Predicting zinc bioavailability to wheat improves by integrating pH dependent nonlinear root surface adsorption

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Abstract

Aim Our aim was to improve the prediction of Zn bioavailability to wheat grown on low-Zn soils. The classical approach that directly relates Zn in a certain soil extract to Zn uptake has been shown to be inadequate in many cases. We tested a stepwise approach where the steps of the uptake process are characterized with, respectively, Zn solid-solution distribution, adsorption of Zn to root surface, Zn uptake into root and Zn translocation to shoot.

Methods Two pot experiments were done with wheat grown on nine low-Zn soils varying widely in pH, clay and organic matter content. Soluble Zn concentrations in two soil extracts (DTPA and $CaCl_2$) were measured. Free Zn ion concentrations in $CaCl_2$ soil extracts were determined with the Donnan Membrane Technique. These Zn concentrations were then related to plant Zn uptake following both the direct and the stepwise approach.

Results In the direct approach, Zn in the DTPA extract was a better predictor for shoot Zn uptake than Zn in the $CaCl_2$ extract. In the stepwise approach, the relationship between Zn in $CaCl_2$ extracts and the root surface adsorbed Zn was pH-dependent and nonlinear. Root surface adsorbed Zn was linearly related to root Zn uptake, and the latter was linearly related to the shoot Zn uptake. The stepwise approach improved the Zn uptake prediction compared to the direct approach and was also validated for different wheat cultivars.

Conclusions The adsorption of Zn on the root surface is pH dependent and nonlinear with respect to the soil Zn concentration, and a useful proxy for bioavailable Zn over a wide range of soils.

Keywords Bioavailability · Zinc · Wheat · pH · Root surface

Abbreviations

ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
DTPA	Diethylenetriamine penta-acetic acid
DMT	Donnan membrane technique
UPW	Ultrapure water

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Introduction

Zinc is an essential element for plants (Sommer and Lipman 1926), but it is toxic at high concentrations (Broadley et al. 2012) so prediction of its bioavailability

is of great importance. Zinc toxicity in crops is far less widespread than Zn deficiency which limits crop production on ~30 % of the world's agricultural soils (Sillanpää and Vlek 1985). Over the past decades, several soil extraction tests have been developed to predict Zn bioavailability in soils (Menzies et al. 2007). Mostly, bioavailability is predicted using the relationship between Zn concentrations in soil extracts and Zn concentrations in the aboveground parts of plants (Fig. 1a) (McLaughlin et al. 2000). There is, however, no agreement on which soil extracted Zn concentration is the best predictor for plant Zn uptake, especially in low-Zn soils (Degryse et al. 2009; Menzies et al. 2007; Tandy et al. 2011).

Soil extraction with diethylenetriamine penta-acetic acid (DTPA) was developed to determine bioavailable Zn in near-neutral and calcareous soils (Lindsay and Norvell 1978) and is considered as one of the standard methods to determine bioavailable metals in non-polluted soils (Tandy et al. 2011). The method is based on the formation of soluble complexes of DTPA with free metal ions, which reduces the activity of the free metal ions in solution. In response, metal ions desorb from soil surfaces or dissolve from labile solid phases to replenish the free metal ions (Lindsay and Norvell 1978). However, there are several studies where the DTPA method did not accurately predict the Zn content in the shoot (Feng et al. 2005; Tandy et al. 2011). This is particularly the case if the soil test is applied under conditions were the method were not developed for, like acid soils, where the buffering capacity (pH 7.3) of the DTPA solution may cause unrealistic concentrations or if the soil test is applied to soils with high metal loadings, which exceed the complexing capacity of the chelate (O'Connor 1988).

Extraction with a $0.01M$ $CaCl_2$ solution utilizes a neutral salt extractant to determine the readily available nutrient concentrations in the solution of a wide range of soils (Houba et al. 1990). This procedure is based on equilibrating the soil with a surplus of cations (Ca^{2+}) which exchanged a certain amount of the metal ions from the soil surfaces by competitive adsorption without influencing the soil pH. The $CaCl_2$ soil extraction has been successfully applied to determine Zn bioavailability in contaminated and non-contaminated soils (Meers et al. 2007; Pueyo et al. 2004). In low-Zn soils, the $CaCl_2$ extraction is sometimes not applicable because the extracted Zn concentrations may not exceed the determination limit ($10^{-7}–10^{-9}M$) of the analytical equipment, like inductively coupled plasma mass spectrometry (ICP-MS) (Duffner et al. 2012).

The free metal ion concentration is often considered a key factor determining metal bioavailability (Parker and Pedler 1997; Weng et al. 2001). Accurate measurement of the free Zn^{2+} ion is difficult, especially at low concentrations (μM range). Various techniques have been applied to experimentally determine the free Zn^{2+} ion concentration in soil solutions, such as the chelation method (Catlett et al. 2002), Permeation Liquid Membrane (PLM) (Gramlich et al. 2012), Diffusive Gradients in Thin-films (DGT) (Tandy et al. 2011), Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) and the Donnan Membrane Technique (DMT) (Chito et al. 2012). The advantage of the DMT is the possibility to measure several free metal ion concentrations simultaneously. The detection limit of this analysis can be decreased (up to 500 times) by making use of ligands for accumulation (Kalis et al. 2006; Weng et al. 2011).

The relationship between bio-uptake and free metal ion concentration, however, was mainly established in aquatic environments (Van Leeuwen et al. 2005) and conceptualized, for example, with the Free Ion Activity Model (FIAM) (Brown and Markich 2000). It is disputable whether this approach applies to metal uptake by terrestrial plants, because metal complexing ligands in the rhizosphere and metal-root cell surface complexes may alter the chemical speciation (Duffner et al. 2012; Parker and Pedler 1997).

