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Does specific parameterization of WHAM improve the prediction of copper competitive binding and toxicity on plant roots?

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Abstract

We aimed at assessing whether the binding and rhizotoxicity of metal cations such as copper that exhibit high affinity for plant roots could be adequately predicted using the Windermere Humic Aqueous Model (WHAM) default parameterization. Accordingly, we first compared the ability of the default parameterization of WHAM and a specific parameterization for terrestrial higher plants (WHAM-THP) to model the competitive binding of copper on wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.) roots. Secondly, in an external dataset, we evaluated the ability of WHAM-THP to predict the copper concentration and toxicity to pea (*Pisum sativum* L.) roots relative to WHAM. WHAM-THP estimates generated a slightly better fit for the competitive binding of copper on wheat and tomato roots ($\log_{10}$ of the root-mean-square error, $RMSE = 0.15$) than WHAM estimates ($RMSE = 0.24$). WHAM-THP estimates slightly better fitted the copper concentration in pea roots ($RMSE \leq 0.49$) than WHAM estimates ($RMSE \leq 0.67$) at low copper exposure and pH ≤ 5. However, WHAM-THP did not at all improve the prediction of copper toxicity to pea roots ($RMSE = 13\%$ as also for WHAM). We thus conclude that, although the default parameterization of WHAM does not neatly predict the binding of metal cations on roots, it could however be used with confidence in predictive ecotoxicology for terrestrial higher plants without any specific parameterization.

Keywords

Biotic ligand model; Complexation; Humic substances; Phytotoxicity; Trace element

Highlights

- WHAM-THP is a specific parameterization of WHAM for terrestrial higher plants
- WHAM-THP much improves the prediction of root acidic properties
- WHAM-THP only slightly improves the prediction of root copper competitive binding
- WHAM-THP does not improve the prediction of copper rhizotoxicity

1. Introduction

Models designed to predict the ecotoxicity of metal cations at the organism level are based on the description of the competitive binding of metal cations to biotic ligands borne to the outer surface of the organism (Di Toro et al. 2001). These models seem particularly promising for modelling the toxicity of metal cations to plant roots, i.e. rhizotoxicity. It was recently suggested that the primary mechanism driving the rhizotoxicity of metal cations is their binding strength to biotic ligands borne by root surfaces (Kopittke et al. 2014). The model's ability to accurately describe metal cation binding on plant roots is therefore a crucial issue.

Accordingly, advanced geochemical models initially designed to model metal cation binding on humic substances have started to be applied in predictive ecotoxicology studies over the past decade (Plette et al. 1996; Tipping et al. 2008). The Windermere Humic Aqueous Model (WHAM) has particularly been used to adequately model the binding and toxicity of metal cations to aquatic and terrestrial biota (Tipping et al. 2008; Antunes et al. 2012; Tipping and Lofts 2013; Qiu et al. 2015 and 2016).

The humic acid (HA) in WHAM was used as a surrogate of biotic ligands (Tipping et al. 2008; Antunes et al. 2012). The amount of metal cations bound to biotic ligands was predicted with WHAM by numerically optimizing the equivalent mass of HA per gram of organism (Tipping and Lofts 2013). The difference between the measurement and the WHAM prediction of the amount of metal cations bound to biotic ligands was hypothetically attributed only to the difference in the density of binding sites between the organism and the HA in WHAM. This hypothesis however overlooks the fact that the binding affinity for metal cations may also differ between organisms and HA in WHAM.

For terrestrial higher plants, a recent investigation showed that WHAM was able to satisfactorily model cadmium (Cd), nickel (Ni), and zinc (Zn) concentrations in pea (*Pisum sativum* L.) roots, but that it overestimated the copper (Cu) concentration in roots (Le et al. 2015). These authors concluded that the default parameterization of HA in WHAM may be not applicable for metal cations having a high affinity for natural organic matter such as Cu.

We recently characterized Cu binding on wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.) roots by combining X-ray absorption spectroscopy with the specific parameterization of a two HA model based on WHAM (Guigues et al. 2016). We showed that Cu binding on wheat and tomato roots was driven by two types of sites, i.e. low- and high-affinity sites respectively corresponding to carboxylic and nitrogen (N) functional groups. As the high-affinity sites parameterized in WHAM presumably correspond to phenolic groups rather than N functional groups, this finding suggested that specific parameterization of WHAM would be necessary for terrestrial higher plants.

Accordingly, we aimed at assessing whether Cu root binding and rhizotoxicity could be adequately predicted with the WHAM default parameterization or if tailored parameterization would be necessary. We first compared the ability of WHAM parameterized with default settings (hereafter referred to as WHAM) and WHAM specifically parameterized for terrestrial higher plants (hereafter referred to as WHAM-THP) to model Cu competitive binding on roots. Wheat and tomato were chosen as model species to be respectively representative of monocots and dicots. Secondly, we evaluated, on an external dataset, the ability of WHAM-THP to predict the Cu concentration in roots and its rhizotoxicity relative to WHAM predictions.

2. Experimental approach

We used only analytical grade reagents unless otherwise stated.

2.1. Plant growth and root recovery

Wheat (cv. Premio) and tomato (cv. Moneymaker) were germinated for 7 days in darkness and then grown for 14 days in hydroponic conditions in a growth chamber under the following climatic conditions (day/night): 25/20°C, 75/70% relative humidity and 16/8 h with a photon flux density of 450 µmol photons m^{-2} s^{-1} during the day (see Guigues et al. 2014 for details). At harvest, roots were separated from shoots, blotted with paper towels, subdivided into homogenous subsamples, and stored frozen.

After thawing, roots were rinsed with 1 mM $Ca(NO_3)_2$ to eliminate vacuolar compounds released due to membrane leakage during freezing. Roots were stirred in HNO_3 solution (trace analysis grade) at pH 3 for 1 h to remove highly bound or precipitated cations (e.g. Fe and Al) and rinsed twice with ultrapure water (18.2 MΩ) for 30 min. Roots were finally oven-dried at 50°C to constant mass.

The plant root material obtained (hereafter referred to as roots) was metabolically inactive. This metabolic inactivity prevented the physiologically-driven absorption of Cu in root cells and enabled us to study only Cu binding onto root surfaces. Roots contained cell walls and plasma membranes, i.e. the two compartments which give roots their cation binding properties (Guigues et al. 2014*)*. Root pretreatment may have partly impacted their binding properties in comparison with live plant roots. However, there is to our knowledge no other reliable analytical technique to distinguish binding from absorption of metal cations in the several dozens of samples necessary to test a model for practical ecotoxicological applications.

2.2. Potentiometric titration

Potentiometric titration of wheat and tomato roots were extensively described by Guigues et al. (2014). Briefly, ca. 0.2 g (dry mass basis) of roots was placed in 100 ml of 10 mM KNO_3 under stirring and flushed with ultra-pure nitrogen. During titration, the pH was first lowered to 2.5 with 0.2 M HNO_3 (trace analysis grade) addition and was then increased step-by-step to 11.5 with the incremental addition of 0.1 M KOH (trace analysis grade) either at a rate of 100 µl in the 2.5-3.5 and 10.5-11.5 pH ranges or at a rate of 20 µl in the 3.5-10.5 pH range.

