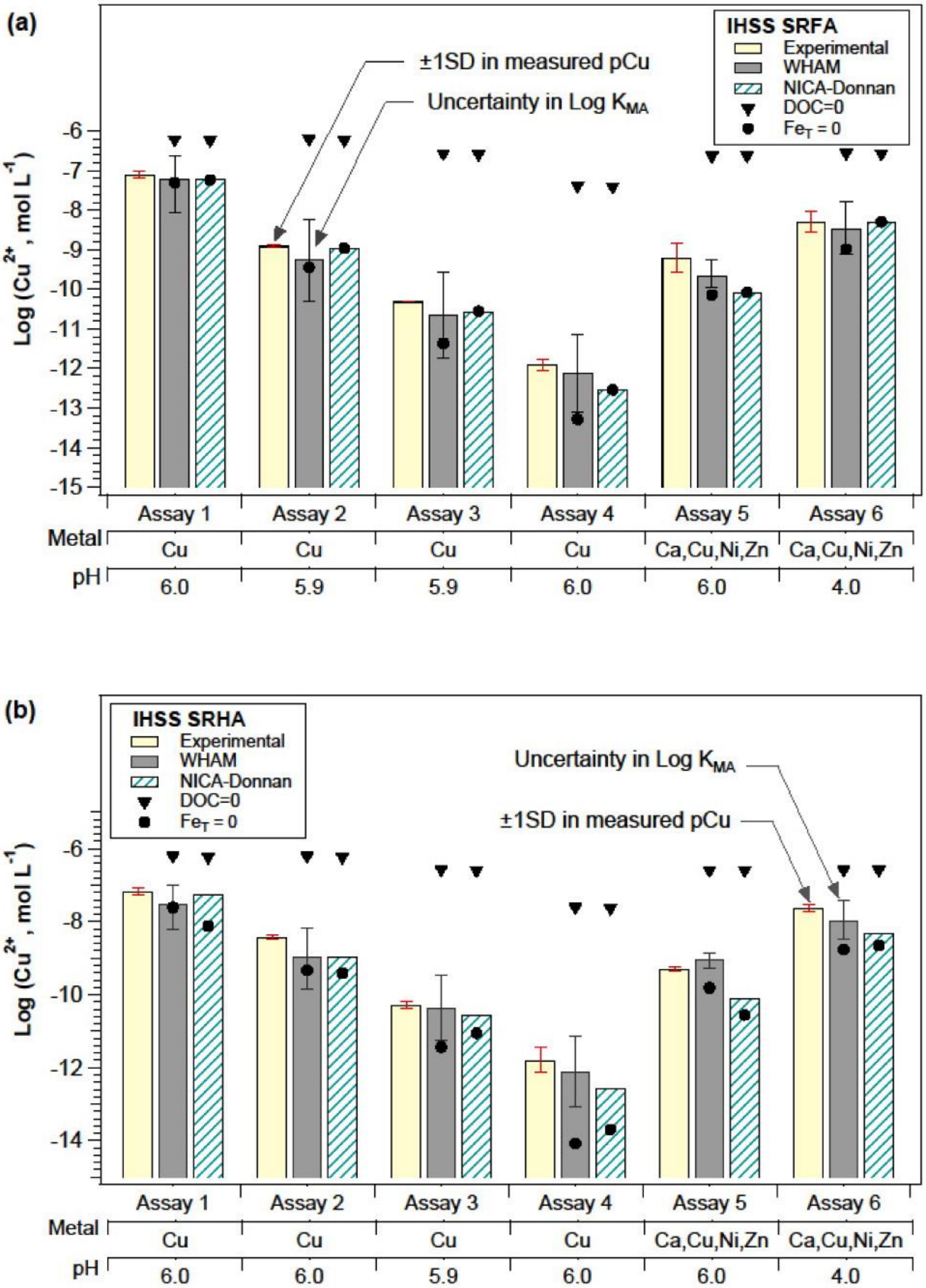

Figure 1. Comparison of ISE-measured pCu with WHAM VII and NICA–Donnan model predictions in samples containing IHSS Suwannee river (a) fulvic acid and (b) humic acid. Additional model predictions were carried out assuming a zero concentration of DOM or total Fe in sample. The error bars represent ±1 standard deviation (SD) in measured pCu and ±1 SD in pCu predicted by WHAM when considering the uncertainty in log K_{MA}. All speciation predictions assumed an active DOC fraction (f) of fulvic or humic acid of 100%.

