New Phytologist

Evaluation of an electrostatic toxicity model for predicting Ni^{2+} toxicity to barley root elongation in hydroponic cultures and in soils

Peng Wang[1], Peter M. Kopittke[2], Karel A. C. De Schamphelaere[3], Fang-Jie Zhao[4], Dong-Mei Zhou[1], Koen Lock[2], Yi-Bing Ma[5], Willie J. G. M. Peijnenburg[6] and Steve P. McGrath[4]

[1]Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China; [2]The University of Queensland, School of Agriculture and Food Sciences, St Lucia, Queensland, 4072, Australia; [3]Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, J. Plateaustraat 22, B-9000 Ghent, Belgium; [4]Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK; [5]Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 12 Southern Street of Zhongguancun, Beijing 10081, China; [6]The Netherlands and National Institute of Public Health and the Environment (RIVM), PO Box 1, NL–3720 Bilthoven, the Netherlands

Author for correspondence:
Dong-Mei Zhou
Tel: +86 25 86881180
Email: dmzhou@issas.ac.cn

Received: *14 April 2011*
Accepted: *26 May 2011*

New Phytologist (2011) **192**: 414–427
doi: 10.1111/j.1469-8137.2011.03806.x

Key words: electrostatic toxicity model (ETM), magnesium (Mg), nickel (Ni), osmotic stress, root growth, surface potential.

Summary

- Assessing environmental risks of metal contamination in soils is a complex task because the biologically effective concentrations of metals in soils vary widely with soil properties.
- The factors influencing the toxic effect of nickel (Ni) on root growth of barley (*Hordeum vulgare*) were re-evaluated using published data from both soil and hydroponic cultures. The electrical potential ($\psi_0{}^o$) and ion activities ($\{I^z\}_0{}^o$) at the outer surfaces of root-cell plasma membranes (PMs) were computed as the basis of the re-evaluation.
- The reanalyses demonstrated that root growth was related to: the Ni^{2+} activity at the PM surface, ($\{Ni^{2+}\}_0{}^o$); calcium (Ca) deficiency (related to $\{Ca^{2+}\}_0{}^o$); osmotic effects; and modification of intrinsic Ni^{2+} toxicity by magnesium (Mg^{2+}; this appeared to exert an intrinsic (specific) ameliorating effect on intrinsic Ni^{2+} toxicity). Electrostatic toxicity models (ETM) were developed to relate root growth to these factors ($R^2 > 0.751$).
- Based on the ETM developed in soil culture and a Ni^{2+} solid–solution partitioning model, critical metal concentrations in soils linked to a biological effect were well predicted for 16 European soils with a wide range of properties, indicating the potential utility of ETM in risk assessment of metals in terrestrial ecosystems.

Introduction

Nickel (Ni) bioavailability and toxicity strongly depend on its speciation and soil characteristics (e.g. pH, organic carbon (OC), soil cation exchange capacity (CEC), and the ionic compositions of the soil solution) (Peijnenburg *et al.*, 1997; Weng *et al.*, 2004; Rooney *et al.*, 2007). For example, the effective concentrations of added Ni in soil causing 50% inhibition (denoted as $EC50[Ni]_{soil}$) for barley (*Hordeum vulgare*) root elongation ranged from 52 to 1929 mg kg^{-1} (a variation of 37-fold) in 16 European soils (Rooney *et al.*, 2007). In this context, empirical models that relate Ni toxicity to a limited number of bulk soil properties, such as CEC and pH, have already been implemented in regulatory frameworks (ECB, 2009). However, our understanding of the mechanism underlying such empirical relationships is still incomplete. Therefore, the present study estimates the toxic effects associated with Ni-contaminated soils on the growth of higher plants and the mechanisms by which soil properties affect Ni toxicity, with the aim of further improving risk assessment and the derivation of soil quality criteria for Ni.