2.3. Copper sorption experiments

A dry mass of 10 (± 0.5) mg of wheat and tomato roots was shaken end-over-end for 24 h at 25°C in 25 ml of solution with varying chemical conditions representative of acidic soil solutions and including the competitive effect of some important major (H and Ca) and minor (Zn) metal cations (Tables S1 and S2). In experiment 1, the initial total Cu concentration of the solution ranged from pCu_{in} 7.3 to 3.0 ($pCu_{in} = -\log_{10}[Cu]_{in}$). The solution ionic strength and pH was set at 30 mM with $NaNO_3$ and 4.7 (± 0.2), respectively.

In experiment 2, the solution ionic strength was set at 0.6 or 300 mM with $NaNO_3$. The initial total Cu concentration was set at pCu_{in} 4.2, 5.2, or 6.2 and the pH was set at 4.7 (± 0.2).

In experiment 3, the solution pH was set at 4.1 (± 0.1) or 6.3 (± 0.1). The initial total Cu concentration was set at pCu_{in} 4.2, 5.2, or 6.2. The ionic strength was set at 30 mM with $NaNO_3$.

In experiment 4, the initial total solution Ca concentration ranged from pCa_T 2.0 to 4.0. The initial total solution Cu concentration, ionic strength and pH was set at pCu_{in} 6.3, 30 mM with $NaNO_3$, and 5.1 (± 0.4), respectively.

In experiment 5, the initial total solution Zn concentration ranged from pZn_T 4.5 to 7.2. The initial total solution Cu concentration, ionic strength and pH was set at pCu_{in} 6.3, 30 mM with $NaNO_3$, and 4.7 (± 0.1), respectively.

The pH was buffered with 1 mM 2-(N-morpholino) ethanesulfonic acid and adjusted with NaOH or HNO_3 (trace analysis grade). Each sorption experiment was performed in duplicate. After a few minutes of root sedimentation, the supernatant was collected for analysis. The copper concentration in the initial (pCu_{in}) and final (i.e. at equilibrium, pCu_{eq}) solutions was determined by inductively coupled plasma mass spectrometry (ICP-MS, NexION 300X Perkin Elmer) to determine the amount of Cu bound to wheat and tomato roots. Blanks and certified reference material (EnviroMAT Driking water EP-L-3 and groundwater ES-H-2) were included in the analyses. The measurement uncertainty was lower than 10%. This procedure was cross-validated with Cu measurements obtained on digested root samples (Guigues et al. 2016).

3. Modelling approach

3.1. Specific parameterization of WHAM

Experimental data were modelled using the humic ion-binding model included in WHAM VII. The formalism of this model was extensively described by Tipping (1998) and Tipping et al. (2011). Briefly, WHAM was designed to simulate the cation binding properties of humic substances depicted as a regular array of two types of binding sites. The density (L_{Hi}, $cmol_c.kg^{-1}$) of type-1 sites is arbitrarily set as twofold higher than the density of the type-2 sites (i.e. $L_{H1} = 2 \times L_{H2}$). Protons and metal cations compete for binding on type 1 and 2 sites. Metal cations are able to form mono-, bi- and tri-dendate complexes.

Proton sorption to humic substances was characterized by two intrinsic proton dissociation constants (pKa_1 and pKa_2) and two distribution terms (ΔpKa_1 and ΔpKa_2) for type 1 and 2 sites, respectively. Metal binding to humic substances was characterized by two intrinsic equilibrium constants ($K_{M,1}$ and $K_{M,2}$) for type 1 and 2 sites, respectively, and one heterogeneity parameter ($\Delta LK2_M$). The parameter $K_{M,2}$ was calculated from $K_{M,1}$, pKa_1, and pKa_2 as follows (Tipping et al. 2011):

$$LogK_{M,2} = LogK_{M,1} \times \frac{pKa_2}{pKa_1} \qquad \text{Eq. 1}$$

Electrostatic effects are accounted for in WHAM by approximating the diffuse layer/bulk solution system with a Donnan model. While WHAM can account for the complexation of the free ionic form and the first hydrolysis product of each metal, we only accounted for Cu^{2+} as a preliminary speciation calculation showed that Cu^{2+} represented > 95% of the total Cu in solution with $pH \leq 6.3$. However, at higher pH the presence of Cu hydroxide should be considered. The partial pressure of CO_2 was assumed to be that of the ambient atmosphere ($10^{-3.5}$ atm) and the temperature was set at 25 °C for the calculations.

To develop a predictive model for ecotoxicological assessment, we aimed at fitting the experimental data as accurately as possible, but with the lowest possible number and the most generic set of parameters for wheat and tomato. Accordingly, we first simulated the root binding properties with a single HA having the default parameterization (pKa_i, ΔpKa_i, $K_{M,i}$, $\Delta LK2_M$) used in WHAM (Tables 1 and 2). The HA concentration was set at the concentration found in dry roots in batch solutions, i.e. 400 mg l^{-1}. The total density of binding sites on HA was set according to the potentiometric titration of wheat and tomato roots reported by Guigues et al. (2014).

The ratio between the density of low-p*Ka* (type-1) and high-p*Ka* (type-2) sites (hereafter referred to as the L/H-p*Ka* ratio) that is arbitrarily assigned at 2 in WHAM was out of line with the L/H-p*Ka* ratios of 0.4 and 0.9 experimentally determined for wheat and tomato roots, respectively (Guigues et al. 2014). In agreement, the L/H-p*Ka* ratios on roots of dicots and monocots found in the literature were also lower than 2, i.e. ranging from 0.5 to 1.7, except for *Lupinus albus* L. that had a ratio of 3.8 (Meychik and Yermakov 1999 and 2001; Ginn et al. 2008; Wu and Hendershot 2009; see Table S3).

To relax the L/H-p*Ka* ratio, we thus mimicked the root binding properties with two HA that were specifically parameterized in WHAM-THP (Tables 1 and 2). The concentration of each HA was set at the concentration established in dry roots in batch solutions, i.e. 400 mg l^{-1}. The total density of binding sites and the distribution between low- and high-affinity sites was set for each HA according to the potentiometric titration of wheat and tomato roots reported by Guigues et al. (2014). The first HA (HA_I) represented the low-p*Ka* sites while the second HA (HA_{II}) represented the high-p*Ka* sites. We first parameterized pKa_1, pKa_2, ΔpKa_1 and ΔpKa_2 for HA_I and HA_{II} to fit the experimental titration curves of wheat and tomato roots (Guigues et al. 2014). We then parameterized $K_{Cu,1}$, $K_{Cu,2}$ and $\Delta LK2_{Cu}$ to fit the Cu sorption data from experiment 1. The ability of WHAM-THP to account for ionic strength and pH effects on Cu binding was verified by predicting Cu sorption data from experiments 2 and 3 without additional parameterization. Finally, the ability of WHAM-THP to account for the competitive effect of Ca and Zn was assessed by parameterizing $K_{Ca,1}$, $K_{Ca,2}$, $\Delta LK2_{Ca}$, $K_{Zn,1}$, $K_{Zn,2}$ and $\Delta LK2_{Zn}$ to fit the Cu sorption data from experiments 4 and 5.