To improve the prediction of bioavailable Zn, an alternative to directly relating plant Zn uptake with soil-
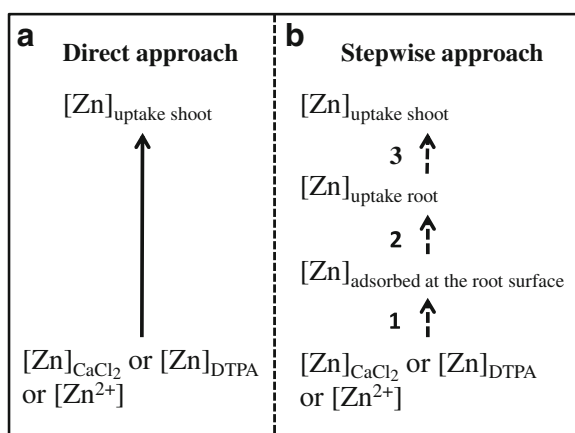

Fig. 1 The direct approach (**a**) and the stepwise approach (**b**) to relate Zn concentrations in soil extracts or Zn^{2+} to Zn bioavailability, expressed as Zn shoot uptake. The numbers 1–3 in **b** indicate the three steps of Zn uptake in the stepwise approach

extracted Zn fractions is to account for the different steps that follow each other in the uptake process, noting that not all of these steps may be linear. Fried and Shapiro (1961) distinguished four steps in ion uptake by plants from a soil: (1) the release of the ion from the solid phase into the soil solution, (2) the transport of the ion from the soil solution to the rhizosphere ("vicinity of the root"), (3) uptake of ions from rhizosphere solution into the root and (4) the translocation of the ion from the root to the shoot. In line with this concept, Kalis et al. (2007) successfully applied this four step approach to predict metal uptake by *Lolium perenne* in contaminated soils. They made the term "vicinity of the root" operational by defining it as the metal ions adsorbed to the root surface. The root surface adsorbed metals can be defined as extracellularly adsorbed metals, i.e. metals bound to one or more negatively charged biotic ligands in the apparent free space of the root cell wall (Santa María and Cogliatti 1988; White 2012; Wu and Hendershot 2009). Even though the adsorption of cations in apparent free space is not a prerequisite for ion uptake across the plasma membrane, the fixed negative charges in the apparent free space can, especially at low ionic activities, determine the cation concentration in the apoplasm (White and Broadley 2003). Zhang et al. (1991) showed that the apparent free space can also serve as a Zn storage pool, which can be mobilized and translocated to the shoot.

The pH is an important factor controlling metal adsorption to the root surface. A decreasing pH increases the competition between the protons and the metal ions at the biotic surface, which can be described by a two-species Freundlich equation (Eq. 1) (Temminghoff et al. 1994; Weng et al. 2003):

$$[Q]_B = K[M^{2+}]^m [H^+]^a \qquad (1)$$

where $[Q]_B$ is the quantity of a metal bound to the biotic surface, K is a constant related to the number of binding sites and their affinity for protons and metal ions, $[M^{2+}]$ represents the free metal ion concentration in the solution, $[H^+]$ is the proton activity, and m and a are empirical parameters. The K value, m and a are surface specific due to the differences of the density and type of binding sites (Plette et al. 1999). Due to the pH dependency of metal adsorption at the root surface the integration of root adsorption was crucial for the success of the stepwise approach of Kalis et al. (2007). They tested this approach on ten soils with moderate and high Zn contents and with a pH range from 4.2 to 7.0.

The objectives of the present study were to improve our understanding of the Zn uptake process from soils that are low in Zn, using a stepwise approach in which the consecutive intermediate Zn pools were considered explicitly. In particular, we considered nonlinearity at the root surface with regard to Zn concentration in different soil extracts and pH.

Material and methods

Soils

Soil samples originated from nine sites located in Turkey (Anatolia), Spain (Xeraco), Italy (Bologna), Western Australia (Dalwallinu and Lancelin), Ethiopia (two sites in Wukro; Wukro-S (sand) and Wukro-C (clay)), India (Patancheru) and the Netherlands (Droevendaal) were used in this study. The soils were named after the location of origin. At each site samples were taken from the topsoil (0–20 cm) except for the Droevendaal soil where samples were taken from the subsoil (60–90 cm). The soils varied in clay content (4–50 %), pH (4.2–7.6), soil organic carbon (SOC) (4–13.7 g kg^{-1}), dissolved organic carbon (DOC) (1.7–12.1 mg L^{-1}) and CaCO$_3$ content (0–154 g kg^{-1}). The total Zn content of the soils ranged from 0.2 mg kg^{-1} to 82.7 mg kg^{-1} (Table 1).

Soil extractions

Two soil extractions were conducted to determine different Zn pools in the soil samples. A 0.005M diethylenetriamine penta-acetic acid (DTPA) extraction was done based on Lindsay and Norvell (1978). Samples of 10 g air-dried soil were placed in 50 mL plastic centrifuge tubes and 20 mL of the DTPA extracting solution adjusted to pH 7.3 was added. The tubes were shaken horizontally for 2 h at 20 °C and centrifuged at 3,000g for 10 min. The supernatant was filtered (0.45 μm) before determining the metal concentrations with ICP-MS (Elan 6000, Perkin Elmer).

