Is the computed speciation of copper in a wide range of Chinese soils reliable?

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ABSTRACT

Free Cu species in soils is a key issue to its bioavailability. However, predictive models for Cu speciation across a wide range of soils were still unavailable. In this study, Cu speciation in 34 contaminated soil samples were investigated via analytical technique and predictive models. The results showed that most of free Cu\(^{2+}\) was underestimated when using default log \(K_{\text{CuFA}}\) and 65% active fulvic acid as inputs in models of WHAM VI and NICA-Donnan. The best prediction was found when using either adjusted active fulvic acid from 10% to 125% for WHAM VI or from 15% to 65% for NICA-Donnan model with the RMSE < 0.32 and \(r^2 > 0.96\). In contrast, NICA-Donnan demonstrated a slightly stronger binding for Cu than WHAM VI due to extra 26% of samples was underestimated. This work presents a comprehensive database of Cu speciation and an effective attempt of free Cu\(^{2+}\) prediction in a wide range of Chinese soils.

1. Introduction

It is well verified that the total metal concentration in soils is not a good indicator for its toxicity [1,2] which is mainly controlled by the metal in soil solution [3]. In solution systems, the free metal ion was hypothesized to control toxicity response of organisms [4]. It is relatively easy to determine the free metal speciation in fresh waters by analytical techniques or chemical models considering that properties of humic substances are comparatively stable and easily measurable [5]. In contrast, metal speciation in soils is more difficult to be confirmed due to the complexity, mainly in nature and sources of soil organic carbon (SOC) [6,7].

Copper (Cu) as an important trace element has been studied carefully in aquatic and terrestrial systems around the world. Also, it is one of the most affinity metals to humic substances compared with others [8–10]. It was reported that humic complexed form accounted for up to 99.9% of total Cu in solution [3,8,11] indicating that Cu speciation is extremely sensitive to humic substances heterogeneity in quantity and quality [12,13]. The dissolved organic matter (DOM) is a complex mixture of reactive molecules and its reactive part is dominated by humic substances, comprising humic acids (HA), fulvic acids (FA) and hydrophilic acid [8], and its effect on Cu speciation in soil systems are still unclear.

Free Cu\(^{2+}\) activity is considered as an important factor on evaluation of Cu toxicity [3,4,14] and could be determined via electrochemical analytical technique [15–17] or chemical model prediction. Given the intrinsic differences among models, the most reliable and well-tested chemical speciation models are recognized as WHAM (V-VII) [18,19] and NICA-Donnan [20] which were developed to describe the electrostatic and specific interactions between the charged humic substances and ions. Although both of models are considered as providing good fit to most data, but due to their different formulations, their predictions do not necessarily converge [21]. Also, a key question about evaluation of chemical speciation models is: how well do model predictions compare against speciation analytical technique? The uncertainties using these models are whether humic substances considered in the modelling are truly representative of the DOM in solution systems.

In the present study, Cu speciation was investigated based on a wide range of true soil pore water samples across China due to Chinese soils ranged widely in soil physicochemical properties [22] and Cu speciation as well as its partition in these soils are scarcely reported. The Visual MINTEQ incorporated with NICA-Donnan [23] and WHAM VI were used to estimate Cu speciation in
soil pore waters and compare with results from a cupric ion-selective electrode (Cu-ISE) technique. The aim of this study is to find a good predictable model for free Cu$^{2+}$ activity in Chinese soils and further applicability to the Cu risk assessment in terrestrial systems. It is very meaningful to undertake this exploration in Chinese soils not only because of the severely increasing pollution situation in China [24] but also presenting a comprehensive database of Cu speciation and modelling worldwide.

2. Materials and methods

2.1. Soil samples

Seventeen soil samples from multiple agricultural locations across China were collected. The bulk soil properties were measured and displayed in Table 1. Total concentrations of Cu in soils were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES; Spectro Flame Modula, Spectro), following digestion with aqua regia [25]. Soil pH was determined in a mixture of 1:5 soil:water (w/v). Total concentrations of carbon were determined using a LECO combustion analyzer (CNS 2000). The cation exchange capacity (CEC) was measured using unbuffered silver-thiourea method. Two contaminated Cu concentrations for each soil were obtained through adding designed Cu chloride (CuCl$_2$) amounts. The concentrations selected were referenced to barley root elongation assay in 17 Chinese soils with toxicity thresholds ranging from EC$_{50}$ (5% inhibition concentration) to EC$_{90}$ (90% inhibition concentration) reported by Li et al. [26], and a lower and higher inhibition concentrations of Cu to barley root elongation were 5 and 45 μm respectively. The Cu-ISE calibration solutions contained 10 grades of alumina (Al$_2$O$_3$) (first polished with 0.3 μm followed by the 0.05 μm) before use. The electrode was conditioned in a 500 ppb Cu standard (approximate pH 4) overnight before measurements for improving electrode sensitivity. The Cu-ISE calibration solutions contained 10 different Cu concentrations as follows: 5 ml of Cu(NO$_3$)$_2$ (1 mmol L$^{-1}$), 5 ml of inominodiacetic acid (0.01 mol L$^{-1}$), 5 ml of potassium acid phthalate (KHC$_8$H$_4$O$_4$) (0.025 mol L$^{-1}$), 5 ml of potassium acid phthalate (KHC$_8$H$_4$O$_4$) (0.025 mol L$^{-1}$), 5 ml of KNO$_3$ (0.1 mol L$^{-1}$) in each standard, 10 of

