**Supporting information to:**

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

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**Table S1**

Initial (pMin) and final/equilibrium (pMeq) concentration of copper, calcium and zinc, pH and ionic strength (I) in the solutions of the five copper sorption experiments on wheat roots.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp. 1****Cu binding affinity** | **pCuin** | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.3 | 7.2 | 7.2 |  | 7.1 | 7.1 | 7.0 | 7.0 | 6.8 | 6.8 | 6.7 | 6.7 | 6.4 |
| **pCueq** | 8.0 | 8.1 | 8.1 | 8.1 | 8.0 | 8.0 | 8.0 | 7.9 | 7.9 | 7.8 | 7.7 |  | 7.8 | 7.7 | 7.6 | 7.5 | 7.4 | 7.3 | 7.1 | 7.1 | 6.9 |
| **pH** | 5.0 | 4.9 | 4.9 | 4.9 | 4.9 | 4.7 | 4.9 | 4.9 | 4.7 | 4.9 | 4.9 |  | 4.9 | 4.8 | 4.9 | 4.9 | 4.9 | 4.9 | 4.9 | 4.9 | 4.9 |
| **I** |  | Set at 30 mM |
| **pCuin** | 6.4 | 6.2 | 6.2 | 6.0 | 6.0 | 5.7 | 5.7 | 5.5 | 5.5 | 5.2 | 5.2 |  | 5.0 | 5.0 | 4.7 | 4.7 | 4.5 | 4.5 | 4.2 | 4.2 | 4.0 |
| **pCueq** | 6.9 | 6.6 | 6.6 | 6.3 | 6.3 | 6.0 | 6.1 | 5.8 | 5.8 | 5.5 | 5.5 |  | 5.2 | 5.2 | 4.9 | 4.9 | 4.6 | 4.6 | 4.3 | 4.4 | 4.1 |
| **pH** | 4.9 | 4.9 | 4.9 | 4.9 | 4.8 | 4.7 | 4.9 | 4.8 | 4.8 | 4.8 | 4.9 |  | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 | 4.7 | 4.8 | 4.8 | 4.8 |
| **I** |  | Set at 30 mM |
| **Exp. 2****Effect of ionic strength** | **pCuin** | 7.0 | 7.0 | 7.0 | 6.2 | 6.2 | 5.2 | 5.2 | 5.2 | 4.2 | 7.0 | 7.0 | 7.0 |  | 6.2 | 6.2 | 6.2 | 5.2 | 5.2 | 5.2 |  |
| **pCueq** | 7.1 | 7.1 | 7.1 | 6.5 | 6.5 | 5.4 | 5.4 | 5.4 | 4.4 | 7.5 | 7.6 | 7.5 |  | 6.9 | 6.8 | 6.9 | 5.5 | 5.5 | 5.5 |  |
| **pH** | 4.8 | 4.8 | 4.8 | 4.8 | 4.9 | 4.8 | 4.9 | 5.0 | 4.8 | 4.7 | 4.8 | 4.8 |  | 4.8 | 4.7 | ∕ | 4.8 | 4.7 | 4.8 |  |
| **I** | Set at 300 mM |  | Set at 0.6 mM |  |
| **Exp. 3****Proton competition** | **pCuin** | 7.0 | 7.0 | 7.0 | 6.2 | 6.2 | 6.2 | 5.2 | 5.2 | 5.2 | 7.0 | 7.0 | 7.0 |  | 6.2 | 6.2 | 6.2 | 5.2 | 5.2 |  |  |
| **pCueq** | 7.3 | 7.3 | 7.3 | 6.4 | 6.4 | 6.5 | 5.3 | 5.4 | 5.3 | 7.4 | 7.3 | 7.4 |  | 6.7 | 6.8 | 6.7 | 5.7 | 5.8 |  |  |
| **pH** | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 6.3 | 6.4 | 6.3 |  | 6.4 | 6.3 | 6.3 | 6.3 | 6.3 |  |  |
| **I** | Set at 30 mM |  | Set at 30 mM |  |  |
| **Exp. 4 and 5****Ca and Zn competition** | **pCuin** | **Ca** | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 |  | **Zn** | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 |
| **pCueq** | 6.6 | 6.6 | 6.6 | 6.6 | 6.6 | 6.6 | 6.5 | 6.6 | 6.5 | 6.6 |  | 6.7 | 6.7 | 6.7 | 6.6 | 6.5 | 6.4 | 6.5 |
| **pMin** | 4.0 | 4.0 | 3.5 | 3.5 | 3.0 | 3.0 | 2.5 | 2.5 | 2.0 | 2.0 |  | 7.2 | 6.0 | 6.0 | 5.2 | 5.2 | 4.5 | 4.5 |
| **pMeq** | 4.7 | 4.7 | 4.2 | 4.2 | 3.7 | 3.7 | 3.1 | 3.1 | 2.2 | 2.3 |  | 7.2 | 6.0 | 6.0 | 6.0 | 6.0 | 4.6 | 4.6 |
| **pH** | 4.9 | 4.9 | 4.8 | 4.9 | 4.9 | 4.9 | 4.8 | 4.8 | 4.6 | 4.8 |  | 4.8 | 4.9 | 4.9 | 4.9 | 4.9 | 4.8 | 4.9 |
| **I** | Set at 30 mM |  | Set at 30 mM |