The extraction with 0.01M CaCl$_2$ was done based on Houba et al. (1990). Samples of 3 g air-dried soil were placed in 50 mL plastic centrifuge tubes and 30 mL 0.01M CaCl$_2$ was added. The tubes were shaken horizontally for 2 h at 20 °C and centrifuged at 3,000g for 10 min. The supernatant (10 ml) was filtered (0.45 μm), acidified (0.1 ml 0.43M HNO$_3$) and stored in precleaned ICP-MS tubes before determining the Zn concentration with ICP-MS.

Table 1 Soil characteristics

Origin/name	Country	Region	pH[a]	Clay g kg^{-1}	CaCO$_3$[b] g kg^{-1}	SOC[c] g kg^{-1}	DOC mg L^{-1}	WHC ml kg^{-1}	Zn (Aqua regia[d]) mg kg^{-1}
Anatolia	Turkey	Anatolia	7.5	220	93	10	6.3	482	33.1
Bologna	Italy	Bologna	7.4	232	144	9	12.1	445	66.6
Dalwallinu	Australia	Western Australia	4.2	72	0	6	1.7	223	2.0
Droevendaal	The Netherlands	Gelderland	6.2	48	0	4	2.5	255	8.0
Lancelin	Australia	Western Australia	5.2	38	0	13	5.1	201	0.2
Patancheru	India	Medak district	7.2	500	14	10	2.0	594	50.0
Wukro-C (Clay)	Ethiopia	Tigray	7.6	401	138	13	1.9	612	48.0
Wukro-S (Sand)	Ethiopia	Tigray	7.3	125	3	8	3.4	261	29.0
Xeraco	Spain	Valencia	7.5	357	154	14	9.4	435	82.7

[a] 0.01M CaCl$_2$, ISO/DIS 10390

[b] ISO 10693

[c] Soil organic carbon, Walinga et al. (1992)

[d] ISO 11466

Both soil extractions were done in triplicate.

Free Zn ion concentration

The free Zn^{2+} concentration was determined in the CaCl$_2$ extracts by means of the Donnan Membrane Technique (DMT), which is described in detail by Temminghoff et al. (2000). Samples of 90 g soil were suspended in 1 L polypropylene centrifugation containers with 900 mL 0.01M CaCl$_2$. Soil suspensions were shaken in a horizontal position on a reciprocal shaker for 2 h at 20 °C and centrifuged for 10 min at 3,000g. Extracts were separated from the soils by decanting supernatants in 1 L polyethylene containers. All DMT cell parts were cleaned by successive washings in 0.1M HNO$_3$ and ultra-pure water (UPW) prior to use. Teflon tubes were rinsed with 0.01M HNO$_3$, UPW and the acceptor solution. The membranes were cleaned and saturated with Ca according to Kalis et al. (2006). The supernatant was used as donor solution. The acceptor solution consisted of 0.01M CaCl$_2$ (17 mL) prepared in UPW and held in 50 mL polypropylene tubes. Based on preliminary experiments, the free Zn^{2+} concentrations in the acceptor were expected to be below the determination limit (0.3 μg L^{-1}) of the analytical technique (ICP-MS). Therefore 10 μM nitrilotriacetic acid (NTA) was added as ligand to the acceptor solution for Zn accumulation (Weng et al. 2011). Donor and acceptor solutions were circulated (7.5 ml min^{-1}) through the DMT cell by a peristaltic pump (Gilson Minipuls 3).

The experiment was carried out at 20 °C, and samples were taken from the donor and the acceptor solution at t=0, 24 and 48 h. In a preliminary experiment we found that the equilibrium was reached after 48 h (data not shown). Samples taken from the acceptor side of the DMT cell (6 mL) at 0 and 24 h were replaced by blank acceptor solution. Donor samples were filtered through a 0.45 μm filter. The pH in the donor and acceptor samples was measured with a combined glass-electrode. The Zn concentrations in the acceptor and donor samples were measured with ICP-MS and Ca concentrations in the acceptor and donor samples were measured with ICP-AES. Dissolved organic carbon (DOC) in the samples was measured at t=0 and 48 h (Sievers 900, GE Analytical Instruments). The DMT experiment was done in triplicate. The free Zn^{2+} concentrations were derived from Zn concentrations in the acceptor solution, which were calculated using the Equilibrium Calculation of Speciation and Transport (ECOSAT) program (Keizer and Van Riemsdijk 1995) by taking into account Zn complexation with NTA. Calcium was used as reference ion to correct for the ionic strength difference between the donor and acceptor. The inorganic ion complexes were calculated based on the reaction constants in the ECOSAT database. Zinc binding to dissolved organic matter was calculated by the Non-Ideal Competitive Adsorption (NICA)–Donnan model (Kinniburgh et al. 1999). We assumed that 30 % of the DOC consisted of fulvic acid and the rest was inert (Weng et al. 2002). Total

metal concentrations in $CaCl_2$ extracts were used as input variables.

Pot experiments

Two pot experiments, a calibration and a validation experiment, were conducted to determine the Zn uptake by wheat (*Triticum aestivum* L.) and the Zn adsorption to the root surface. Both experiments were conducted in the greenhouse at Wageningen University (photoperiod of 15 h (6:00–21:00 h), light intensity 400 W m^{-2} (16 SON-T agro lamps), day/night temperature 21/19 °C, rel. humidity 60 %). Both pot experiments were done in triplicate.