3.2. Application of WHAM to an external dataset

To evaluate the extent to which WHAM-THP improves the prediction of Cu accumulation into roots and Cu rhizotoxicity in comparison with WHAM, we concomitantly applied the two models to the dataset obtained by Wu and Hendershot (2010). These authors measured the Cu concentration in roots and the root length of pea seedlings exposed to solutions with varying Cu (0 to 24.8 µM) and Ca (0.04, 0.18, and 1.92 mM) concentrations at pH 4, 5, or 6.

The prediction of root Cu concentration with WHAM and WHAM-THP was done by considering a factor of 0.044 g HA.g^{-1} DW to convert a quantity of Cu per g of HA in a quantity of Cu per g of dry roots, as described by Le et al. (2015) on the same dataset. The relative root elongation (*RRE*, %) was calculated from the root length (*RL*, mm):

$$RRE = \frac{RL}{RL_{max}} \times 100 \qquad \text{Eq. 2}$$

with RL_{max} being the maximal *RL* measured. The *RRE* was predicted with a log-logistic dose-response curve:

$$RRE = \frac{100}{1+\left(\frac{Ftox}{Ftox_{50}}\right)^{\beta}} \qquad \text{Eq. 3}$$

and

$$Ftox = \sum \alpha_i \times \nu_i = \alpha_H \times \nu_H + \alpha_{Cu} \times \nu_{Cu} \qquad \text{Eq. 4}$$

with *Ftox* being the toxicity function of a mixture of metal cations (H and Cu herein) calculated by summing the products of the metal-specific toxicity coefficient (α_i) and the concentration of metal bound to HA (ν_i, mol.g^{-1} HA) calculated with WHAM or WHAM-THP, $Ftox_{50}$ is the value of *Ftox* inducing a 50% reduction in the *RRE*, and β is a shape parameter. The α_H was set at 1 according to Tipping and Lofts (2013).

3.3. Parameter optimization

The quality of the fits obtained with WHAM and WHAM-THP were determined by calculating the root mean square error (*RMSE*) between the experimental data and model outputs (Table S4). *RMSE* was calculated from untransformed data for potentiometric titrations as some experimental data were negative. *RMSE* was calculated from $\log_{10}$-transformed data for Cu sorption experiments to balance the weight of the highest values. The *RRE* was predicted by optimizing the α_{Cu}, $Ftox_{50}$, and β to minimize the *RMSE* between measured and predicted values.

4. Results and Discussion

4.1. WHAM-THP substantially improves the modeling of acidic properties of wheat and tomato roots

Despite the fact that the total density of binding sites has been fitted to the experimental data (Guigues et al. 2014), WHAM estimates failed to fit the titration data for wheat and tomato (*RMSE* = 12.7 cmol$_c$.kg^{-1}; Figure 1; Figure S1). WHAM estimates overestimated the density of binding sites observed experimentally over the whole investigated pH range. The shape of the WHAM simulation curves also differed substantially from that of the experimental data. The inadequacy of WHAM estimates to fit the experimental data was partly due to the L/H-p*Ka* ratio arbitrarily assigned at 2 in WHAM (Table 1), which differs substantially from the L/H-p*Ka* ratios determined experimentally in previously published studies, and particularly for wheat and tomato roots in our study (see 3.1 for rationale).

Relaxing the L/H-p*Ka* ratio by using two HA but with the default parameterization of WHAM for the two HA (e.g. not as with WHAM-THP for which the two HA were specifically parameterized) generally improved the fit of the titration data for wheat and tomato ($RMSE$ = 4.7 and 5.2 $cmol_c.kg^{-1}$), but this only satisfactorily fitted the experimental data for tomato at $pH \leq 6$ (data not shown). The inadequacy of WHAM estimates to fit the experimental data was thus also partly due to the default parameterization of proton dissociation (p*Ka* and Δp*Ka*) of HA in WHAM.

The acidic properties of plant roots are so different from those of HA that a specific parameterization of HA for terrestrial higher plants in WHAM-THP is required (Table 1). By contrast with WHAM estimates, WHAM-THP estimates accurately fitted the titration data for wheat and tomato ($RMSE$ = 1.6 $cmol_c.kg^{-1}$; Figure 1; Figure S1).

4.2. WHAM-THP slightly improves the modelling of copper competitive binding on wheat and tomato roots

Over the five experiments of Cu sorption for wheat and tomato, WHAM estimates fitted 79% of the experimental data within twofold (Figure 2A). However, WHAM estimates almost systematically overestimated Cu binding by a factor 1.6 ($RMSE$ = 0.24). Le et al. (2015) showed that the WHAM default parameterization also overestimated the Cu concentration in pea roots. The overestimation increased as the Cu concentration in roots decreased.

WHAM-THP estimates better fitted the experimental data ($RMSE$ = 0.15) than WHAM estimates, with 91% of the experimental data within twofold and without any systematic bias (Figure 2B). The comparison of the results obtained with WHAM and WHAM-THP in each of the five Cu sorption experiments is detailed below in sections 4.2.1 to 4.2.4. The specific parameterization of WHAM-THP (Tables 1 and 2) thus slightly improved the goodness of fit for the modelling of Cu competitive binding relative to the WHAM default parameterization. This suggests that WHAM-THP should also improve modelling of the concentration in roots and the rhizotoxicity of Cu.

4.2.1. Experiment 1 on the copper binding affinity

For wheat, WHAM estimates overestimated Cu binding (*RMSE* = 0.23), with an increase in the overestimation as the Cu concentration in solution increased (Figure S2A). By contrast, WHAM-THP estimates neatly fitted (*RMSE* = 0.04) Cu binding on wheat roots throughout the investigated Cu concentration range.

For tomato, WHAM-THP estimates adequately fitted (*RMSE* = 0.03) Cu binding for $pCu_{in} > 4.5$ in solution (i.e. $pCu_{eq} > 5$; Table S2) (Figure S2B). WHAM-THP estimates fitted Cu binding for $pCu_{in} > 4.5$ better than WHAM estimates (*RMSE* = 0.09), which slightly but systematically overestimated the experimental data. WHAM and WHAM-THP estimates showed a twofold deviation with the experimental data at $pCu_{in} \leq 4.5$. This deviation increased with increasing Cu concentration. These high Cu concentrations (equivalent to $pCu_{eq} < 5$, i.e. $Cu_{eq} > 10$ µM) however exceeded the concentration range that usually generates acute Cu rhizotoxicity (Kopittke et al. 2010 and 2011). We thus did not attempt to specifically parameterize WHAM-THP to fit the experimental data at $pCu_{in} \leq 4.5$.