2.2. Extraction and analysis of soil pore water

Soil pore-water was extracted for contaminated soils using the method of Thibault and Sheppard [27]. The soils were rewetted to maximum water holding capacity (MWHC) with Milli-Q deionized water and incubated for 24 h at 25 °C in 25-ml filtration tubes. Soils were then centrifuged at 3500 RCF for 45 min with the filtration tube inside a 50-ml centrifuge tube that contained a small spacer in the bottom [1]. Extracted solutions were then centrifuged at 12,500 RCF for 45 min and filtered through a 0.45 μm filter. The total number of filtration tubes required generally varied from 10 to 15, giving a total volume of 60–100 ml, which was combined and then split into two duplicate samples for Cu-ISE and subsequent analysis. The extracted solutions were stored at 4 °C prior to analysis. The analysis of the solutions included dissolved organic carbon (DOC) (FormacsTOC/TNAnalyser), major cation concentration by ICP-OES [25], and major inorganic anion concentrations (i.e. CO$_3^{2-}$, Cl$^-$, NO$_3^{-}$ and SO$_4^{2-}$) by ion chromatography (IC; Dionex4000i, AS9-HC column).

2.3. Measurement of free Cu$^{2+}$ activity in pore water

The free Cu$^{2+}$ activity was determined with an Orion 94–29 Cu-ISE combination electrode with an AgS/CuS double junction reference electrode at 20 °C. The CuS crystal of the cupric electrode was polished for 2 min with 2 grades of alumina (Al$_2$O$_3$) (first polished with 0.3 μm followed by the 0.05 μm) before use. The electrode was conditioned in a 500 ppb Cu standard (approximate pH 4) overnight before measurements for improving electrode sensitivity. The Cu-ISE calibration solutions contained 10 of different Cu concentrations as follows: 5 ml of Cu(NO$_3$)$_2$ (1 mmol L$^{-1}$), 5 ml of inominodiacetic acid (0.01 mol L$^{-1}$), 5 ml of potassium acid phthalate (KHC$_8$H$_4$O$_4$) (0.025 mol L$^{-1}$), 5 ml of KNO$_3$ (0.1 mol L$^{-1}$) in each standard, 10 of

| No. | Site            | Location (longitude, latitude) | pH (1:5) | EC (μS cm$^{-1}$) | CEC (cmol+ kg$^{-1}$) | SOC (%) | Clay (%) <2 μm | Total Cu (mg kg$^{-1}$) |
|-----|-----------------|--------------------------------|----------|------------------|----------------------|---------|----------------|------------------------|
| S1  | Haikou          | 19°55′N,111°29′E              | 4.93     | 111              | 4.77                 | 1.51    | 66             | 50.5                   |
| S2  | Qiyang          | 26°45′N,111°52′E              | 5.31     | 74               | 5.52                 | 0.87    | 46             | 22.2                   |
| S3  | Hailun          | 47°28′N,126°57′E              | 6.54     | 153              | 29.5                 | 3.03    | 40             | 18.3                   |
| S4  | Jiaxing         | 30°77′N,120°76′E              | 6.70     | 159              | 21.4                 | 1.42    | 39             | 31.3                   |
| S5  | Hangzhou        | 30°26′N,120°25′E              | 6.80     | 203              | 14.9                 | 2.46    | 41             | 47.2                   |
| S6  | Chongqing       | 30°26′N,106°26′E              | 7.12     | 71               | 26.7                 | 0.99    | 27             | 10.7                   |
| S7  | Guangzhou       | 23°10′N,113°18′E              | 7.20     | 137              | 10.0                 | 1.47    | 25             | 10.8                   |
| S8  | Lingshan        | 39°55′N,116°8′E               | 7.48     | 93               | 25.4                 | 4.28    | 20             | 14.2                   |
| S9  | Hulunber        | 46°03′N,122°03′E              | 7.66     | 888              | 24.1                 | 2.66    | 37             | 14.7                   |
| S10 | Gongzhuling     | 42°40′N,124°88′E              | 7.82     | 147              | 29.1                 | 2.17    | 45             | 21.4                   |
| S11 | Shiijiahuang    | 38°03′N,114°26′E              | 8.19     | 302              | 16.8                 | 1.00    | 10             | 16.5                   |
| S12 | Urumchi         | 43°95′N,87°46′E               | 8.72     | 227              | 14.8                 | 0.87    | 25             | 26.5                   |
| S13 | Yangling        | 34°19′N,108°0′E               | 8.83     | 83               | 14.4                 | 0.62    | 16             | 21.5                   |
| S14 | Langfang        | 39°31′N,116°44′E              | 8.84     | 5.7              | 10.5                 | 0.6     | 21             | 9.51                   |
| S15 | Zhenghoudo      | 34°47′N,112°40′E              | 8.86     | 109              | 13.3                 | 1.57    | 28             | 13.4                   |
| S16 | Zhangye         | 38°56′N,100°27′E              | 8.86     | 152              | 13.1                 | 1.02    | 20             | 27.6                   |
| S17 | Dezhou          | 37°20′N,116°29′E              | 8.90     | 112              | 14.3                 | 0.69    | 18             | 15.0                   |
various volume of NaOH (0.02 mol L⁻¹) (i.e. 1, 4, 6, 8, 9, 9.2, 9.3, 9.4, 9.7 and 10 mL) and corresponding Milli-Q water for a total volume of 50 mL to obtain 10 different concentrations of Cu standards with gradually decreased pH (between 9.3 and 4.3). The 10 standard solutions for the calibration were deemed sufficient to get a representative linear regression equation with $r^2$ ≥ 0.99, and prepared the previous day to allow chemical equilibration overnight.