Calibration experiment

Nine soils (Table 1) and one wheat cultivar (*Triticum aestivum* L. *var.* Minaret) were used to calibrate the direct and the stepwise approach (Fig. 1b). One kg of air-dried and sieved (2 mm) soil was mixed with a nutrient solution and filled in 1 L plastic pots to a bulk density of 1.2 g cm^{-3}. The final nutrient concentration in the soil was: 150 mg N kg^{-1} (as $Ca(NO_3)_2 \cdot 4H_2O$), 80 mg P kg^{-1} (as KH_2PO_4), 101 mg K kg^{-1} (as KH_2PO_4) and 1.8 mg Fe kg^{-1} (as Fe-HBED, (Chaney 1988)). In four of the nine soils (Anatolia, Xeraco, Bologna and Droevendaal) Zn (10 mg Zn (as $ZnSO_4$) kg^{-1}) was also applied in an additional treatment to test the plant growth response to Zn application.

The soil was adjusted to 60 % of its water holding capacity every day with deionised water. The moist soils were allowed to equilibrate for 2 weeks in the greenhouse before sowing. In each pot twenty seeds were sown and were thinned to sixteen plants after germination. The plants were harvested 7 weeks after germination. Roots were washed thoroughly with distilled water to remove all soil particles. Afterwards, the roots were washed for 1 min with 100 mL of 0.01M disodium ethylenediaminetetraacetic acid (Na_2H_2-EDTA) to remove Zn ions that were adsorbed to the root surface (Kalis et al. 2007). The roots were then washed with UPW. The roots and shoots were dried at 70 °C for 3 days to determine their dry weight and milled (Planetary mill with agate milling balls) and digested with nitric acid, hydrofluoric acid and hydrogen peroxide in a closed-system microwave according to the procedure described by Novozamsky et al. (1996). The Zn concentrations in digests and the EDTA

solution from root washing were measured with ICP-MS.

Validation experiment

The Anatolia and Droevendaal soils and five different wheat cultivars (*T. aestivum* L. *var.* Eskischir, Italyan, Karachow, Kose and Sandomir) were used to validate the stepwise approach (Fig. 1b). Different cultivars were used to test whether relationships within the approach were cultivar specific. In each pot fifteen seeds were sown and were thinned to ten plants after germination. After 8 weeks the plants were harvested. Except for the plant density, the experimental setup, plant treatment and analysis were the same as in the calibration experiment (see calibration experiment).

Statistical analysis

Statistical analysis of data was performed with SPSS analytical software (SPSS Inc., Chicago, IL, USA; version 19). Both linear and nonlinear stepwise regressions were used to relate the Zn concentrations in the soil extracts with plant Zn uptake steps (Fig. 1). The variation among data was homogeneous (Levene's test).

Results

Soil extractions and free Zn ion concentration

The 0.01M $CaCl_2$ extractable Zn was low in all soils and varied by one order of magnitude among the different soils (0.1–1.2 μmol kg^{-1}) (Table 2). The Zn concentrations in the DTPA extracts showed more variation. The DTPA extractable Zn concentrations of the Anatolia, Dalwallinu and Lancellin soil were in the range of the $CaCl_2$ extracted Zn concentrations, whereas those of the Xeraco and Wukro-S soil were around 100 times higher (Table 2).

The free Zn ion (Zn^{2+}) concentration in the $CaCl_2$ extracts ranged from $10^{-7.10}M$ to $10^{-9.65}M$ (Table 2). The highest and lowest Zn^{2+} concentrations were found in the Dalwallinu and Patancheru soil, respectively. The highest and lowest proportion of Zn^{2+} on the total Zn in the $CaCl_2$ extract were found in the Dalwallinu (~80 %) and the Wukro-C (~0.3 %) soils (Table 2).

Table 2 Zn concentrations in the DTPA and the CaCl$_2$ soil extracts, Zn^{2+} concentration and the proportion of Zn^{2+} concentration to the total Zn concentration (means and standard error; n=3)

| Soil | 0.005 M DTPA | 0.01 M CaCl$_2$ | | |
	[Zn] μmol kg^{-1}	[Zn] μmol kg^{-1}	$-\log[Zn^{2+}]$[a] M	$[Zn^{2+}]/[Zn]$[b] %
Anatolia	1.7 (0.1)	0.18 (0.00)	9.3 (0.4)	2.7
Bologna	5.8 (0.2)	0.43 (0.02)	8.9 (0.4)	3.0
Dalwallinu	0.8 (0.0)	0.98 (0.03)	7.1 (0.3)	80.7
Droevendaal	7.0 (1.0)	1.02 (0.04)	7.8 (0.2)	16.1
Lancelin	1.0 (0.0)	0.55 (0.02)	8.0 (0.3)	17.8
Patancheru	8.3 (0.3)	0.12 (0.01)	9.7 (0.2)	1.8
Wukro-C	7.6 (1.2)	1.24 (0.05)	9.5 (0.5)	0.3
Wukro-S	34.0 (0.7)	0.73 (0.03)	8.6 (0.3)	3.2
Xeraco	99.6 (4.0)	0.15 (0.00)	9.0 (0.3)	6.5

[a] Determined with the Donnan Membrane Technique

[b] Proportion of Zn^{2+} concentration to the total Zn concentration in the CaCl$_2$ extract

Calibration experiment

This pot experiment was used to calibrate the direct and the stepwise approaches (Fig. 1). Wheat plants grown in the Xeraco and the Wukro-C soil had the highest root and shoot dry matter production (Table 3). Biomass production in the two Australian soils (Dalwallinu and Lancelin) was about 50 % less.