There is increasing evidence that plant growth responses to ions are often dependent upon their activities at the plasma membrane (PM) surface rather than the activities in the root-bathing medium (Kinraide, 2006; Wang *et al.*, 2008;

Kinraide & Wang, 2010; Kopittke *et al.*, 2010). Because of the electrical potential at the outer surfaces of PMs (ψ_0^o), arising from surface charges, the concentrations or activities of ions at the PM surfaces often differ significantly from those in the contacting bulk medium. The ψ_0^o is often sufficiently negative (relative to the bulk solution) to enrich cations and to deplete anions at the PM surface by > 10-fold relative to the bulk-phase medium. Cations in the bulk medium, such as aluminium (Al^{3+}), Ni^{2+}, calcium (Ca^{2+}), magnesium (Mg^{2+}) and hydrogen (H^+), reduce the negativity of ψ_0^o by charge screening and ionic binding (Kinraide *et al.*, 1998; Tatulian, 1999). This reduction in the negativity of ψ_0^o caused by the addition of cations decreases the activity of ions at the PM surface (for example, reducing the surface activity of Ni^{2+}). This reduction in ψ_0^o, and the resultant reduction in cation activities at the PM surface, is a nonspecific effect. The anionic components (commonly Cl^- or SO_4^{2-}) generally have small effects because of their weak binding to the PM surface and small surface concentrations because of electrostatic repulsion. Although the cell wall is negatively charged, it has small effects upon ion activities at the PM surface (Kinraide, 2004).

Ions may inhibit plant growth through three main mechanisms: induced Ca deficiency, osmotic stress; and direct phytotoxicity (Munns, 2002; Kopittke *et al.*, 2011). Calcium is essential for root elongation and a crucial regulator of growth and development (Hanson, 1984). Elevated concentrations of other cations such as Al^{3+}, H^+ and Mg^{2+} may induce Ca deficiency by displacing Ca^{2+} from the PM surface (Kinraide, 1998; Munns, 2002; Wang *et al.*, 2010; Kopittke *et al.*, 2011). For example, Kopittke *et al.* (2011) reported that 1.9 mM Ca^{2+} at the membrane surfaces ($\{Ca^{2+}\}_0^o$) was required for optimal elongation of roots of *Vigna unguiculata*. Second, an increase in the osmotic potential of cultures results in a decrease in growth as a result of water stress (Kinraide, 1999; Munns, 2002; Kopittke *et al.*, 2011). About 300 mM osmolarity resulted in a 50% decrease of elongation of *V. unguiculata* root (Kopittke *et al.*, 2011). Indeed, the apparent decrease in toxicity with the leaching and aging of metal salt-amended soils may be partly attributable to the leaching of ions and concomitant reduction in osmolarity (Stevens *et al.*, 2003). Separation of these multiple toxic effects in soils is not straightforward and few studies have systematically investigated the factors affecting toxicity in soils. This lack of systematic investigations also results from the limitations inherent in the assays of metal toxicities to soil organisms, namely the intercorrelations among variables (e.g. pH and soluble Al^{3+}, Kinraide (2003); osmolarity, Ni^{2+} and Ca^{2+} in this study). By contrast, hydroponic cultures may be used to overcome the complications of variable intercorrelations by systematically varying one of the covarying parameters.

Based on a reanalysis of published data, this study was conducted to (1) investigate the mechanisms of Ni toxicity to barley root elongation in soils, giving particular consideration of plant cell membrane electrical phenomena and ion activities at the membrane surface, and (2) construct electrostatic toxicity models (ETMs) to predict the toxicity and effective concentration of Ni (inhibition of root elongation) for potential utility in risk assessment in soils with a wide range of properties. To assist in this process, interactions of Ni^{2+} with Ca^{2+}, Mg^{2+} and H^+ were first examined in solution culture (where intercorrelation between variables is less problematic) in order to provide a theoretical basis for understanding the factors that influencing Ni toxicity in soils.

Materials and Methods

Data for barley (*Hordeum vulgare* L.) root elongation in response to Ni^{2+} and other cations (H^+, Ca^{2+}, Mg^{2+}, etc.) were compiled from two hydroponic cultured experiments (Lock *et al.*, 2007; Li *et al.*, 2009) and one soil cultured experiment with 16 European soils that had a wide range of properties (Rooney *et al.*, 2007). The barley root elongation was assessed by the cultivation of barley seedlings in soil or hydroponic cultures amended with Ni according to International Organization for Standardization (ISO) 11269-1 (1993).