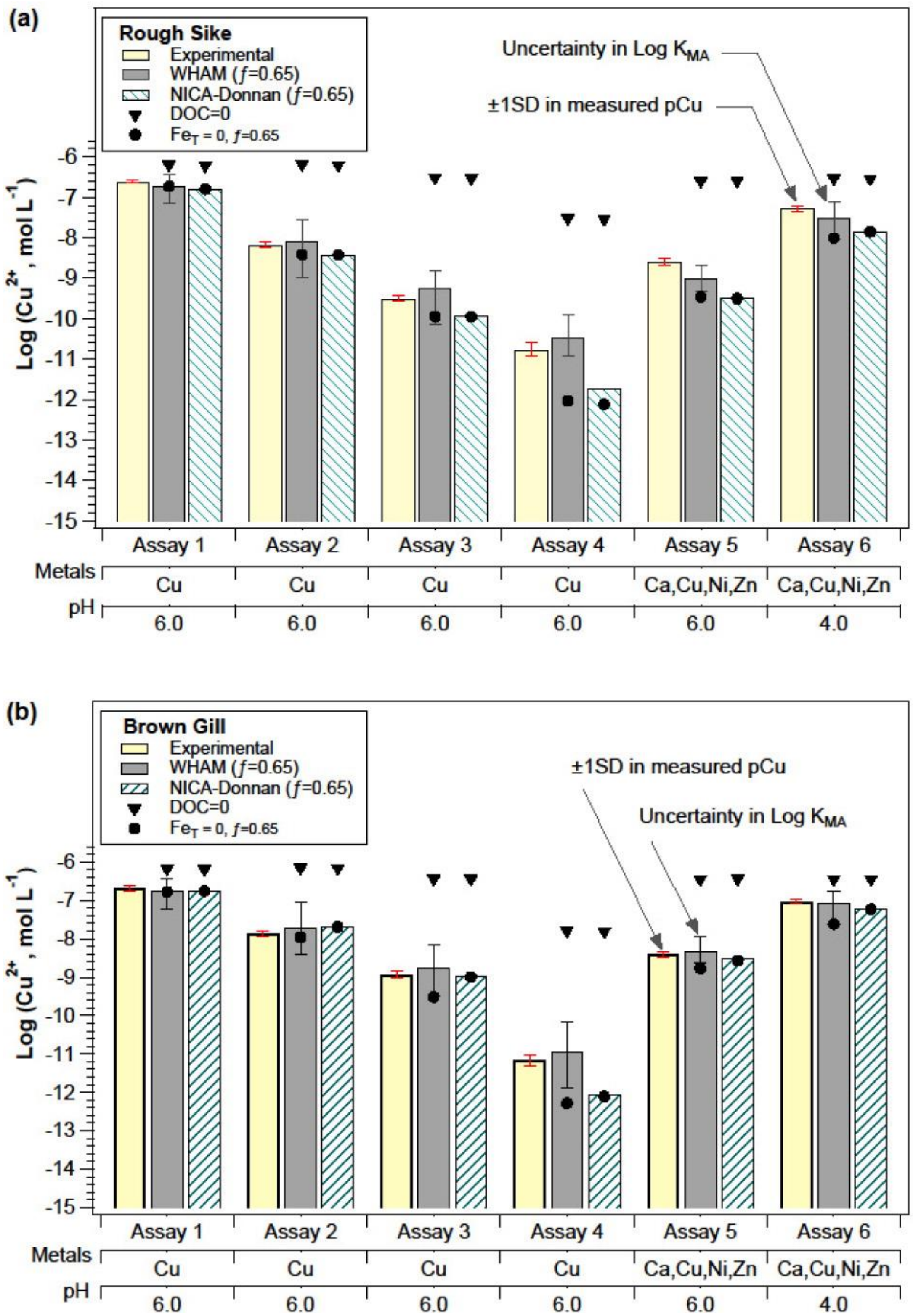

Figure 2. Comparison of ISE-measured pCu with WHAM VII and NICA–Donnan model predictions in samples containing DOM isolated from (a) Rough Sike and (b) Brown Gill. Additional model predictions were carried out assuming a zero concentration of DOC or total Fe in sample. All speciation predictions assumed an active DOC fraction (f) of fulvic acid of 65%. Error bars have the same meaning as in Figure 1.