The highest root tissue Zn concentration (57 μg g^{-1}dw) was found in the Xeraco soil, whereas the root tissue Zn concentration of the Bologna and Wukro-C soil were about 3–4 times lower. The highest shoot tissue Zn

concentration (56 μg g^{-1}dw) was also found in the Xeraco soil. The tissue Zn concentration in the shoots grown in the Anatolia, Lancelin and Wukro-C soil were about 3–4 times smaller as in the Xeraco soil (Table 3).

Four soils (Anatolia, Xeraco, Bologna and Droevendaal) received a Zn application (10 mg Zn kg^{-1}) to determine the corresponding plants' response. The tissue Zn concentrations in the shoot and the root increased on average by 68 % and 56 %, respectively (Fig. 2). The plants grown in the Anatolia soil showed the strongest response, whereas the plants grown in the Xeraco soil showed the lowest response. The response of the shoot dry weight to the Zn

Table 3 Root and shoot dry weight of the harvested plants per pot (16 plants) and root and shoot tissue concentrations (μg g^{-1}) in the calibration experiment (means and standard error; n=3)

| Soil | Dry weight (g pot^{-1}) | | Zn concentration (μg g^{-1}) | |
	Roots	Shoot	Roots	Shoot
Anatolia	1.60 (0.12)	5.09 (0.26)	14 (0.0)	12 (0.9)
Bologna	1.31 (0.05)	5.76 (0.11)	17 (1.7)	19 (0.9)
Dalwallinu	0.99 (0.09)	2.50 (0.04)	24 (4.4)	36 (5.8)
Droevendaal	1.38 (0.07)	4.10 (0.21)	21 (0.9)	27 (0.3)
Lancelin	0.90 (0.08)	2.80 (0.17)	12 (0.3)	15 (0.9)
Patancheru	1.27 (0.04)	4.28 (0.13)	11 (0.7)	21 (1.2)
Wukro-C	1.59 (0.14)	6.86 (0.44)	11 (0.3)	17 (0.3)
Wukro-S	1.39 (0.09)	5.14 (0.59)	27 (1.9)	35 (1.7)
Xeraco	1.71 (0.07)	5.92 (0.06)	57 (1.0)	56 (5.7)

application was positive and ranged from 3 % to 20 % increase (Fig. 2).

Relating soil extracted Zn and free Zn^{2+} with Zn plant uptake

In line with the direct approach (Fig. 1a) linear regression analyses between soil Zn (concentrations or fractions) and shoot Zn uptake were done. This resulted in the following equations:

$$[Zn]_{Shoot} = 0.072[Zn]_{DTPA} \quad (r^2 = 0.79, \quad P = 0.001) \tag{2}$$

$$[Zn]_{Shoot} = 1.29[Zn]_{CaCl_2} \quad (r^2 = 0.42, \quad P = 0.044) \tag{3}$$

$$[Zn]_{Shoot} = 1.50[Zn^{2+}]_{CaCl_2} \quad (r^2 = 0.08, \quad P = 0.42) \tag{4}$$

where $[Zn]_{Shoot}$ is the shoot Zn uptake expressed as nmol plant^{-1} and soil $[Zn]_{DTPA}$, $[Zn]_{CaCl_2}$ and $[Zn^{2+}]_{CaCl_2}$ as nM. Both $[Zn]_{DTPA}$ and $[Zn]_{CaCl2}$ related significantly to shoot Zn uptake, whereas there was no significant relationship between $[Zn^{2+}]_{CaCl2}$ and shoot Zn uptake.
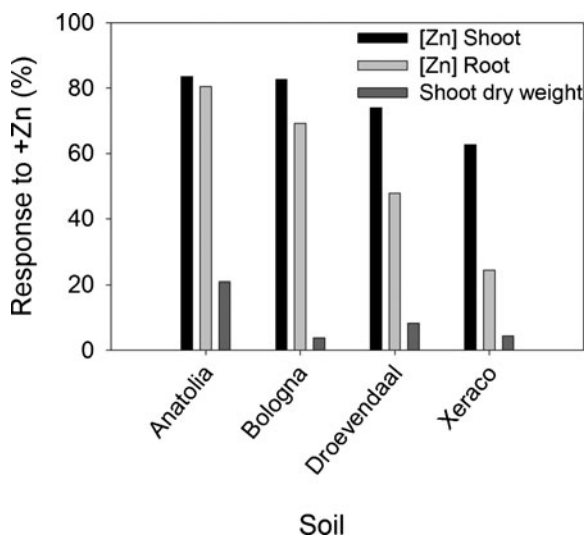
Fig. 2 Relative response to Zn application of the shoot tissue Zn concentration, root tissue Zn concentration and shoot dry weight grown in four selected soils

In the first step of the stepwise approach (Fig. 1b), Zn concentrations in the various soil extracts or free Zn^{2+} in $CaCl_2$ were related to root surface adsorbed Zn, by nonlinear regressions that account for pH dependency, assuming a two-species-Freundlich relationship, resulting in the following equations:

$$[Zn]_{RS} = 5.3[Zn]_{DTPA}^{0.22} \ [H^+]^{-0.19} (r^2 = 0.17, \quad P = 0.31) \tag{5}$$

$$[Zn]_{RS} = 18.7[Zn]_{CaCl_2}^{0.29} \ [H^+]^{-0.14} \quad (r^2 = 0.84, \quad P = 0.001) \tag{6}$$

$$[Zn]_{RS} = 35.1[Zn^{2+}]_{CaCl_2}^{0.55} \ [H^+]^{-0.21} \quad (r^2 = 0.29, \quad P = 0.19) \tag{7}$$

where $[Zn]_{RS}$ is the Zn adsorbed at the root surface expressed as μmol kg^{-1} root dw, $[Zn]_{DTPA}$, $[Zn]_{CaCl_2}$ and $[Zn^{2+}]_{CaCl_2}$ as nM and $[H^+]$ as μM. Total Zn in the $CaCl_2$ extract ($[Zn]_{CaCl2}$) and the root surface adsorbed Zn were significantly related (r^2=0.84) (Eq. 6) though DTPA extractable Zn (Eq. 5) and $[Zn^{2+}]_{CaCl2}$ (Eq. 7) did not relate significantly to root surface adsorbed Zn.

