Supplementary material

Diffusion limitation of zinc fluxes into wheat roots, PLM and DGT devices in the presence of organic ligands

A. Gramlich^{A,D}, S. Tandy^A, E. Frossard^B, J. Eikenberg^C and R. Schulin^A

^AInstitute of Terrestrial Ecosystems, ETH Zurich, Universitätstraße 16, CH-8092 Zürich, Switzerland.

^BInstitute for Plant, Animal and Agroecosystems Sciences, ETH Zurich, Switzerland.

^CRadioanalytics Group, Paul Scherrer Institute, Villigen, Switzerland.

^DCorresponding author. Email: anja.gramlich@env.ethz.ch

Determination of dissociation rate constants of Zn-complexes with EDTA, citrate and histidine *Method*

Chelex20 resin was pre-treated over night in a solution containing 100 mM Ca(NO₃)₂ and 5 mM MOPS (pH 7.2). The experimental solutions consisted all of 20 μ M ZnSO₄, 400 μ M Ca(NO₃)₂, 500 μ M KNO₃, 2.5 mM MOPS (pH 7.2, adjusted with NaOH). Additionally, the solutions contained either 20.2 μ M EDTA, 1450 μ M potassium–citrate or 1300 μ M L-histidine, except for the ligand free control treatment. According to MINEQL calculations more than 99 % of the Zn was complexed with the respective ligands in all treatments. Experiments were carried out in duplicates. Fifty millilitres of each solution was used and stirred at 500 rpm. Then 2.5 g of Chelex was added and 2-mL samples were taken at following times: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 min. Samples were filtered through 0.45- μ m filters and total Zn concentrations in the solution samples were measured using ICP-OES (Vista-MPX, Varian). Dissociation rate constants were determined using Eqn S1^[1,2]:

$$C_{ML}(t) = C_{ML}^0 \times e^{-k_d t} \quad (S1)$$

where $C_{ML}(t)$ is the Zn concentration in the solution at time *t*, C_{ML}^{0} is the initial metal concentration in the solution and k_d the dissociation rate constant of the respective complexes. The equation was fitted to the data with least-square fit using the software R.^[3]

Results

 k_d values in the presence of EDTA were, under our experimental, conditions in the same order of magnitude as determined in other studies.^[4] <u> k_d </u> values of Zn–citrate and Zn–histidine complexes were too weak to be determined with this method, as their dissociation and subsequent Zn complexation with Chelex20 was nearly as fast as in ligand free treatments (Table S1, Fig. S1).

Table S1. log *k_d* values of Zn with the organic ligands EDTA, citrate and histidine under the experimental conditions (20 μM ZnSO₄, 400 μM Ca(NO₃)₂, 500 μM KNO₃, 2.5 mM MOPS (pH 7.2, adjusted with NaOH), plus either 20.2 μM EDTA, 1450 μM potassium–citrate or 1300 μM L-histidine)

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Zn-Ligand		$\operatorname{Log} k_d(s^{-1})$
No ligand		-1.75
Zn-EDTA		-4.32
Zn-citrate		≥-1.75
Zn-histidine		≥-1.75

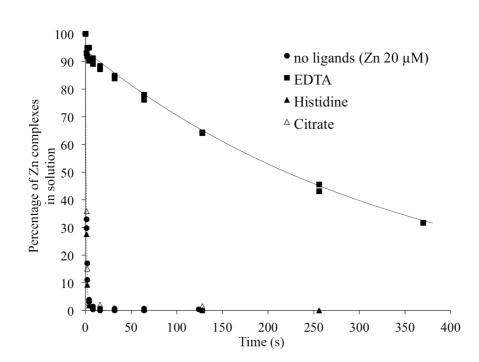


Fig. S1. Percentages of $[Zn]_{tot}$ remaining in the experimental solutions over time after the addition of Chelex20. The solid line represents least-square fit of Eqn S1 in the EDTA treatment and the dotted line is the least-square fit in ligand free treatments.

Calculation of C_{DGT}

Table S2. Calculation of C_{DGT} using the formula: $C_{DGT} = M \times g/(D \times A \times T)^{[5]}$

M is the amount of Zn accumulated in the resin, *g* is the thickness of the diffusive gel plus the filter thickness (0.092 cm), *D* the diffusion coefficient of the metal in the gel at 24 °C (5.92×10^{-6} cm² s⁻¹),

A the window area of the DGT device (3.14 cm^2) and T the exposure time (s) (24 h). Numbers in

parentheses represent s.e.

	Citrate	Histidine	EDTA high	EDTA low	No ligand
$C_{DGT Stirred}(\mu M)$	15.22 (0.8)	17.62 (0.2)	0.82 (0.1)	0.21 (0.01)	2.53 (0.2)

Root to shoot translocation

Table S3.Root to shoot translocation factors (65Zn activity in shoots + (65Zn activity in shoots + 65Zn activity in roots)) for the stirred, non-stirred and agar treatments

Standard errors are given in parentheses. Different superscript letters indicate significant differences

Treatments Translocation factor		Translocation factor	Translocation factor
	stirred	non-stirred	agar
Citrate	$0.24 (0.02)^{a}$	$0.19 (0.004)^{a}$	$0.11 (0.01)^{b}$
Histidine	$0.13 (0.01)^{a}$	$0.10(0.01)^{a}$	$0.06(0.004)^{b}$
EDTA high	$0.27 (0.02)^{a}$	$0.35 (0.03)^{a}$	$0.20 (0.01)^{a}$
EDTA low	$0.22 (0.01)^{a}$	$0.33 (0.03)^{a}$	$0.16 (0.02)^{a}$
No ligand	$0.12(0.01)^{a}$	$0.17 (0.02)^{a}$	0.16 (0.01) ^a

within one row (P < 0.05)

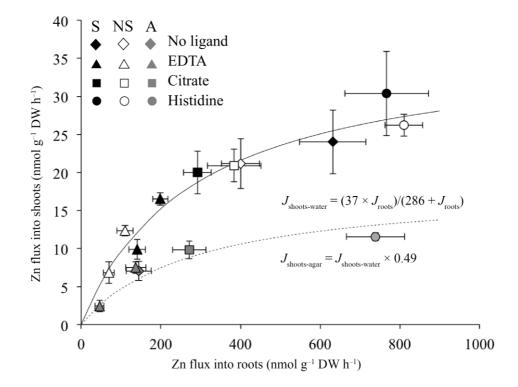


Fig. S2. Zn translocation into shoots *v*. Zn flux into the roots. S, stirred; NS: non-stirred, A, agar. The solid line represents best-fit Michaelis–Menten kinetics under stirred and non-stirred conditions. The dashed line represents best-fit through $J_{\text{shoots-water}}$ multiplied by a reduction factor. Error bars represent s.e.

Zn adsorption at the root surface

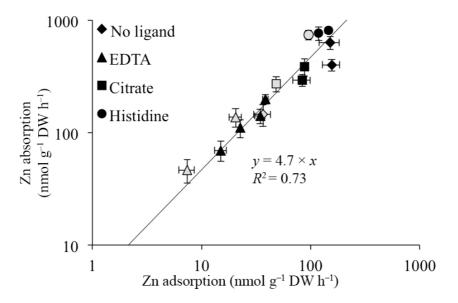


Fig. S3. Zn absorption by the plant roots v. Zn adsorption at the root surfaces. Black data points represent the stirred and non-stirred treatments, grey data points represent the agar treatments with the respective ligands.

References

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