**Table S2**

Initial (pMin) and final/equilibrium (pMeq) concentration of copper, calcium and zinc, pH and ionic strength (I) in the solutions of the five copper sorption experiments on tomato roots.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp. 1****Cu binding affinity** | **pCuin** | 7.3 | 7.3 | 7.3 | 7.3 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.1 | 7.1 | 7.1 | 7.1 | 7.0 | 7.0 | 6.8 | 6.8 | 6.6 | 6.6 |
| **pCueq** | 8.0 | 8.1 | 8.1 | 8.0 | 8.0 | 8.0 | 8.0 | 7.9 | 8.0 | 7.9 | 7.8 | 7.8 | 7.8 | 7.8 | 7.6 | 7.7 | 7.5 | 7.6 | 7.3 | 7.4 |
| **pH** | 4.5 | 4.6 | 4.6 | 4.7 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 | 4.5 | 4.5 | 4.5 | 4.6 | 4.6 | 4.6 | 4.6 |
| **I** | Set at 30 mM |
| **pCuin** | 6.4 | 6.4 | 6.2 | 6.2 | 6.0 | 6.0 | 5.7 | 5.7 | 5.5 | 5.5 | 5.2 | 5.2 | 5.0 | 5.0 | 4.7 | 4.7 | 4.5 | 4.5 | 4.2 | 4.2 |
| **pCueq** | 7.2 | 7.1 | 7.1 | 7.1 | 6.7 | 6.8 | 6.5 | 6.5 | 6.1 | 6.2 | 5.9 | 5.9 | 5.7 | 5.6 | 5.3 | 5.4 | 5.1 | 5.0 | 4.8 | 4.8 |
| **pH** | 4.6 | 4.6 | 4.5 | 4.5 | 4.6 | 4.5 | 4.5 | 4.6 | 4.6 | 4.6 | 4.5 | 4.5 | 4.5 | 4.6 | 4.6 | 4.5 | 4.5 | 4.5 | 4.6 | 4.5 |
| **I** | Set at 30 mM |
| **pCuin** | 4.0 | 4.0 | 3.6 | 3.6 | 3.3 | 3.3 | 3.0 | 3.0 |  |  |  |  |  |  |  |  |  |  |  |  |
| **pCueq** | 4.5 | 4.5 | 3.8 | 3.8 | 3.5 | 3.5 | 3.1 | 3.2 |  |  |  |  |  |  |  |  |  |  |  |  |
| **pH** | 4.4 | 4.4 | 4.7 | 4.6 | 4.2 | 4.4 | 4.3 | 4.1 |  |  |  |  |  |  |  |  |  |  |  |  |
| **I** | Set at 30 mM |  |  |  |  |  |  |  |  |  |  |  |  |
| **Exp. 2** **Effect of ionic strength** | **pCuin** | 6.2 | 6.2 | 5.2 | 5.2 | 4.2 | 4.2 | 6.2 | 6.2 | 5.2 | 5.2 | 4.2 | 4.2 |  |  |  |  |
| **pCueq** | 6.6 | 6.4 | 5.6 | 5.5 | 4.4 | 4.6 | 7.4 | 7.3 | 6.3 | 6.3 | 4.9 | 4.9 |  |  |  |  |
| **pH** | 5.0 | 5.0 | 4.9 | 4.9 | 4.8 | 4.8 | 4.5 | 4.6 | 4.6 | 4.3 | 4.2 | 4.2 |  |  |  |  |
| **I** | Set at 300 mM | Set at 0.6 mM |  |  |  |  |
| **Exp. 3** **Proton competition** | **pCuin** | 6.2 | 6.2 | 5.2 | 5.2 | 4.2 | 4.2 | 6.2 | 6.2 | 5.2 | 5.2 | 4.2 | 4.2 |  |  |  |  |