The second step of the stepwise approach relates root surface adsorbed Zn and root Zn uptake (Fig. 1b), and was both significant and strong:

$$[Zn]_{Root} = 0.41[Zn]_{RS} \quad (r^2 = 0.90, \quad P = \ < 0.001) \tag{8}$$

where $[Zn]_{Root}$ and $[Zn]_{Root \ surface}$ is expressed as nmol plant^{-1} and μmol kg^{-1}, respectively.

In the third and last step, root Zn uptake was related to shoot Zn uptake by a linear regression, which showed a strong and significant relationship (Eq. 9):

$$[Zn]_{Shoot} = 4.42[Zn]_{Root} (r^2 = 0.92, \quad P = \ < 0.001) \tag{9}$$

where $[Zn]_{Shoot}$ and $[Zn]_{Root}$ are both expressed as nmol plant^{-1}.

The predicted results were in good agreement with the measured values as is shown by the 1:1 line in Fig. 3. The shoot Zn uptake prediction with the direct approach (Eq. 2) and the stepwise approach (combining Eqs. 6, 8 and 9) were compared with each other. Predicting the shoot Zn uptake with the stepwise approach was done
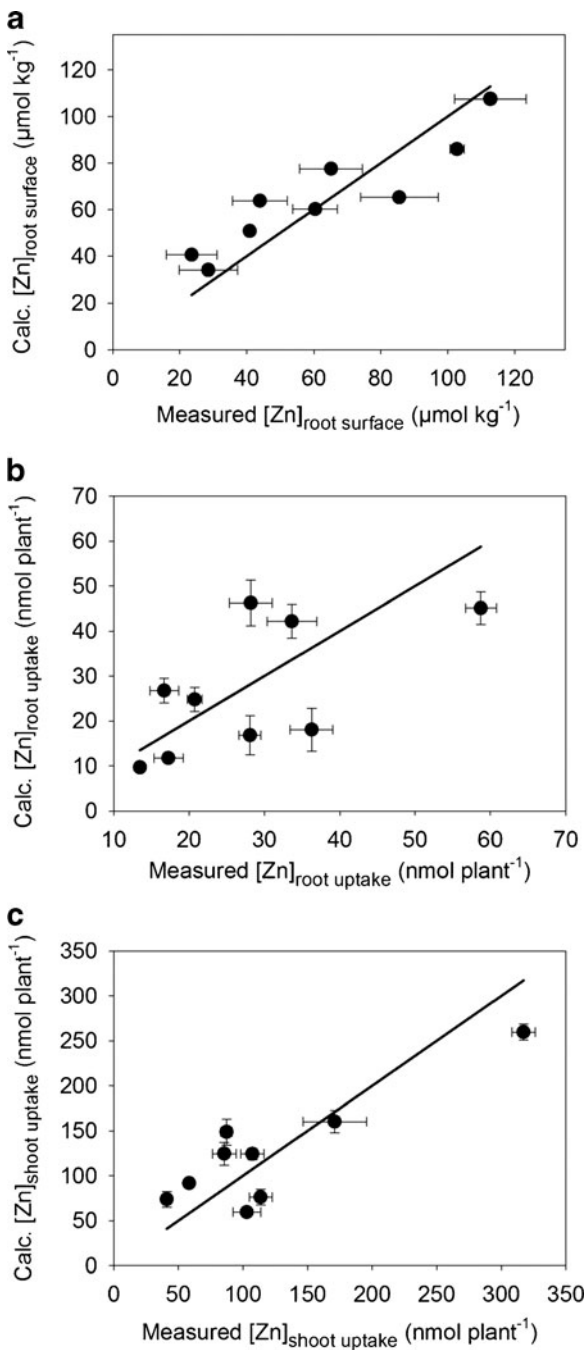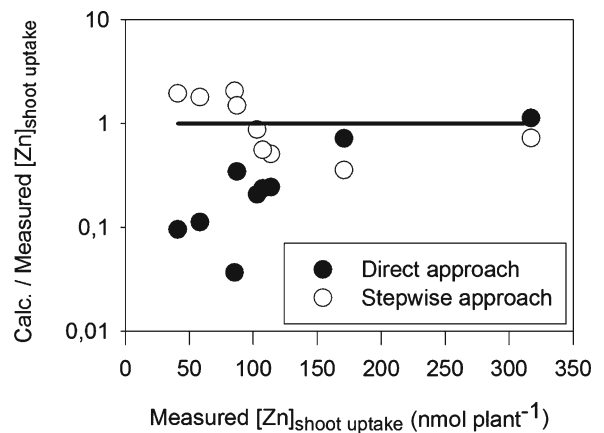
Fig. 4 Measured shoot Zn uptake of the calibration experiment plotted against the quotient (log scale) of the calculated and the measured shoot Zn uptake. The calculated shoot Zn uptake is based on the outcome of the direct approach (Eq. 2) and the stepwise approach (Eqs. 6, 8 and 9), respectively. The calculated root surface adsorbed Zn, using $CaCl_2$ soil extracted Zn and soil pH, (Eq. 6) was used as input to calculate the root Zn uptake (Eq. 8). The calculated root Zn uptake (Eq. 8) was used as input to calculate the shoot Zn uptake (Eq. 9). The black solid line represents the 1:1 line