The free Cu²⁺ activities in pore waters were measured immediately after extraction, and in order of increasing free Cu²⁺ ion for better reproducibility and faster analysis. The pH was measured using micro electrode pH (Thermo Fisher Scientific Inc., MA, USA) [28]. The electrode potential (EP, mV) determined by Cu-ISE was then plotted against free Cu²⁺ activity (expressed as logarithm-transformed base) calculated by Visual MINTEQ which was particularly added stability constants of iminodiacetic acid according to Rachou et al. [16]. This showed a linear relationship with the following regression equation:

$$\log(\text{freeCu}^{2+}\text{activity}) = 0.0349 \times \text{EP} - 10.265$$

($n = 10, r^2 = 0.997$)

The slope of the electrode response obtained in the calibration (28.6 mV/free Cu²⁺) was close to the theoretical Nernstian slope of 29.08 at 20 °C.

### 2.4. Calculation of Cu speciation by models

#### 2.4.1. WHAM VI

The program was used to calculate free Cu²⁺ activities in soil pore water using measured parameters of pH, DOC concentration (g L⁻¹), total soluble cations (i.e. Cu, Ca, Mg, K and Na) and anions (i.e. Cl⁻, CO₃²⁻, NO₃⁻, and SO₄²⁻) as inputs. Default parameters for active proton and metal-binding by fulvic acid (FA, 50% C) were used and 100% DOM in soil solutions was defined as FA. In order to improve the prediction of free Cu²⁺ activity, reasonable assumptions on DOM active fraction (%AFA) in models and binding constants of FA to Cu (log $K$) were taken, which was actually a recalibration of the model localization [29]. The best agreement between measured and predicted free Cu²⁺ activity, expressed as log (free Cu²⁺ activity) was obtained according to the minimal root mean squared error (RMSE). The 65% AFA (35% FA as inert) as a starting value [30] for modelling, the FA input (g L⁻¹) for running the models was thus obtained by DOX × 2 × %AFA and dissolved cations and anions were expressed in total molar concentration (mol L⁻¹). As colloidal Fe(OH)₃ and Al(OH), can easily pass 0.45 μm filters, which result in measured ‘dissolved’ Fe and Al higher than the true condition that can interact with DOM and other ligands [12,30], consequently, free Fe³⁺ and Al³⁺ activities were used as input and estimated from the solubility constants of colloidal Al(OH)₃ ($\log K_{\text{sol}} = 8.5$) and Fe(OH)₃ ($\log K_{\text{sol}} = 3.0$) at 20 °C [31]. The temperature of 293 K and the atmospheric partial pressure of CO₂ ($P_{\text{CO}_2}$) of 10⁻¹⁵ atm were used in models.

#### 2.4.2. NICA-Donnan model

To ensure consistency, thermodynamic data (for inorganic metal complexes) from MINTEQA2 version 4 (http://www.epa.gov/ceampubl/mmedia/minteq/supple1.pdf) was used in databases of both humic substances. The temperature and $P_{\text{CO}_2}$ was used the same values in the two models. The default stability constants and 65% AFA were also used as the start values for Cu speciation computation. Similar to WHAM VI, the %AFA and stability constants were adjusted to get the best agreement between measured and predicted free Cu²⁺ activity.

### 3. Results

#### 3.1. Soil solid-phase properties

Selected soil solid-phase properties were shown in Table 1. The analytical data showed that there was a wide variation in soil characteristics. The soil pHs ranged from 4.93 to 8.90 (70% of soil with pH > 7.0), soil organic carbon content (SOC) from 0.60 to 4.28%, CEC from 4.77 to 29.5 cmol+ kg⁻¹, and clay content from 10 to 66%. The Cu concentration varied from 31 to 1485 mg kg⁻¹ with 48-fold difference (Table 2).

#### 3.2. Soil pore water properties and Cu distribution

Selected soil pore water properties were listed in Table 2. Soil pore water pH varied from 4.39 to 8.07. The concentrations of DOC ranged from 102 to 765 mg L⁻¹ with an average of 299 mg L⁻¹. Total concentrations of soluble Cu changed within two orders of magnitude, from 1.10

| Soluble cation (mmol L⁻¹) | Log (free Cu²⁺ activity) (mol L⁻¹) | %free Cu²⁺ activity | Log (K) (L kg⁻¹) |
|---------------------------|----------------------------------|---------------------|------------------|
| Ca²⁺ | Mg²⁺ | K⁺ | Na⁺ | Total Cu (μmol L⁻¹) | Total Cu (μmol L⁻¹) |
| Minimum | 30.8 | 6.89 | 9.49 | 1148 | 102 | 2.10 | 0.63 | 0.07 | 0.48 | 1.10 | −5.30 | 0.001 | 2.10 |
| Quartile 1 | 151 | 7.42 | 2683 | 246 | 11.04 | 1.73 | 0.21 | 1.08 | 5.83 | −7.80 | 0.006 | 2.50 |
| Median | 301 | 7.42 | 3367 | 246 | 14.46 | 4.83 | 0.38 | 2.13 | 10.2 | −8.48 | 0.006 | 2.50 |
| Mean | 450 | 7.11 | 4816 | 246 | 17.32 | 6.77 | 0.68 | 11.4 | 14.1 | −8.54 | 2.73 | 2.69 |
| Quartile 3 | 674 | 7.80 | 5091 | 246 | 21.47 | 6.79 | 1.10 | 6.14 | 19.7 | −9.69 | 0.006 | 2.50 |
| Maximum | 1485 | 8.07 | 17260 | 246 | 42.68 | 37.20 | 2.58 | 12.0 | 48.9 | −11.5 | 39.8 | 3.31 |
to 48.9 μmol L\(^{-1}\) for 34 samples. The free Cu\(^{2+}\) activity (log (free Cu\(^{2+}\))) varied from −11.5 to −5.30 by up to six orders of magnitude. The percentage of free Cu\(^{2+}\) activity to total soluble Cu in solution was diverse from a value of <0.1 to 39.8% with an average of 2.73%. Most of fractions were less than 0.5% except for a number of acidic soils with soil pore water pH < 6.5.