| **pCueq** | 6.9 | 6.9 | 5.7 | 5.8 | 4.6 | 4.4 | 6.8 | 6.9 | 6.0 | 5.9 | 4.7 | 4.7 |  |  |  |  |
| **pH** | 4.2 | 4.2 | 4.2 | 4.2 | 4.1 | 4.1 | 6.3 | 6.2 | 6.2 | 6.3 | 6.1 | 6.2 |  |  |  |  |
| **I** | Set at 30 mM | Set at 30 mM |  |  |  |  |
| **Exp. 4 and 5****Ca and Zn competition** | **pCuin** | **Ca** | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | **Zn** | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 | 6.2 |
| **pCueq** | 6.8 | 6.8 | 6.8 | 7.1 | 7.2 | 6.9 | 6.9 | 6.8 | 6.7 | 6.9 | 7.0 | 7.0 | 6.9 | 7.0 | 6.9 | 6.7 | 6.7 | 6.7 |
| **pMin** | 3.9 | 3.9 | 3.5 | 3.5 | 3.0 | 3.0 | 2.5 | 2.5 | 2.0 | 2.0 | 6.9 | 6.6 | 6.0 | 5.2 | 5.2 | 5.2 | 4.5 | 4.5 |
| **pMeq** | 3.9 | 4.0 | 4.1 | 4.0 | 3.4 | 3.4 | 2.8 | 2.8 | 2.2 | 2.2 | 7.1 | 6.9 | 6.4 | 5.6 | 5.6 | 5.6 | 4.9 | 5.0 |
| **pH** | 5.2 | 4.9 | 4.7 | 4.6 | 5.5 | 5.6 | 5.5 | 5.8 | 5.6 | 5.7 | 4.7 | 4.7 | 4.6 | 4.6 | 4.6 | 4.6 | 4.5 | 4.7 |
| **I** | Set at 30 mM | Set at 30 mM |

**Table S3**

Literature review of ratios between the density of low-p*Ka* (p*Ka* < 7.5) and high-p*Ka* (p*Ka* ≥ 7.5) sites (L/H ratios) measured on the roots of terrestrial higher plants.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Species1 | Plant age (days) | L/Hratio | Reference |
| Dicots | *Pisum sativum* L. | 10 | 1.7 | Wu and Hendershot 2009 |
| 35 | 0.5 | Meychik and Yermakov 1999 and 2001 |
| *Solanum lycopersicum* L. | 21 | 0.9 | Guigues et al. 2014 |
| 21 | 1.1 | Unpublished data2 |
| *White Lupin* L. | 10 | 3.8 | Meychik and Yermakov 1999 and 2001 |
| Monocots | *Festuca rubra* L. | 30 | 1.4 | Ginn et al. 2008 |
| *Lolium perenne* L. | 21 | 2.9 | Unpublished data |
| *Triticum aestivum* L. | 10 | 0.4 | Meychik and Yermakov 1999 and 2001 |
| 21 | 0.5 | Guigues et al. 2014 |
| 38 | 0.4 | Meychik and Yermakov 1999 and 2001 |

1 All plant species were grown in hydroponic conditions except for *F. rubra*, which was grown in pots using commercially supplied potting soil.