The $\log K_{Cu,1}$ of HA_I sites in WHAM set at 2.4 by default was intermediate between the $\log K_{Cu,1}$ (equal to 2.2) and the $\log K_{Cu,2}$ (equal to 2.7) of HA_I sites specifically parameterized in WHAM-THP (Table 2). Similar to the low-p*Ka* sites of HA_I parameterized by default in WHAM that presumably correspond to carboxyl groups (Tipping 1998), our recent spectroscopic analyses on wheat and tomato roots showed that the low-p*Ka* sites involved in Cu binding on wheat and tomato roots corresponded to carboxyl groups (Guigues et al. 2016). Considering the identical nature of the binding sites, it is thus hard to attribute the better fits obtained with WHAM-THP as compared to those obtained with WHAM to the specific parameterization of Cu binding affinity of HA_I in WHAM-THP.

The $\log K_{Cu,1}$ (equal to 6.0) and the $\log K_{Cu,2}$ (equal to 6.7) of HA_{II} sites specifically parameterized in WHAM-THP was about one order of magnitude higher than the $\log K_{Cu,2}$ of HA_I sites in WHAM set at 5.1 by default (Table 2). While the high-p*Ka* sites of HA parameterized by default in WHAM presumably correspond to phenolic groups (Tipping 1998), our recent spectroscopic analyses showed that the high-p*Ka* sites involved in Cu binding on wheat and tomato roots corresponded to N functional groups (Guigues et al. 2016). Since N functional groups have a higher affinity for Cu than phenolic groups (Fry et al. 2002), the involvement of N functional groups in Cu binding on wheat and tomato roots supports the higher $\log K_{Cu}$ fitted for the high-p*Ka* sites of HA_{II} in WHAM-THP. The better fits obtained with WHAM-THP than with WHAM could thus be attributed to the specific parameterization of Cu binding affinity of HA_{II} in WHAM-THP.

The heterogeneity parameters, i.e. $\Delta LK2_{Cu,I}$ in WHAM, set at 2.3 by default were much higher than the $\Delta LK2_{Cu,I}$ and $\Delta LK2_{Cu,II}$ that were fitted to 0 in WHAM-THP (Table 2). This heterogeneity parameter accounts for binding sites that occur at a low density and that exhibit a particularly high affinity for Cu (Tipping 1998). With our dataset, increasing the $\Delta LK2_{Cu}$ from 0 to 3 led to overestimation of the binding of Cu at low concentration (i.e. pCu > 7) by up to four orders of magnitude (data not shown). The much lower $\Delta LK2_{Cu}$ fitted in WHAM-THP than in WHAM thus likely explains why WHAM estimates overestimated Cu binding on wheat and tomato roots at low Cu concentration compared to WHAM-THP estimates.

4.2.2. Experiment 2 on the effect of ionic strength

The increase in ionic strength from 0.6 to 300 mM decreased the amount of Cu bound to wheat and tomato roots by 43% on average (Figure S3). As commonly described in the literature, the higher the ionic strength, the higher the tendency for major cations (for a negatively-charged sorbent such as roots) to accumulate in the diffuse layer (Vidali et al. 2011). This accumulation of major cations thus masks the negative charges on root surfaces and thus decreases the ability of binding sites to attract and bind metal cations such as Cu (Wang et al. 2011).

At the two ionic strengths, WHAM estimates better fitted the experimental data for tomato (*RMSE* = 0.18) than for wheat (*RMSE* = 0.38) (Table S4). At 300 mM ionic strength, WHAM estimates more particularly overestimated Cu binding on wheat and tomato roots, except at the highest Cu concentration (i.e. pCu_{in} 4.2). WHAM-THP estimates better fitted the experimental data for the two ionic strengths (*RMSE* = 0.11 and 0.21 for tomato and wheat, respectively) than WHAM estimates (Table S4). These results show that the specific parameterization of WHAM-THP better accounted for the effect of the ionic strength on Cu binding on roots than the default parameterization.

4.2.3. Experiment 3 on proton competition

The increase of pH from 4 to 6 boosted the amount of Cu bound to wheat and tomato roots by 41 and 16% on average, respectively (Figure S4). As commonly described in the literature, an increase in pH increases the dissociation of protons from negatively-charged surfaces such as roots, thus decreasing the competition between protons and metal cations such as Cu for binding on roots (Ginn et al. 2008; Bulgariu and Bulgariu 2012).

At pH 6, WHAM and WHAM-THP estimates similarly overestimated Cu binding on wheat and tomato roots (*RMSE* = 0.19 and 0.14, respectively) (Figure S4). At pH 4, WHAM estimates overestimated Cu binding on wheat and tomato roots (*RMSE* = 0.45 and 0.13, respectively). In comparison, WHAM-THP estimates underestimated Cu binding on wheat and tomato roots (*RMSE* = 0.38 and 0.20, respectively).

In the 4 to 6 pH range, WHAM modelled a very weak competitive effect of protons and thus consistently overestimated the amount of Cu bound on wheat and tomato roots (Figure S4). By contrast, WHAM-THP accounted for a substantial competitive effect of protons and overestimated it. This led WHAM-THP to overestimate the decrease in Cu bound to roots from pH 6 to 4. The WHAM-THP estimates closely fitted the potentiometric data between pH 3.5 and 10.5 (Figure S1), which should theoretically enable us to describe the increase in the proportion of the total binding sites available for metal binding as the pH increases. This indicated that the binding site affinity for Cu decreased from pH 4 to 6 and was consequently underestimated at pH 4 and overestimated at pH 6 in comparison with the initial optimization made at pH 5. Faced with the same issue, Wu and Hendershot (2010) chose to lower the logK_{Cu} by approximately 1 unit to fit the Cu binding on pea roots from pH 4 to 6. Overall, proton competition was the only effect for which WHAM-THP estimates (*RMSE* = 0.31 and 0.17 for wheat and tomato, respectively) did not improve the fit of the experimental data obtained with WHAM estimates (*RMSE* = 0.38 and 0.18) (Table S4).

4.2.4. Experiments 4 and 5 on calcium and zinc competition

While the Ca concentration range exceeded that of Cu by more than four orders of magnitude, Ca only had a weak competitive effect on Cu binding on roots (Figure S5). For wheat, the amount of Cu bound to roots decreased by only 15% as the Ca concentration increased. For tomato, the amount of Cu bound to roots did not significantly decrease as the Ca concentration increased. The competitive effect of Zn on Cu binding on wheat and tomato roots was also weak, but it was higher than the competitive effect of Ca (Figure S5). The amount of Cu bound to roots decreased by 30% and 18% for wheat and tomato, respectively, as the Zn concentration increased.