The data in Figure 1 showed that total soluble Cu in soil pore water was correlated significantly to total Cu concentrations in soil with \(r^2\) value of 0.55 and DOC in soil pore water to a lesser extent with \(r^2\) of 0.45. The stepwise regression analysis showed that the combined factors of total Cu concentrations in soils and DOC contents in soil pore water could explain 63% variances of soluble Cu in soil pore water (Equation 1 in Table 3).

The distribution of Cu between the solid and aqueous phases was described by a partition coefficient, \(K_d\) (solid phase concentration/solution phase concentration) \([32]\) were calculated and shown in Table 2. The range of \(K_d\) values varied from 126 to 2064 L kg\(^{-1}\). The regression analysis between \(K_d\) and soil or soil pore water properties based on logarithmic transferred data showed that dissolved Cu content and CEC were two important factors affecting \(K_d\) with an \(r^2\) value of 0.42 (Equation 3 in Table 3) and it was difficult to find a better correlation while took all soil pHs into consideration. However, there was an improved empirical prediction on \(K_d\) using total Cu concentrations in soil, CEC and DOC concentration when only analyzed the soils with pH > 7.2 (Equation 4 in Table 3). As soluble Cu was controlled by total Cu concentrations in soil and DOC in soil pore water, therefore, it was basically consistent for prediction on \(K_d\) for Equations 3 and 4.

### 3.4. Empirical prediction of free Cu\(^{2+}\)activity using soil or soil pore water properties

The free Cu\(^{2+}\) activities were influenced mainly by two factors: soil pore water pH and total Cu concentration (Equations 5 and 6, Table 3). The regressions in Table 3 explained reasonable proportions (65%) of the variability in free Cu\(^{2+}\). Other soil parameters, including SOM, CEC and clay content were excluded in explaining the variability in free Cu\(^{2+}\). The percentage of free Cu\(^{2+}\) to total soluble Cu was negatively related to soil pore water pH with \(r^2\) of 0.64 (Equation 7 in Table 3), which explained why the soils with low pH values had a higher percentage of free Cu\(^{2+}\) in pore water (3.64–39.8%). Also, Lofts et al. \([33]\) showed a strong relationship between log (free Cu\(^{2+}\) activity) and pH, which is similar to the results in our study. These results from Sauvé et al. \([34]\) (c.f. log (free Cu\(^{2+}\)) = −1.40 pH + 1.70 log (total Cu) – 3.42, \(r^2 = 0.85\)) and Vulkan et al. \([14]\) (c.f. log (free Cu\(^{2+}\)) = −1.79 pH + 1.47 log (total Cu) + 0.53, \(r^2 = 0.89\)) also confirmed the effect of pH and total Cu content on free Cu\(^{2+}\), despite a slight difference on coefficients of equations.

### 3.4. Prediction of Cu speciation using models

#### 3.4.1. WHAM VI

The agreement between calculated and measured free Cu\(^{2+}\) activity was evaluated using match degree of corresponding log (free Cu\(^{2+}\) activity) values due to free Cu\(^{2+}\) activity varied up to 6 orders of magnitude in soil pore water and almost 80% of free Cu\(^{2+}\) activity accounted for less than 0.5% of total soluble Cu. As the data shown in Figure 2(a), when 65% of AFA and default binding constants log \(K_{CuFA}\) as input values, 56% of free Cu\(^{2+}\) activity in soil pore water were severely underestimated by model especially for soils with lower pH values. In order to get the better agreement of free Cu\(^{2+}\) activity, adjusting AFA ratio between 10 and 30% for these 19 samples under-rated and maintaining 65% AFA for the other 15 samples, the regression between measured and calculated free Cu\(^{2+}\) activity was significantly improved with decreased RMSE 0.42 and increased \(r^2\) value of 0.94. Also, there were 4 in 34 samples (i.e. S7 and S11) overestimated by model for free Cu\(^{2+}\) activity (see Figure 2(a)), enhancing the AFA.
from 10 to 65% (36% on average) for samples with pore water pH 6.5–7.5, from 30 to 125% (69% on average) for samples with pore water pH > 7.5. For the three samples using 125% AFA as inputs for modelling, free Cu²⁺ activity was all lower than 10⁻¹⁰ mol L⁻¹.

Meanwhile, in order to weaken the binding of Cu to DOM for samples undervalued, log \( K_{CuFA} \) was decreased from 2.1 to 1.0 and to intensify Cu affinity to DOM for samples overvalued, log \( K_{CuFA} \) was increased from 2.1 to 2.3. The best agreement between measured and predicted free Cu²⁺ activity was achieved using adjusted AFA ratio ranging from 10 to 125% for all samples with the smallest RMSE of 0.23 and the highest \( r^2 \) of 0.98 (see Figure 2(b) and Table 4).