Fig. 3 Measured and calculated root surface adsorbed Zn (a), root Zn uptake (b) and shoot Zn uptake (c) based on the equations (Eqs. 6, 8 and 9) of the (non) linear regression analysis with the nine different soils (calibration experiment), respectively. The black solid line represents the 1:1 line. Error bars represent standard errors; $n=3$

by calculating the root surface adsorbed Zn (Eq. 6), using $CaCl_2$ soil extracted Zn and soil pH. The

calculated root surface adsorbed Zn was used to calculate root Zn uptake (Eq. 8) and calculated root Zn uptake (Eq. 8) was used to calculate shoot Zn uptake (Eq. 9). The direct approach underestimated the shoot Zn uptake, particularly in soils with low shoot Zn uptake (values below the 1:1 line), whereas the values of the stepwise approach were distributed around the 1:1 line (Fig. 4).

Validation experiment

This experiment was used to validate the equations developed for the stepwise approach. The calculations for Zn adsorbed to the root surface (Eq. 6) of the five different wheat cultivars grown in the Anatolia and the Droevendaal soils were in good agreement (i.e. within ± 2 x standard errors around the 1:1 line) with the measured values (Fig. 5). Measured Zn uptake by root and shoot was also in good agreement with the values calculated by Eqs. 8 and 9 (Fig. 6).

Discussion

Including the pH dependent, nonlinear Zn adsorption at the root surface and root Zn uptake as intermediate steps improved the prediction of the shoot Zn uptake significantly compared with directly relating the Zn
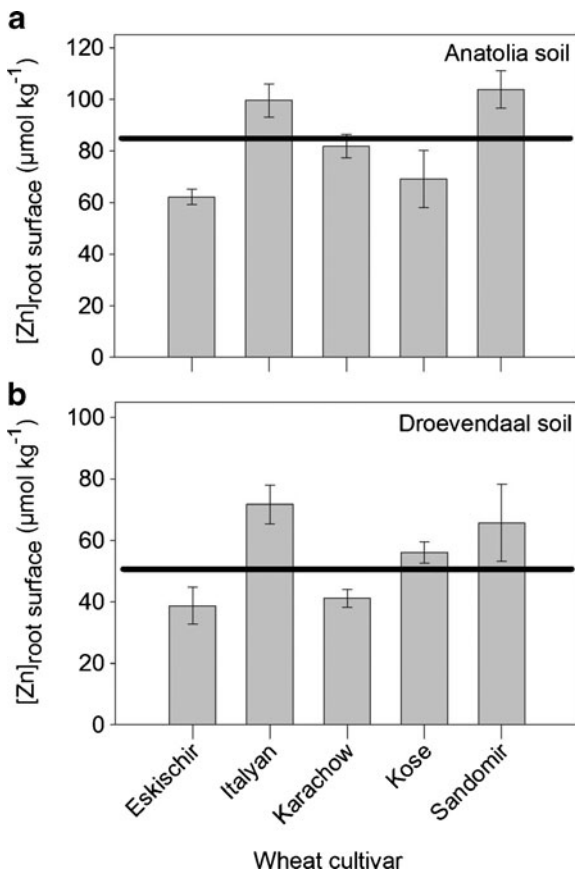
Fig. 5 Measured root surface adsorbed Zn of the five wheat cultivars grown in the Anatolia (a) and the Droevendaal (b) soil in the validation experiment. The black solid line represents the calculated values based on Eq. 6. Error bars represent standard errors; n=3
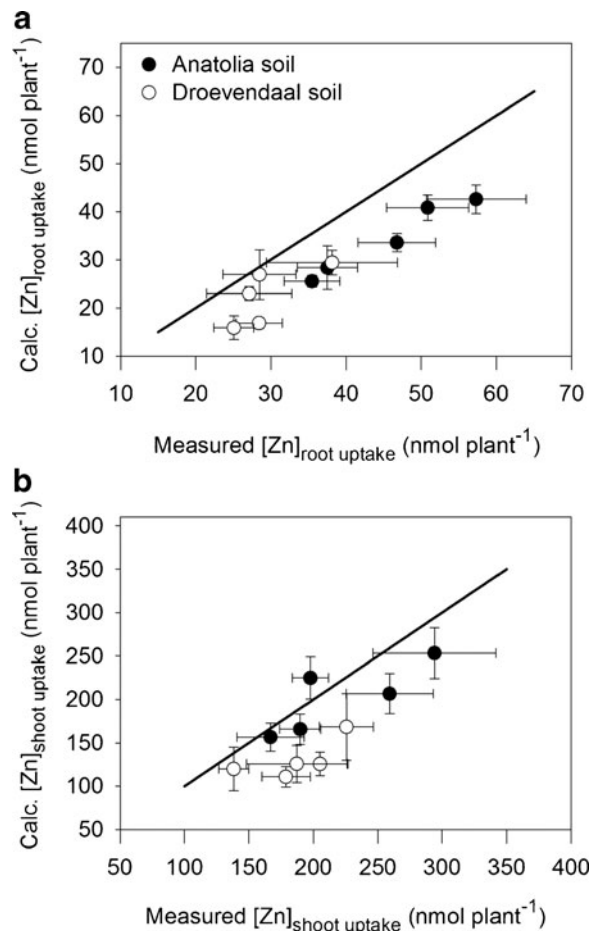

Fig. 6 Measured root Zn uptake (a) and shoot Zn uptake (b) of the five wheat cultivars grown in the Anatolia and the Droevendaal soil in the validation experiment plotted against the values calculated with Eqs. 8 and 9, respectively. The black solid line is the 1:1 line. Error bars represent standard errors (n=3)

concentrations in the $CaCl_2$ extracts with the shoot Zn uptake. This was also observed by Kalis et al. (2007) in contaminated soils.