When determined for a given species (monocots or dicots) under similar experimental conditions for Cu, Ca, and Zn, the logK_{Cu} in roots was reported to be higher than the logK_{Zn} by 1.9 to 3.4 orders of magnitude (Vulkan et al. 2004; Wang et al. 2010 and 2012; Le et al. 2013) and were reported to be higher than the logK_{Ca} by 0.9 to 4.4 orders of magnitude (Cheng and Allen 2001; Luo et al. 2008; Wu and Hendershot 2010; Wang et al. 2012). The logK_{Zn} also remained higher than the logK_{Ca} by 1 to 2 orders of magnitude (Wang et al. 2010; Kinraide 2009). The literature thus supports our findings, which showed that the competitive effect of Ca and Zn on Cu binding on wheat and tomato roots was weak, but that Zn was a stronger competitor than Ca.

WHAM modelled almost no competitive effect of Ca and Zn and hence overestimated Cu binding on roots in the presence of Ca or Zn for wheat (*RMSE*= 0.28) and tomato (*RMSE* = 0.12) (Figure S5; Table S4). In contrast, WHAM-THP estimates correctly fitted (*RMSE* = 0.03-0.04) the competitive effect of Ca and Zn on Cu binding on wheat and tomato roots (Figure S5). To obtain this good fit, the $\log K_{Ca}$ and $\log K_{Zn}$ of HA_{II} sites in WHAM-THP was increased by 2 orders of magnitude compared to the default $\log K_{Ca}$ and $\log K_{Zn}$ of type-2 sites in WHAM (Table 2). However, the difference between the corresponding log*K* for Cu and Ca (Δ = 1.0-1.2 for HA_I and Δ = 2.0-2.2 for HA_{II}) or for Cu and Zn (Δ = 0.2 for HA_I and Δ = 0 for HA_{II}) in WHAM-THP remained approximately the same as in WHAM (Table 2). This suggests that the better fits obtained with WHAM-THP than with WHAM could be attributed to the specific parameterization of the acidic properties of wheat and tomato roots rather than to the specific parameterization of the binding affinity of Cu, Ca, and Zn.

4.3. WHAM-THP only slightly improves the prediction of copper concentration in pea roots

WHAM and WHAM-THP estimates exhibited the same goodness of fit for the Cu concentration in roots when considering the whole dataset of Wu and Hendershot (2010) (*RMSE* = 0.69 and 0.70, respectively), with almost all data points predicted within one order of magnitude. The deviation between root Cu concentrations measured and those modelled with WHAM increased as the root Cu concentration decreased (Figure 3), indicating that WHAM estimates tends to overestimate root Cu concentration at low Cu exposure levels.

It is noteworthy that the prediction of the root Cu concentration with WHAM could be improved (*RMSE*= 0.56) by setting the *ΔLK2* at 0 for Ca and Cu (data not shown). This suggests that the deviation observed with WHAM estimates at low root Cu concentration was mainly due to a too strong contribution of low density sites exhibiting a particularly high affinity for metal cations in the default parameterization of WHAM. In WHAM-THP, the *ΔLK2* has been set at 0 for Ca and Cu. Accordingly, WHAM-THP estimates better fitted the root Cu concentration than WHAM estimates for root Cu concentrations approximately lower than 10^{-5} $mol.g^{-1}$ at pH 4 and 5 10^{-6} $mol.g^{-1}$ at pH 5 (Figure 3A and B).

WHAM estimates were very sensitive to pH as the *RMSE* between measured and modelled data increased from 0.42 at pH 4 to 0.90 at pH 6 (Figure 3). WHAM-THP estimates were less sensitive to pH than WHAM estimates at pH 4 and 5 as the *RMSE* between measured and modelled data were 0.33 and 0.49, respectively (Figure 3A and B). However, at pH 6, WHAM-THP estimates deviated more from the measured data than WHAM estimates (Figure 3C). This was in agreement with the results obtained in the Cu sorption experiment 3, which showed that WHAM-THP estimates tended to overestimate the proton competition in comparison with WHAM estimates (see 4.2.3; Figure S4).

4.4. WHAM-THP does not improve the prediction of copper rhizotoxicity

Estimates of WHAM and WHAM-THP combined with *Ftox* fitted very similarly the measured *RRE* (R^2 = 0.73 and *RMSE* = 13 %; Figure 4A and B). The *Ftox* values calculated with WHAM-THP were closely correlated with those of WHAM (R^2 = 0.94) although the *Ftox* values calculated with WHAM-THP were about twofold higher. As the $\bullet_{Cu}$ optimized in WHAM and WHAM-THP simulations was very similar (3.3 and 3.6, respectively), the difference between the *Ftox* values calculated with WHAM and WHAM-THP comes, according to Eq. 4, from the stronger complexation calculated with WHAM-THP (i.e. higher v_{Cu} and v_H) than that calculated with WHAM.

Accordingly, WHAM and WHAM-THP estimates combined with *Ftox* predicted very similar *RRE* (R^2 = 0.97; Figure 4C), even at low root Cu concentration for which WHAM-THP better predicted the root Cu concentration than WHAM (data not shown). This may have been due to the fact that the prediction of *RRE* with *Ftox* values involved the optimization independently for WHAM and WHAM-THP of three supplementary parameters, i.e. $Ftox_{50}$, β, and α_{Cu}. This supplemental optimization may enable correction of the small deviation observed between WHAM and WHAM-THP estimates of Cu concentration in roots so as to finally achieve similar *RRE* predictions with WHAM and WHAM-THP.

Although WHAM-THP slightly improved the prediction of Cu binding on roots (see 4.2) and the prediction of Cu concentration in roots at low root Cu concentration (see 4.3) relative to WHAM, WHAM-THP hence did not at all improve the prediction of Cu rhizotoxicity. Considering this result and how time-consuming it would be to complete the parameterization of WHAM-THP for all metal cations of interest, we conclude that, although the default parameterization of WHAM does not neatly predict the binding of metal cations on roots, it could be used with a good level of confidence in predictive ecotoxicology for terrestrial higher plants without any specific parameterization.

Supporting information

Supporting information (Tables S1 to S4; Figures S1 to S5) can be found at http:…

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Figure captions

Fig. 1. Measured versus modelled acidic properties (expressed in charge Q corrected by the initial charge Q_0) of wheat (crosses) and tomato (circles) roots. Green and red symbols correspond to data modelled with the Windermere Humic Aqueous Model either parameterized by default (WHAM) or specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively. The root mean square errors (*RMSE*) pool data for wheat and tomato (*n* = 176). The solid line refers to 1:1 line and dashed lines refer to a factor ± 2.

Fig. 2. Measured versus modelled binding of copper (Cu) to wheat (crosses) and tomato (circles) roots. Green and red symbols correspond to data modelled with the Windermere Humic Aqueous Model either parameterized by default (WHAM, A) or specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP, B), respectively. The root mean square errors (*RMSE*) pool data for wheat and tomato in the Cu sorption experiments 1 to 5 (*n* = 183). The solid line refers to 1:1 line and dashed lines refer to a factor ± 2.

