Electronic Supplementary Information for the article:

Thlaspi arvense binds Cu(II) as a bis-(L-histidinato) complex on root cell walls in an urban ecosystem

Supplementary Results: EXAFS characterization of the Cu-histidine double chelate

Histidine can form three types of chelate with Cu(II) in solution as shown in Figure 3:[1-3] a histamine-like tridentate chelate, denoted him-him, in which the two L-histidine ligands are coordinated equatorially to Cu(II) by the amino and the imidazole nitrogens; a bisglycine-like chelate, denoted gly-gly, with two glycine bidentate ligands bound equatorially by the amino nitrogen and carboxylate oxygen atoms and with two water molecules at the axial positions; and, a mixed gly-him chelate. These low-molecular-weight chelates are in equilibrium in solution, and their relative amounts vary as a function of pH (see, e.g., Figure 9 of Mesu *et al.*[3]).

The EXAFS spectrum of the him-him chelate at pH 7.4 represents this species because the species is dominant above neutral pH. At pH 4.8, Cu^{2+} is bound mainly as a gly-him chelate. At pH 3-4, the gly-gly chelate occurs in equilibrium with the gly-him chelate and the monoglycine-like chelate. Furthermore, at acidic pH with glycine only, the bis(glycinato)copper(II) complex (gly-gly chelate) co-exists with the mono(glycinato)copper(II) complex and hydrated Cu^{2+}, similarly to the distribution of Cu species in solution with histidine only[4], whereas at circumneutral pH (e.g. pH 7.4) the proportion of the gly-gly chelate is > 99%. In our study, the pure Cu(II) gly-gly chelate was obtained in solution by mixing Cu(II) and glycine at pH 7.4. We also note that a neutral pH $CuGly_2$ solution and a pH 4 solution containing 60% $CuGly_2$ and 40% CuGly have nearly identical XANES spectra.[4]

The EXAFS spectra and Fourier transforms for the three types of Cu-histidine double chelates are shown in Figure S3. Results show that EXAFS is sensitive to the coordination of Cu with O and N ligands. Two higher coordination shells are evident in the cell wall spectrum: an O shell from an axial carboxylate group at 2.30 Å and a carbon shell at 2.90 Å (Fig. 4b and S3d). In the gly-him chelate, the axial O_c and H_2O molecule are less strongly bonded at 2.34 Å and the carbon atoms are distributed over a wider range of distances, at least partly because of the dynamic equilibrium between several coordination configurations of the Cu^{2+}-his moieties at pH 4.8. Also, molecular packing, as governed by van der Waals and hydrogen-bond interaction energies, may not be equivalent in the aqueous histidine complexes and the cell wall.

Supplementary Figures

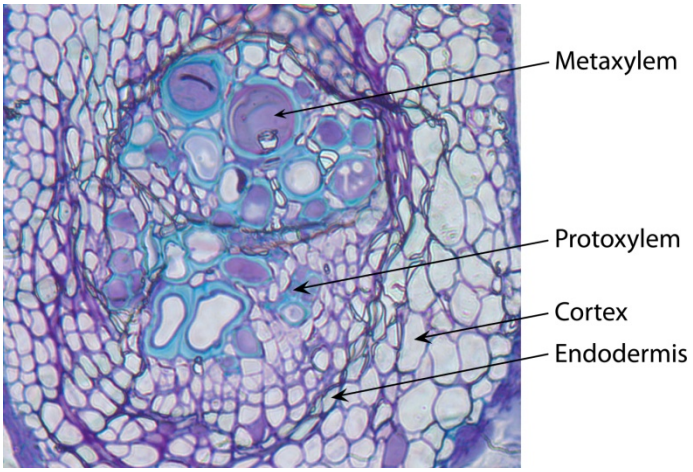

Figure S1. Two micron-thick cross-section of a secondary root of *T. arvense* stained with toluidine blue. Turquoise and dark blue represent lignification of the cell walls. The xylem vessels have thickened secondary walls (turquoise zones) encrusted with lignin. Note the dark-blue stained inner surface layer in the secondary walls of some xylem vessels.[5] These regions are enriched in Cu and Zn. The endodermis, which supports the Casparian strip, is weakly lignified.

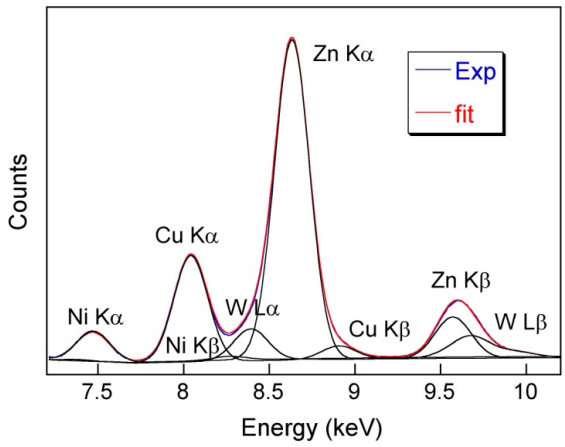

Figure S2. Decomposition of X-ray emission lines used to image Cu and Zn. Typical nano-SXRF spectrum plotted in the 7.2-10.2 keV fluorescence interval to show the decomposition of emission lines used to image Cu and Zn. The fluorescence lines were fitted with Hypermet functions.[6]