2 Plants corresponding to the *sitiens* mutant of the wild-type Moneymaker cultivar of *S. lycopersicum* (see Curvers et al. 2010 for further information)

**Table S4**

Root mean square error a (*RMSE*) between measured and modelled data of potentiometric titrations and copper (Cu) sorption experiments (see Table S1 and S2 and section 2.3) for wheat and tomato roots. Modelled data were obtained with the Windermere Humic Aqueous Model parameterized by default (WHAM) or specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP). *RMSE* was calculated from untransformed data for potentiometric titrations as some experimental data were negative. *RMSE* was calculated from log10-transformed data for Cu sorption experiments to balance the weight of the highest values.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ***n* b** | **WHAM** | **WHAM-THP** |
| Potentiometric titrations (cmolc.kg-1) | Wheat |  65 |  9.0 | 1.0 |
| Tomato | 111 | 14.4 | 1.8 |
| Cu sorption exp. 1: Cu binding affinity | Wheat |  40 | 0.23 | 0.04 |
| Tomato c |  48 | 0.14 | 0.14 |
| Cu sorption exp. 2: Effect of ionic strength | Wheat |  19 | 0.38 | 0.21 |
| Tomato |  12 | 0.18 | 0.11 |
| Cu sorption exp. 3: Proton competition | Wheat |  17 | 0.35 | 0.31 |
| Tomato |  12 | 0.14 | 0.17 |
| Cu sorption exp. 4 and 5: Ca and Zn competition | Wheat |  17 | 0.28 | 0.04 |
| Tomato |  18 | 0.12 | 0.03 |

a $RMSE= \sqrt{\frac{\sum\_{i=1}^{n}(x\_{i,exp}-x\_{i, model})^{2}}{n}}$ where $x\_{i,exp}$ is an experimental datum, $x\_{i, model}$ its corresponding modelled datum and *n* the number of experimental data

b Number of experimental data

c When removing experimental data for pCuin ≤ 4.5, *RMSE* for Cu binding on tomato roots were equal to 0.09 and 0.03 for WHAM and WHAM-THP, respectively (see section 4.2.1 for rationale).

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**Figure S1** Potentiometric titration of wheat (crosses) and tomato (circles) roots expressed in charge (Q) corrected by the initial charge (Q0). Dotted and solid lines correspond to simulations performed with the Windermere Humic Aqueous Model parameterized by default (WHAM) and specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively.

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**Figure S2** Copper binding on wheat (crosses, a) and tomato (circles, b) roots at pH 4.7 (± 0.2) and an ionic strength of 0.03 M. Dotted and solid lines correspond to simulations performed with the Windermere Humic Aqueous Model parameterized by default (WHAM) and specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively.

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**Figure S3** Copper binding on wheat (crosses, a) and tomato (circles, b) roots at an ionic strength of 0.6 mM (dark symbols and lines) or 300 mM (red symbols and lines) and at pH 4.7 (± 0.2). Dotted and solid lines correspond to simulations performed with the Windermere Humic Aqueous Model parameterized by default (WHAM) and specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively.

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**Figure S4** Copper binding on wheat (crosses, a) and tomato (circles, b) roots at pH 6.3 (± 0.1) (dark symbols and lines) and pH 4.1 (± 0.1) (red symbols and lines) with an ionic strength of 0.03 M. Dotted and solid lines correspond to simulations performed with the Windermere Humic Aqueous Model parameterized by default (WHAM) and specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively.

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**Figure S5** Copper binding on wheat (crosses, a) and tomato (circles, b) roots in the presence of Ca (red symbols and lines) and Zn (dark symbols and lines) at an ionic strength of 0.03 M and pH 4.9 (± 0.3). Dotted and solid lines correspond to simulations performed with the Windermere Humic Aqueous Model parameterized by default (WHAM) and specifically parameterized with two humic acids for terrestrial higher plants (WHAM-THP), respectively.

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