Fig. 3. Measured versus modelled copper (Cu) concentration in pea roots exposed to solutions at pH 4 (A), 5 (B), and 6 (C) with varying calcium (0.04, 0.18, and 1.92 mM) and Cu (0 to 24.8 µM) concentrations. Green and red symbols correspond to data modelled with the Windermere Humic Aqueous Model either parameterized by default (WHAM) or specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively. The deviation between measured and modelled data is expressed as the root mean square errors (*RMSE*). The solid line refers to 1:1 line and dashed lines refer to a factor ± 10.

Fig. 4. Relative root elongation (*RRE*) measured for pea as a function of the exposure to toxic cations (proton and copper) in solutions at pH 4, 5 or, 6 and with varying calcium (0.04, 0.18, and 1.92 mM) and copper (0 to 24.8 µM) concentrations (A and B). The proton and copper toxicity is calculated by combining a toxicity function (*Ftox*, see 3.2 for rationale) with the Windermere Humic Aqueous Model either parameterized by default (WHAM, green circles, A) or specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP, red circles, B). The solid line refers to the *RRE* predicted with WHAM (A) or WHAM-THP (B) and dashed lines refer to a deviation of ± 10%. The deviation between measured and modelled data is

expressed as the root mean square errors (*RMSE*). Relative root elongation predicted with WHAM-THP versus *RRE* predicted with WHAM (C). The solid line refers to 1:1 line.

Does specific parameterization of WHAM improve the prediction of copper competitive binding and toxicity on plant roots?

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Tables = 2

Tables

Table 1

Proton dissociation constants (pKa_i) and distribution terms (ΔpKa_i) of wheat and tomato roots as parameterized in the Windermere Humic Aqueous Model by default (WHAM) for one humic acid (HA) and specifically parameterized for terrestrial higher plants (WHAM-THP) for two HA. Total site densities (L_{Hi}, cmol$_c$.kg^{-1}) were determined experimentally by Guigues et al. (2014).

		HA_I						HA_{II}						$L_{H,total}$
		Type 1			Type 2			Type 1			Type 2			
		L_{H1}	pKa_1	ΔpKa_1	L_{H2}	pKa_2	ΔpKa_2	L_{H1}	pKa_1	ΔpKa_1	L_{H2}	pKa_2	ΔpKa_2	
WHAM	Wheat	24.4	4.1	2.6	12.2	8.3	3.1	–	–	–	–	–	–	36.6
	Tomato	64.9			32.5									97.4
WHAM-THP	Wheat	6.3	4.2	1.5	3.3	5.2	2.0	18.0	9.8	0	9.0	8.8	1.5	36.6
	Tomato	31.3			15.7			33.6			16.8			97.4

Table 2

Intrinsic equilibrium constants ($K_{M,,i}$) and heterogeneity parameters ($\Delta LK2_{M},_{i}$) of copper (Cu), calcium (Ca) and zinc (Zn) binding on wheat and tomato roots as parameterized in the Windermere Humic Aqueous Model by default (WHAM) for one humic acid (HA) and specifically parameterized for terrestrial higher plants (WHAM-THP) for two HA.

		HA_I			HA_{II}		
		Type 1	Type 2		Type 1	Type 2	
		Log$K_{M,1}$	Log$K_{M,2}$	$\Delta LK2_{M,I}$	Log$K_{M,1}$	Log$K_{M,2}$	$\Delta LK2_{M,II}$
Cu	WHAM	2.4	5.1	2.3	–	–	–
	WHAM-THP	2.2	2.7	0	6.7	6.0	0
Ca	WHAM	1.3	2.3	0	–	–	–
	WHAM-THP	1.2	1.5	0	4.5	4.0	0
Zn	WHAM	1.9	4.1	1.3	–	–	–
	WHAM-THP	2.0	2.5	0	6.7	6.0	0

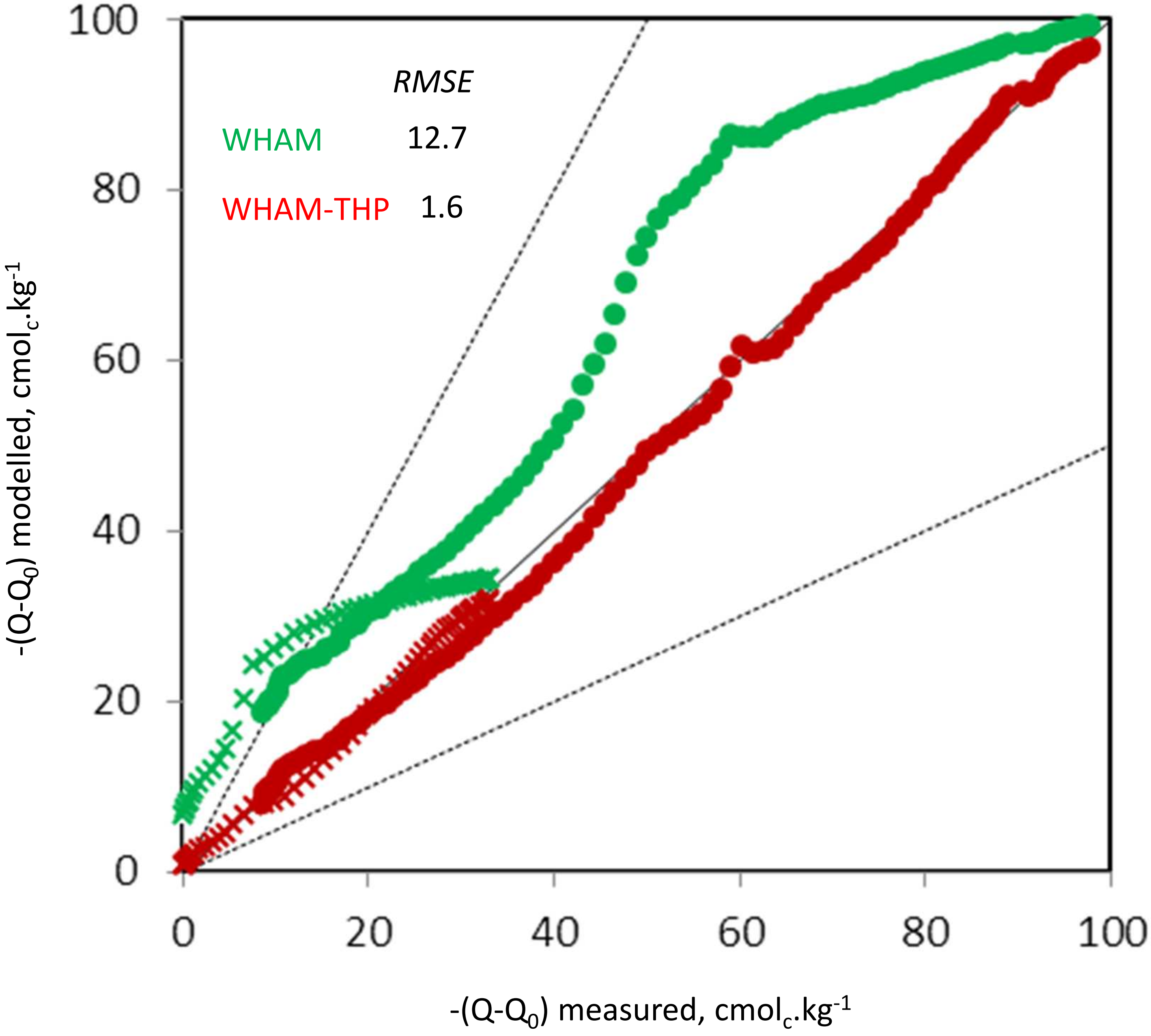
RMSE
WHAM 12.7
WHAM-THP 1.6
100
80
60
40
20
0
0
20
40
60
80
100
-(Q-Q$_0$) modelled, cmol$_c$.kg^{-1}
-(Q-Q$_0$) measured, cmol$_c$.kg^{-1}