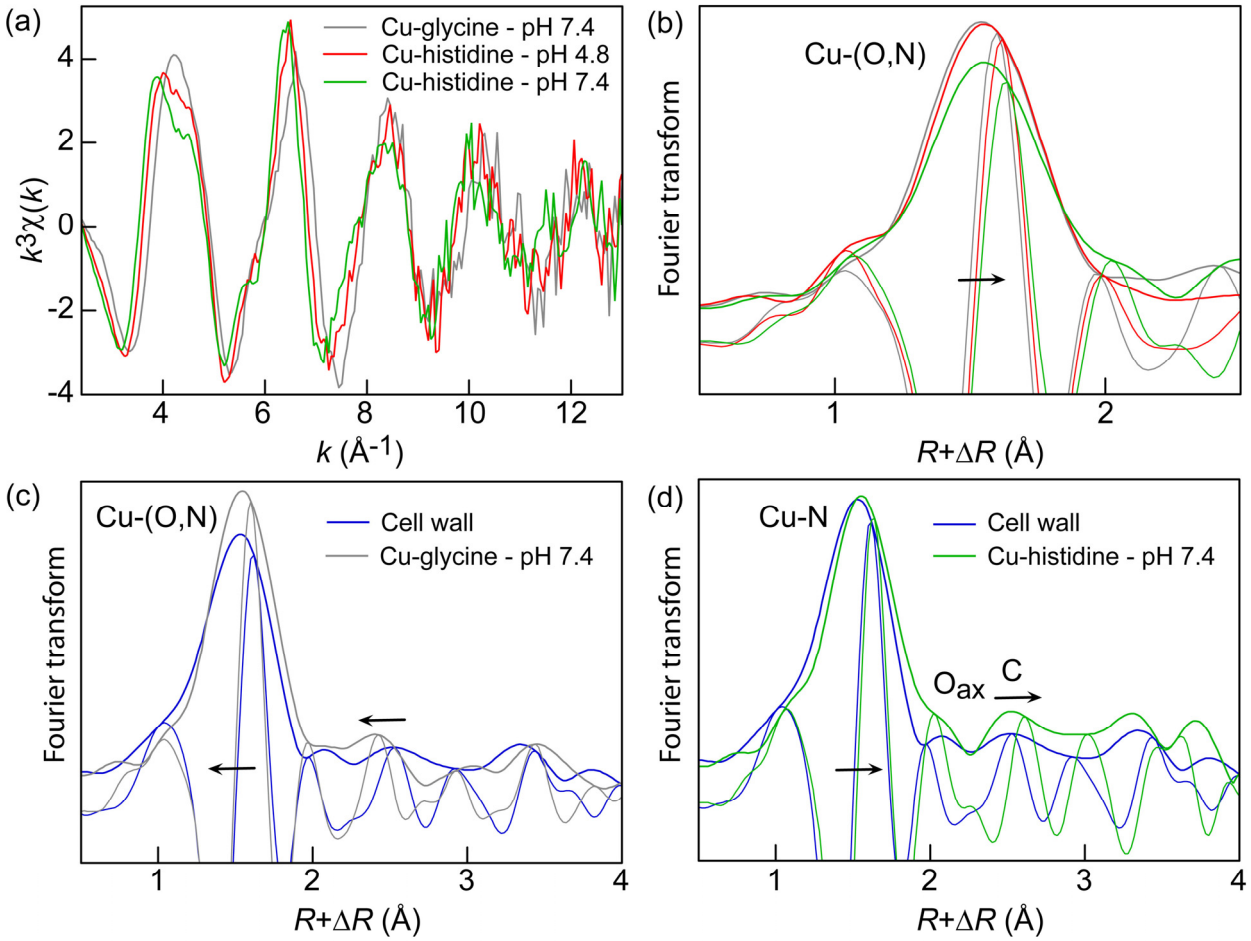

Figure S3. EXAFS spectra and Fourier transforms of the cell wall and Cu-histidine complexes in frozen solution. (a,b) EXAFS spectra and Fourier transforms of the gly-gly (Cu-glycine pH 7.4), gly-him (Cu-histidine pH 4.8), and him-him (Cu-histidine pH 7.4) chelates showing the variation of the signal frequency with the relative number of O and N ligands. The average Cu-L distance in the CuL_4 square plane is 1.94 Å for L = 4O,[7] 1.95 Å for L = 2O+2N (gly-gly), 1.96 Å for L = 1O+3N (gly-him), and 1.98 Å for L = 4N.[3] (c) In the gly-gly configuration, the first C shell is at 2.80 Å, and the average Cu-O_{ax} distance is 2.33 Å. (d) In the him-him configuration, the first C shell is at 2.98 Å, and the average Cu-O_{ax} distance is 2.37 Å. The average Cu-C distance measured by EXAFS increases with the size of the chelate ring.

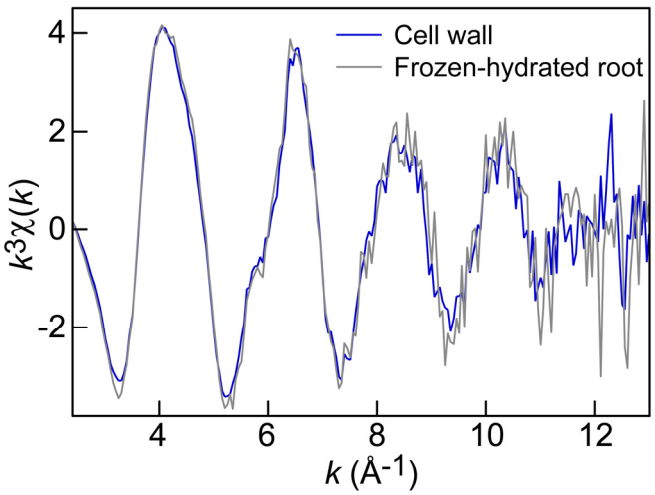

Figure S4. Micro-EXAFS spectrum of the cell wall and bulk spectrum of the frozen-hydrated roots. No difference was observed between the two spectra, nor among individual micro-EXAFS, which indicates that other ligands, such as glycine, are subordinate, if they exist.

References

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