Table 3. The regression equations between Cu speciation and soil or soil pore-water properties.

| No. | Regression equations                                                                 | \( r^2 \) | \( N \) |
|-----|--------------------------------------------------------------------------------------|----------|--------|
| 1   | \( \log(\text{soluble Cu}) = 0.515 \log(\text{total Cu in soil}) + 1.223 \log(\text{DOC}) - 2.976 \) | 0.63     | 34     |
| 2   | \( \log(K_d) = 0.475 \log(\text{total Cu in soil}) - 0.666 \log(\text{DOC}) + 3.124 \) | 0.28     | 34     |
| 3   | \( \log(K_d) = -0.452 \log(\text{soluble Cu}) + 0.808 \log(\text{CEC}) + 2.179 \) | 0.42     | 34     |
| 4   | \( \log(\text{free Cu}^{2+} \text{ activity}) = -2.617 \log(\text{soluble Cu}) - 1.391 \log(\text{DOC}) + 1.545 \log(\text{CEC}) + 2.549 \) for soils with pH > 7.20 | 0.88     | 20     |
| 5   | \( \log(\text{free Cu}^{2+} \text{ activity}) = -2.617 \text{ pH} + 1.639 \log(\text{total Cu in soil}) - 4.303 \) | 0.65     | 34     |
| 6   | \( \log(\text{free Cu}^{2+} \text{ activity}) = -1.310 \text{ pH} + 1.60 \log(\text{soluble Cu}) - 0.783 \) | 0.65     | 34     |
| 7   | \( \log(\%\text{free Cu}^{2+} \text{ activity}) = -1.216 \text{ pH} + 7.134 \) | 0.64     | 34     |

Figure 2. Comparison of WHAM VI calculated free Cu²⁺ ion and measured free Cu²⁺ ion in 34 samples of soil pore water. Free Cu²⁺ activity was predicted with WHAM VI using either (a) 65% AFA and default log \( K_{CuFa} \), (b) adjusted AFA rate from 10% to 125% and default log \( K_{CuFa} \), (c) 65% AFA and adjusted log \( K_{CuFa} \) from 1.0 to 2.3.

Note: The thin solid line represents 1:1 line and dot line represents 1 order of magnitude either side of the 1:1 line. The thick solid line represents regression line.

from 65 to 100% or 125% for the four samples could benefit the fit further (Table 4). The best agreement between measured and predicted free Cu²⁺ activity was achieved using adjusted AFA ratio ranging from 10 to 125% for all samples with the smallest RMSE of 0.23 and the highest \( r^2 \) of 0.98 (see Figure 2(b) and Table 4). Generally, it was found that %AFA was positively correlated to soil pore water pH despite a few exceptions. It varied from 10 to 30% (15% on average) for samples with pore water pH < 6.5, from 10 to 65% (36% on average) for samples with pore water pH 6.5–7.5, from 30 to 125% (69% on average) for samples with pore water pH > 7.5. For the three samples using 125% AFA as inputs for modelling, free Cu²⁺ activity was all lower than 10⁻¹⁰ mol L⁻¹.

Meanwhile, in order to weaken the binding of Cu to DOM for samples undervalued, log \( K_{CuFa} \) was decreased from 2.1 to 1.0 and to intensify Cu affinity to DOM for samples overvalued, log \( K_{CuFa} \) was increased from 2.1 to 2.3.
Due to contaminated concentrations of soils at between toxicity thresholds EC 5 and EC 90 according to Cu inhibition in barley root elongation, the fractions of Cu species to total soluble Cu were calculated by WHAM VI using the optimized AFA rate (10–125%) to analyze the impact of free Cu²⁺ concentration and its percentage on ECx (see Figure 3). It was found that Cu bound to DOM was the predominant species (>98%) in the majority of soil pore waters obtained in the present study except for a number of low-pH soils (i.e. S1, S2, S3 and S6). The percentage of FA-Cu across 34 samples was insignificantly correlated to Cu inhibition to barley root elongation. In Figure 3, inhibition rate of barley root elongation at 5 and 90%, the log (free Cu²⁺) reached the maximum −5.3 (S3) and the minimum −11.5 (S7) mol L⁻¹, respectively. The correlative relationship between inhibition rate (%) of barley root elongation and log (free Cu²⁺ activity) or percentage of free Cu²⁺ concentration showed that these two factors could explain variances of inhibition rate (%) by 43% and 15%, respectively.

### 3.4.2. NICA-Donnan model

Similarly to WHAM VI, free Cu²⁺ activity was underestimated for 82% of samples (Figure 4(a)) by NICA-Donnan model when using 65% AFA and default constants as inputs. Decreasing %AFA to 30% for samples underestimated, the prediction was improved significantly (see Table 4), but there were still some values far away from the 1:1 line. To continue to decrease %AFA to 15% for the 47% of samples with larger RMSE, the regression was extremely improved with the smallest RMSE 0.32 and the highest $r^2$ 0.96 (Figure 4(b)). Differently to WHAM VI, adjustment of binding constant of log $K_{CuFA2}$ could not benefit the improvement on prediction of free Cu²⁺ activity. The correlation between estimated and measured free Cu²⁺ activity also improved significantly with RMSE 0.49 and $r^2$ 0.91 (Figure 2(c) and Table 4). Nevertheless, there was no consistent match between predicted and measured free Cu²⁺ activity on premise of adjusting site heterogeneity ($\Delta L K_2$) from 1.4 to 2.34.