Fig. 1

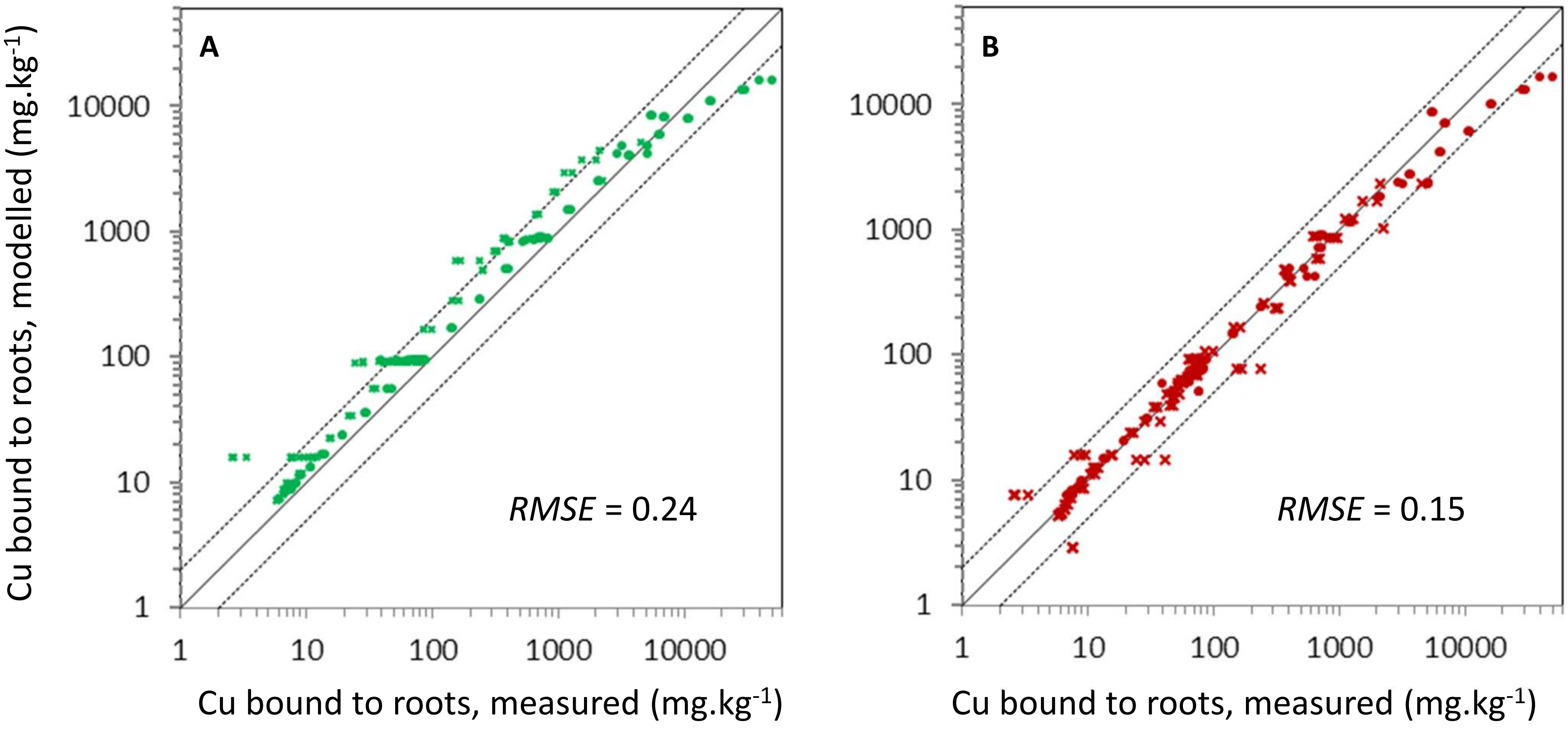
A
B
Cu bound to roots, modelled (mg.kg-1)
Cu bound to roots, measured (mg.kg-1)
Cu bound to roots, measured (mg.kg-1)
1
10
100
1000
10000
RMSE = 0.24
RMSE = 0.15