With increasing pH the Zn adsorption at the root surface increased (Eqs. 6 and 7). This is contrasting to the study of Plette et al. (1999), where they showed that with increasing soil pH, less copper was bound to the maize root cell walls because at high pH soil ligands most probably outcompete the maize ligands. However, Von Wirén et al. (1996) showed that phytosiderophores are also able to form complexes with Zn (in addition to Fe) and this complexes can be adsorbed by maize roots. Such mechanisms were also observed for other graminaceous plant species (Arnold et al. 2010). Therefore exuded ligands from the wheat root probably outcompete the soil ligands when forming complexes with Zn.

That is also in line with our outcome that the free Zn ion concentration (Zn^{2+}) in the $CaCl_2$ extract was only weakly related to the root surface adsorbed Zn (Eq. 7). That indicates that depending on the pH most probably other Zn species like $Zn(OH)^+$, $ZnCl^+$ or phytosiderophore-chelated Zn were adsorbed to the root surface or diffused into the apparent free space of the root cell wall (Broadley et al. 2012; Chairidchai and Ritchie 1990; Papanicolaou and Nobeli 1977; Sadiq 1991; Von Wirén et al. 1996). Gramlich (2013) showed with plant experiments that Zn uptake by wheat was enhanced in the presence of citrate and histidine compared to treatments with solutions of the same free Zn concentrations buffered by EDTA. This shows that the Free Ion Activity Model

(FIAM) is not valid in the presence of ligands. These results also indicate that the dissociation of these complexes or the adsorption of the whole complex can play an important role for Zn uptake.

Root surface adsorbed Zn seems to be a more accurate proxy variable for the bioavailable Zn than the Zn concentrations in the different soil extracts, which was shown by the significant linear relationship between root uptake and root surface adsorbed Zn (Eq. 8). This is in line with the conclusion of Degryse et al. (2009) that the Zn uptake obeys Michaelis–Menten kinetics also at low Zn activities at the root surface, because if the metal activity is lower than the Michaelis constant the Michaelis–Menten equation can be reduced to a linear relationship. Our results indicate that the root surface adsorbed Zn is taken up via the apoplastic pathway. This is in line with findings of Redjala et al. (2010) who showed the importance of Ni adsorption on root apoplast, which represented 81–95 % of the total root uptake in maize plants grown in nutrient solutions. They concluded that this uptake process might act as a driving force to extract the metal from the soil. Similar observations were made for Cd uptake (Redjala et al. 2009). This shows that the root surface adsorbed Zn acts as a proxy of the Zn that is available for plant uptake.

Root Zn uptake showed also a strong linear relationship with shoot Zn uptake (Eq. 9). That suggests that Zn was translocated from the root to the shoot without reaching a maximum, which was also observed by Kalis et al. (2007) in contaminated soils and recently by Gramlich (2013) under low Zn conditions in nutrient solution experiments. That shows that the translocation from root to shoot is not related to the Zn concentration in the soil solution, but only to root Zn content regardless whether the soil is low in Zn or contaminated with Zn.

The strong pH dependency of Zn adsorption to the root surface most probably also explains the weak relationship between DTPA extractable Zn and root surface adsorbed Zn (Eq. 5), because the DTPA extraction solution is adjusted and buffered at pH 7.3, which excludes the pH effect. The fact that the direct approach based on DTPA extractable Zn underestimated shoot Zn uptake in soils with low Zn uptake, whereas the predictions of the stepwise approach were around the 1:1 line (Fig. 4) means that the stepwise approach based on $CaCl_2$ extractable Zn is more universally applicable compared to the direct approach based on DTPA-extracted Zn, even though both approaches had similar coefficients of determination. The improved applicability is an important

aspect considering the variety of soils with low Zn bio-availability. The nine soils in our work are characteristic for this variation which could be divided into two groups: the highly weathered Australian soils (Dalwallinu and Lancelin) with a small but labile total Zn pool and low pH and the other soils with a larger but relatively less soluble total Zn pool and higher pH (Tables 1 and 2).

Our validation experiment confirmed that the stepwise approach was also valid for wheat cultivars that were not used for the calibration (Figs. 5 and 6). The differences between the measured and the calculated root surface adsorbed Zn (Fig. 5) could possibly be explained by variation of the root surface area per plant among the cultivars (data not shown). Rengel and Wheal (1997) showed that Zn-efficient wheat cultivars had a greater proportion of fine roots than Zn inefficient wheat cultivars. Accounting for the root surface area as an additional parameter may therefore further improve the accuracy of the stepwise approach.

In conclusion, unravelling the uptake process in distinct steps demonstrated that the Zn bioavailability prediction is nonlinear due to pH dependent adsorption of Zn on the root surface. Zn uptake was shown to be strongly and linearly related to Zn adsorption to the root surface. Including these processes at the root surface improves the prediction of the Zn uptake based on Zn concentrations in soil extracts and pH. Integrating these root surface interactions most probably also improves the prediction of the bioavailability of other trace metals, since this has already been shown for other biotic surfaces (e.g. bacteria cell wall (Plette et al. 1996)).

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