### Table 4. Summary of the parameterization of WHAM 6 and NICA-Donnan parameters based on goodness-of-fit of measured free Cu²⁺ activity data.

| WHAM 6 parameters | Model fit indices |
|--------------------|-------------------|
| $\log K_{CuFA}$ | $\Delta L K_2$ | % AFA | RMSE | Slope | $r^2$ |
| Default values | 2.1 | 2.34 | 65 | 1.23 | 0.44 | 0.54 |
| 2.1 | 2.34 | 30 | 1.06 | 0.43 | 0.53 |
| 2.1 | 2.34 | 20 | 1.18 | 0.43 | 0.55 |
| 2.1 | 2.34 | 10–30 | 0.84 | 0.63 | 0.81 |
| 2.1 | 2.34 | 10–65 | 0.42 | 0.83 | 0.94 |
| 2.1 | 2.34 | 10–125 | 0.23 | 0.92 | 0.98 |

Adjusting binding constant

| 1.8 | 2.34 | 65 | 0.84 | 0.59 | 0.72 |
| 1.0 | 2.34 | 65 | 0.77 | 0.71 | 0.74 |
| 1.0–2.3 | 2.34 | 65 | 0.49 | 0.89 | 0.91 |

Decreasing site heterogeneity

| 2.1 | 1.80 | 65 | 1.04 | 0.49 | 0.64 |
| 2.1 | 1.40 | 65 | 0.96 | 0.53 | 0.67 |

NICA-Donnan parameters | Model fit indices

| $\log K_{CuFA1}$ | $\log K_{CuFA2}$ | %AFA | RMSE | Slope | $r^2$ |
| Default values | 0.26 | 8.24 | 65 | 1.34 | 0.72 | 0.78 |
| 0.26 | 8.24 | 30 | 0.78 | 0.67 | 0.77 |
| 0.26 | 8.24 | 20 | 0.79 | 0.64 | 0.76 |
| 0.26 | 8.24 | 15–30 | 0.61 | 0.78 | 0.84 |
| 0.26 | 8.24 | 15–65 | 0.32 | 0.94 | 0.96 |

Decreasing binding constant

| 0.26 | 8.00 | 65 | 1.14 | 0.64 | 0.55 |
| 0.26 | 7.50 | 65 | 1.09 | 0.56 | 0.50 |

Note: The goodness-of-fit was evaluated by RMSE of free Cu²⁺ activity, and the slope of the linear regression line of measured v. predicted free Cu²⁺ activity. The best fit is shown in bold.

### Figure 3. The proportions of Cu species calculated by WHAM 6 and measured free Cu²⁺ activity (log mol L⁻¹) in soil pore water for 34 samples at toxicity thresholds of ECx (x ranged from 5 to 90). A lower and higher inhibition concentrations of Cu to barley root elongation were adopted for each soil, respectively.
activity to a significant level. Across the adjusted %AFA of 34 samples for the best fit, it was 15% for samples with pH < 7.0, and 15–65% (35% on average) for samples with pH > 7.0.

4. Discussion

4.1. Distribution of Cu in soil pore water and soil solid

It is showed in Table 3 that soluble Cu was only correlative to total Cu in soil and DOC in soil pore water, which is partly similar to the result from Lamb et al. [35] (c.f. log (soluble Cu) = −0.0261 log (total Cu in soil) + 0.254 pH + 0.635 log (SOC) − 2.813, \( r^2 = 0.72 \)) because pH was excluded from the regression equation in our study. Howbeit, Luo et al. [36] also concluded that only SOC in soil could control soluble Cu content in soil and pH played a negligible effect on it. Similarly, all of these results confirmed the influence of SOC (or expressed as DOC) on soluble Cu concentrations in soils.

For solid-solution distribution of Cu, it was very difficult to obtain a linear relationship between \( K_d \) and soil pore water pH across 34 samples (Table 3), which is dis-similar to studies from Sauvé et al. [32] (c.f. log (\( K_d \)) = 0.21 pH − 0.51 log (SOM) + 1.75) and Vulkan et al. [14] (c.f. log (\( K_d \)) = 0.34 pH − 0.58 log (DOC) + 1.74) due to pH as the top important factor to \( K_d \) in their studies. However, Luo et al. [36] (c.f. log (\( K_d \)) = 1.04 log (total Cu) − 0.91 log (SOC) + 0.70, \( r^2 = 0.57 \)) and Lamb et al. [35] (\( K_d \) of Cu was only correlated with DOC, \( r^2 = 0.72 \)) also determined pH was not an indispensible factor to Cu distribution, which is generally consistent to the results in our study. Also, Antoniadis and Golia [37] expressed that \( K_d \) of Cu was significantly related to soil CEC instead of soil pH and SOC due to soil samples were strongly wreathed, given that CEC was also an effective predictor on \( K_d \) of Cu in our study (see Equations 3 and 4 in Table 3), therefore, Cu distribution is supposed to be strongly soil-dependent.

4.2. Prediction of free Cu\(^{2+} \) activity using models

For both of models, the best prediction on free Cu\(^{2+} \) was obtained only when adjusting AFA rate to the bigger values with increase of soil pore water pH, which is possibly due to competition between H\(^+ \) and Cu\(^{2+} \) ion for DOM binding sites resulting in most of DOM in acidic soils inefficient [38,39]. The free Cu\(^{2+} \) activity at EC\(_x\) varied by more than four orders of magnitude among the soils tested and was related to Cu concentration in tomato shoots with \( r^2 \) of 0.41 [3], which is quite similar to these results in our study. Although there was a significant correlation between free Cu\(^{2+} \) concentration and its toxicity to barley root elongation, however, free Cu\(^{2+} \) activity alone was not a good predictor of plant toxicity due to it only explained approximately 50% of variance of toxicity. Given most of soluble Cu as DOM-complexed form in soil pore water, presumably, a small proportion of Cu-organic complexes could be toxic along with free Cu\(^{2+} \). Guo et al. [40] found that weakly bound Cu-organic complexes, such as malate-complexed Cu, was nearly 0.5-fold toxic to plant in contrast to free Cu\(^{2+} \) ion; and strongly bound Cu-organic complexes, such as EDTA-complexed Cu was completely nontoxic. Due to affinity of Cu to EDTA, malate and FA was 20.49 (log \( K_d \)) in Visual MINTEQ, and 2.16 (in WHAM VII), therefore, a small
amount of FA-bound Cu could be possibly toxic to barley root elongation to explain another half variances of toxicity response. As for how much proportion of DOM-complexed Cu could contribute to its toxicity to plant should be further considered.