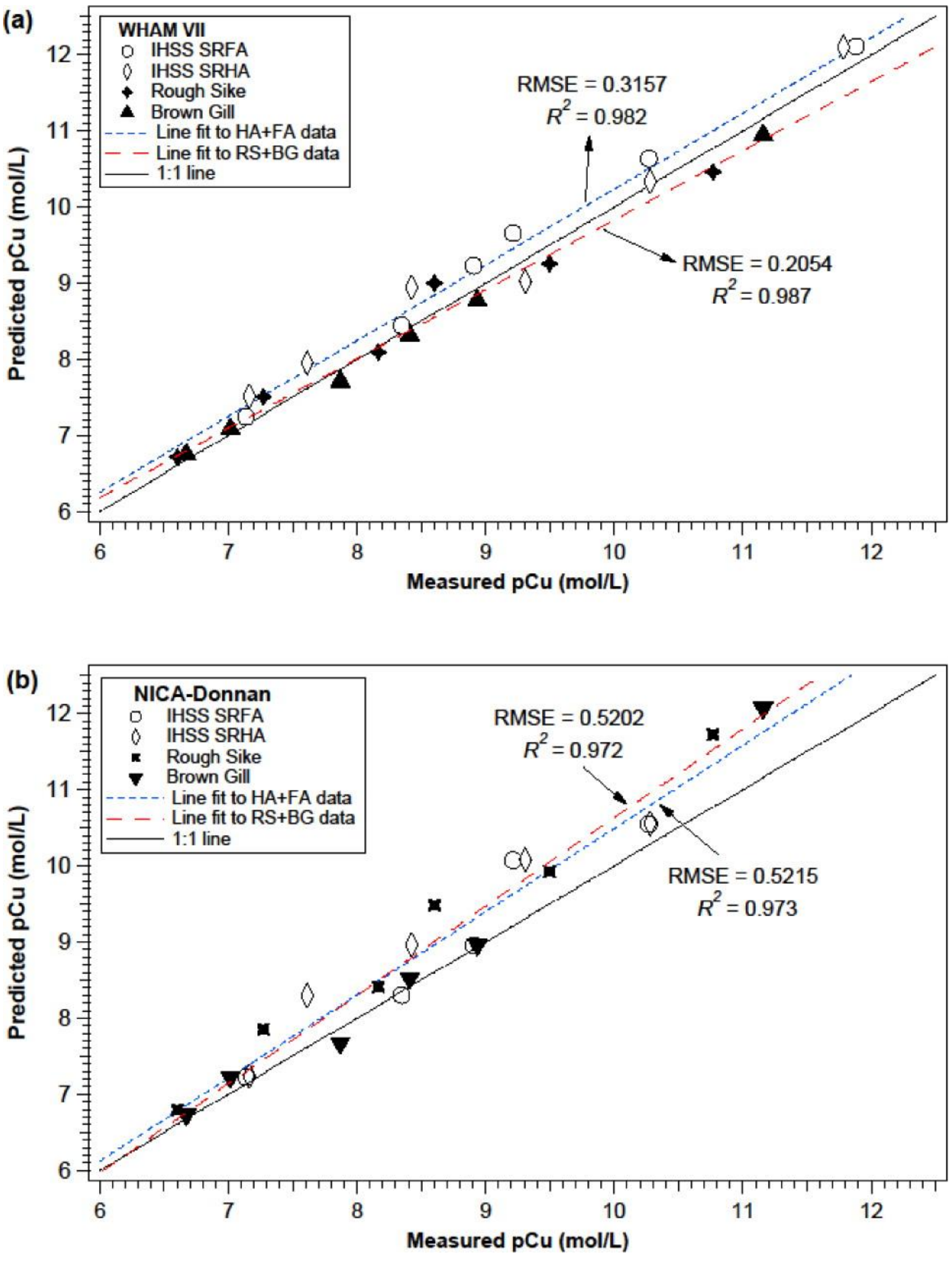

Figure 3. (a) WHAM VII predicted versus measured pCu and (b) NICA–Donnan predicted versus measured pCu. The solid line is the ideal 1:1 fit. The dotted and dashed lines represent the best-fit linear regressions to data for IHSS reference materials (i.e., SFRA and SRHA) and isolated DOM (i.e., Brown Gill and Rough Sike).