Fig. 2

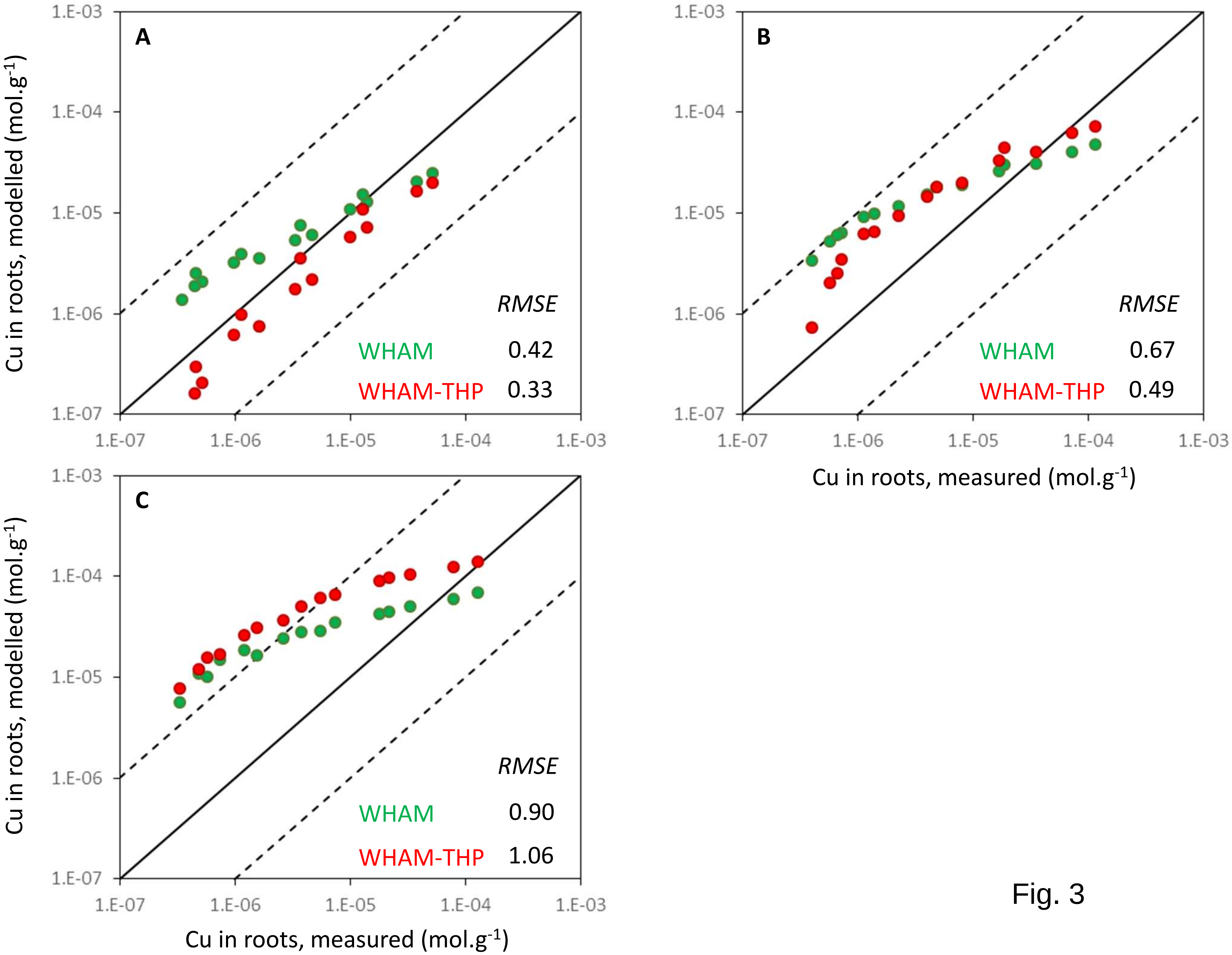
A
B
C
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
Cu in roots, modelled (mol.g^{-1})
Cu in roots, measured (mol.g^{-1})
RMSE
WHAM 0.42
WHAM-THP 0.33
WHAM 0.67
WHAM-THP 0.49
WHAM 0.90
WHAM-THP 1.06

Fig. 3

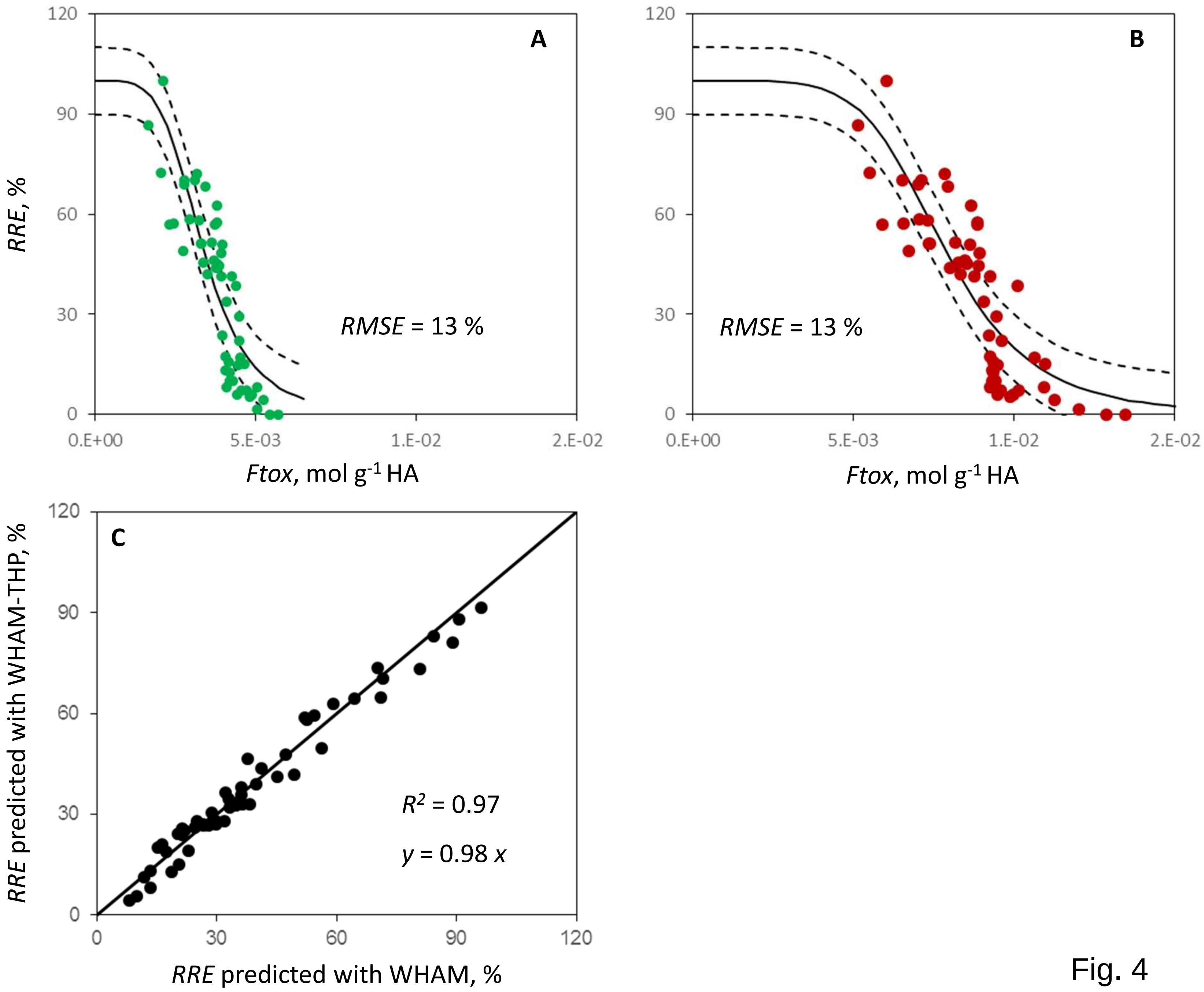
A
B
C
RRE, %
Ftox, mol g-1 HA
RMSE = 13 %
RMSE = 13 %
0
30
60
90
120
0.E+00
5.E-03
1.E-02
2.E-02
RRE predicted with WHAM-THP, %
RRE predicted with WHAM, %
R2 = 0.97
y = 0.98 x

Fig. 4

Does specific parameterization of WHAM improve the prediction of copper competitive binding and toxicity on plant roots?

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Highlights

- WHAM-THP is a specific parameterization of WHAM for terrestrial higher plants
- WHAM-THP much improves the prediction of root acidic properties
- WHAM-THP only slightly improves the prediction of root copper competitive binding
- WHAM-THP does not improve the prediction of copper rhizotoxicity