Dissolved Al and Fe concentrations in soil pore water were lower than the limit of detection of 0.25 mg L⁻¹ and 0.5 mg L⁻¹, respectively, except S1 and S2 with total soluble Al ranging from 0.58 to 1.06 mg L⁻¹. Due to dissolved Al³⁺ could be considered as a critical parameter in free metal speciation [41] and most of Al in Ferrosol with pH < 4.5 existed as free Al³⁺ ion [42], the Cu²⁺ speciation was recalculated for both S1 and S2 using measured Al concentrations instead of calculated Al³⁺ activity from Al(OH)₃ colloid as inputs in WHAM VI. The results showed that free Cu²⁺ activity derived from measured Al was approximately 3-times higher than measured values when using adjusted %AFA (10–30%) as active DOM. Thus, the effect of Al³⁺ ion on free Cu²⁺ species in acidic S1 and S2 should be considered carefully. It was reported that for samples with >99% of Cu as DOM complex, the fraction of Cu species associated with AlO(OH) was negligible [11]. In our study, most of samples with high percentage of FA-bound Cu (>98%), hence, impacts of Al and Fe on prediction of Cu speciation are extremely limited.

A large number of studies adopted between 50% and 100% AFA as assumption of active DOM to compute free Cu²⁺ concentration in solution [14,30,43–45], it was changeable depending on DOM nature and quantity in systems. Amery et al. [44] found that 65% AFA in WHAM VI could fit measured FA by UV-absorbance at 254 nm to a great extent, but Stockdale et al. [17] concluded that 65% AFA in WHAM VII could cause free Cu²⁺ more than one order of magnitude higher than predicted values for 38% of samples. Vulkan et al. [14] found that 69% AFA used in WHAM VI could ensure predicted free Cu²⁺ in soil solution closer to measured values, but this ratio was only consistent to that for alkaline soils, not acidic soils in our study. Some studies also used certain %HA as active DOM [10,46] to predict Cu speciation. For example, Zhu and Guéguen [11] adjusted FA:HA ratio from 1:0 to 1:1 to compute free Cu²⁺ concentration via WHAM VII, and found a very small effect on it when Cu bound to DOM was >99%. However, Ponthieu et al. [10] concluded that FA:HA ratio ranging from 1:0 to 0:1 influenced up to an order of magnitude of free Cu²⁺ concentration due to HA with log $K_{CuHA}$ (2.38) complexed more Cu than FA with log $K_{CuFA}$ (2.16). In our study, assuming HA in WHAM VI as active DOM instead of FA at the best optimized %AFA (10–125%), the predicted free Cu²⁺ activity was approximately 1.6 orders of magnitude lower than measured values even for these samples with higher proportion of organic complexes. Given that HA proportion in soil solutions was quite low [8], hence, assuming FA as active DOM for modelling rather than HA is more realistic in this study.

In our study, the frequently-used ratio of 65% AFA generally caused the underprediction of free Cu²⁺ for both of models, and 10–125% AFA for WHAM VI and 15–65% for NICA-Donnan were the optimized ratio for best agreement of free Cu²⁺ activity. Groenenberg et al. [47] found the humic substances fractions of DOM in the soil solution varied between 14% and 63% depending on the samples. Ren et al. [8] also reported only 16–42% of DOM was humic substances and consisted mainly of FA as the most crucial affecting factor for Cu speciation. These results are very similar to those from NICA-Donnan predictions in this study. As to 125% AFA as input in WHAM VI for the three samples with measured free Cu²⁺ activity < $10^{-16}$ mol L⁻¹, it was possibly related to either high pHs (7.77 and 7.79) for 2 samples and lower total soluble Cu (2.84 μmol L⁻¹) for 1 sample. The model did not predict any concentrations to be below $10^{-13}$ mol L⁻¹, but measured values could be as low as $10^{-16}$ mol L⁻¹ [19], adjusting %AFA to a range (>100%) could be a recalibration of the model intrinsically for the sample with quite low free Cu²⁺ activity. Besides, Amery et al. [44] estimated that active DOM ranged from 28% to 152% in soil solutions in a soil profile collected over time and Djæe et al. [45] found that free Cu²⁺ speciation in 55 soil solutions was mainly overestimated when using default constant and 65% AFA in WHAM VII, and using an optimized AFA percentage (35–215%) and log $K_{CuFA}$ (1.84–2.46) could make the fit better. In this study, the adjusted AFA (10–125%) and binding constant log $K_{CuFA}$ (1.0–2.3) in WHAM VI also ensured the prediction maximally optimized. Differently, the parameters were mainly adjusted downward in our study but upward in study of Djæe et al. [45] from the start value of 65% AFA and default constants for the better estimation.

However, the difference in optimization of parameters in these models was connected to nature/heterogeneity of samples along with various analytical issues but more likely attributed to the DOM isolation procedure. The DOM affinity for metals could be affected by extraction procedures and soil pre-treatment methods [48]. In this study, DOC concentrations (300 mg L⁻¹ on average) were approximately 3-times higher than the values (100 mg L⁻¹ on average) in study of Nolan et al. [13] using different soil pre-treatment methods despite similar SOC variations. Meanwhile, Nolan et al. [1] also found that predicted free Cu²⁺ by model using 70% AFA for Australian soils was underestimated about three orders of magnitude compared with measured results when adopted similar DOC extraction procedure (217 mg L⁻¹ on average) to the present study. One-month soil incubation could decrease DOC concentration by 2–3 times and elevated its affinity for metals by 1–1.5-fold in contrast to 4 days’ incubation [44]. All of soils were incubated only 24 h before extraction in our study, which
probably accounted for the high DOC concentrations but low affinity capacity for Cu in soil pore water.

Generally, free Cu\(^{2+}\) prediction is more complicated than other trace element, e.g. Zn, Ni, Cd, and Co\([7,17,19]\) due to a large proportion (up to 99.9%) of soluble Cu was DOM-complexed form\([11]\). The DOM nature is extremely crucial to Cu speciation and how to select a reliable technique to measure its nature is another issue. Amery et al.\([44]\) studied that specific UV-absorbance of DOM at 254 nm could represent aromaticity of DOM, and was positive proportionally to %AFA. Ahmed et al.\([21]\) used the three-dimensional fluorescence excitation emission matrix spectroscopy to evaluate the fractions of FA and HA in DOM and found using these fractions improved model predictability in contrast to using conventional 65% AFA assumption. Ren et al.\([8]\) adopted a rapid DAX-8 resin technique to fractionate DOM mainly into FA and HA, and found that measured FA concentration incorporated with NICA-Donnan to calculate free Cu\(^{2+}\) could fit the measured values much better than an assumed FA ratio. Further, the selection of analytical techniques for free Cu\(^{2+}\) activity could play a role on its modelling correction especially for samples with low (Cu)/(DOC) ratios\([5,19,21]\). Tipping and Lofts\([19]\) described that Donnan membrane technique was more consistent to the model prediction than voltammetric technique (overrated) and ion exchange column method (underrated). However, Stockdale et al.\([17]\) assessed that voltammetry yielded the greatest fraction of agreement for Cu\(^{2+}\) activity than ISE technique and the competitive ligand method. There are substantial variations among different analysis techniques but no systematic bias from the model was observed across techniques.

### 4.3. Comparison between methods

Although both of models obtained excellent fit after adjusting %AFA or binding constant, there was a slight difference on free Cu\(^{2+}\) predictability for them. The NICA-Donnan model caused slightly higher bias for free Cu\(^{2+}\) prediction when using 65% AFA and default constants as input values for all samples in this study. Also, NICA-Donnan appeared to predict much stronger binding of DOC to Cu\(^{2+}\) than WHAM VI, because more than three-quarter samples were underestimated for NICA-Donnan and only half samples for WHAM VI when using 65% AFA as input. The AFA ratio was 125% as the maximum in WHAM VI but 65% in NICA-Donnan model to fulfill the prediction. Weng et al.\([30]\) compared free Cu\(^{2+}\) activity obtained with WHAM VI and NICA-Donnan with a variation of DOM composition, and concluded that WHAM VI was slightly better than NICA-Donnan with lower RMSE. Ahmed et al.\([21]\) found that NICA-Donnan showed 2-times higher RMSE values for free Cu\(^{2+}\) prediction than WHAM VII especially for samples with low (Cu)/(IDOM) ratio. Sierra et al.\([49]\) confirmed that WHAM VII was especially practical for Cu speciation calculation where chemical speciation was driven basically for organic matter.

Besides, the difference in free Cu\(^{2+}\) prediction was further analyzed when using 65% AFA and default constant as inputs, it was less than 0.7 orders of magnitude between two models for the samples with pH <7.7, and close to one order of magnitude for samples with pH >7.7. Similarly, Ponthieu et al.\([10]\) evaluated the impact of DOM composition in Cu speciation in 36 calcareous soil solutions (pH 7.0–8.4) using WHAM VI and NICA-Donnan, and difference of free Cu\(^{2+}\) predicted by two models was enlarged with increase of pH. The difference of free Cu\(^{2+}\) prediction might be related to the distinction of intrinsic property in these two humic substances models. The NICA-Donnan model uses a bimodal, continuous distribution of affinities for protons and metal ions, whereas WHAM VI is based on a discrete set of sites, and phenolic contents for the two models are also negatively correlated\([18,20]\). As a whole, predictability of models for Cu speciation is strongly dependent on humic substances nature and quantity in soil solution.

### 5. Conclusion

The Cu concentrations and free Cu\(^{2+}\) activities in pore waters for 34 Chinese soil samples were measured in order to determine its controlling factors and predictive models. Total Cu in soils and DOC in soil pore water were two key properties explaining soluble Cu content in soil pore water and its distribution. The free Cu\(^{2+}\) activity in soil pore water was controlled mainly by pore water pH and total Cu concentrations.

The predictability of WHAM VI and NICA-Donnan models for free Cu\(^{2+}\) was evaluated, which indicated that both models overestimated combination of DOM and Cu resulting in the lower free Cu\(^{2+}\) activities. While using varied AFA ratio from 10% to 125% for WHAM VI and from 15% to 65% for NICA-Donnan these two models provided preferable estimates of free Cu\(^{2+}\) activity with \(r^2 > 0.96\). Also, NICA-Donnan model showed a slightly stronger affinity for Cu\(^{2+}\) in pore water than WHAM VI considering that extra 26% of samples were undervalued on free Cu\(^{2+}\) activity. The impact of the composition of the DOM on the free Cu\(^{2+}\) prediction by models could vary up to two orders of magnitude depending on what ratio of AFA was used to represent DOM. Despite DOM nature was not measured by analytical techniques in this study, this work made a good attempt on Cu speciation prediction in a wide range of Chinese soils.

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