PM surface electrical potential and ion surface activities

The activities of all ion species in hydroponic solutions or soil solutions were presented in all three studies (Lock *et al.*, 2007; Rooney *et al.*, 2007; Li *et al.*, 2009). In the present study, these values were recalculated using up-to-date equilibrium constants using Visual MINTEQ 3.0 chemical speciation model (www2.iwr.kth.se/English/oursoftware/vminteq/) based on the ion composition in soil solutions. Metal binding to humic substance is simulated by the NICA-Donnan model (Kinniburgh *et al.*, 1999). It was assumed that 65% of dissolved organic matter (DOM) is fulvic and 35% inert, and default parameters for generic fulvic were used (Weng *et al.*, 2002). The DOM was set as dissolved organic carbon (DOC)/2. The values obtained agreed closely to those presented in the original studies. Values for ψ_0^o were computed with a Gouy–Chapman–Stern (GCS) model (Yermiyahu *et al.*, 1997; Kinraide *et al.*, 1998; Kinraide, 2006) (see the Supporting Information, Notes S1 and S2). The ion surface activity can be calculated from ψ_0^o with the Nernst Equation $\{I^Z\}_0^o = \{I^Z\}_b \exp[-ZF\psi_0^o/(RT)]$, where Z, F, R and T are the charge on the ion, the Faraday constant, the gas constant and temperature, respectively ($F/(RT)$ = 1/25.7 at 25°C for ψ_0^o expressed in mV) (Notes S3). The subscript 0 in $\{I^Z\}_0^o$ denotes the ion activity at the PM outer surface; the subscript b in $\{I^Z\}_b$ indicates the activity in the bulk-phase medium.

Osmolarity

Osmolarity (mOsM) was calculated using the formula: osmolarity = $\sum \varphi_i C_i$, where φ is the osmotic coefficient (being 1.86 for NaCl, 1.85 for KCl, 2.58 for $MgCl_2$, 2.56 for $CaCl_2$ and 2.57 for $NiCl_2$ (Robinson & Stokes, 2002)) and C is the concentration of solute i (mM). The organic solutes will also contribute to osmolarity. However, their concentrations in the current experimental systems are small compared with those of the inorganic solutes, and their contribution to the osmolarity is negligible.

$$\begin{aligned} \mathrm{RER} &= \mathrm{RER_C} \times \mathrm{pRER_{(Ca)}} \\ &= \mathrm{RER_C} \times [1 - 1/\exp(a_2\{\mathrm{Ca^{2+}}\}_0{}^{o})] \end{aligned} \quad \text{Eqn 2}$$

where a_2 (mM^{-1}) is a strength coefficient. Eqn 1 may be expanded to incorporate the secondary effects of $\psi_0{}^{o}$ on Ni^{2+} toxicity by expending a_1 into $(1 + a_{12}\psi_0{}^{o})$ (Wang *et al.*, 2011), osmotic stress (osmolarity, mOsM) and an ameliorant such as Ca^{2+} (Kinraide, 1998; Kopittke *et al.*, 2011; Wang *et al.*, 2010, 2011). Independent effects can be expressed as the product

$$\begin{aligned} \mathrm{RER} &= \mathrm{RER_C} \times \mathrm{pRER_{(Ca)}} \times \mathrm{pRER_{(Ni)}} \times \mathrm{pRER_{(Osm)}} \\ &= \mathrm{RER_C} \times [1 - 1/\exp(a_2\{\mathrm{Ca^{2+}}\}_0{}^{o})]/\exp[(a_1(1 + a_{12}\psi_0{}^{o})\{\mathrm{Ni^{2+}}\}_0{}^{o})^{b1} + (a_3\mathrm{Osmolarity})^{b3}] \end{aligned} \quad \text{Eqn 3}$$

Analysis of root elongation rate

Barley root elongation assays were conducted for 4 d in the studies of Rooney *et al.* (2007) and Lock *et al.* (2007) and for 5 d in Li *et al.* (2009). Root elongation was evaluated as root elongation rate (RER, mm h^{-1}). When growth responds to measures of toxicant intensity, such as $\{Ni^{2+}\}_0{}^{o}$ (μM), the resulting curves (e.g. RER vs $\{Ni^{2+}\}_0{}^{o}$) often exhibit the downwardly sigmoidal shape and can be expressed by the following equation:

$$\begin{aligned} \mathrm{RER} &= \mathrm{RER_C} \times \mathrm{pRER_{(Ni)}} \\ &= \mathrm{RER_C}/\exp[(a_1\{\mathrm{Ni^{2+}}\}_0{}^{o})^{b1}] \end{aligned} \quad \text{Eqn 1}$$

where RER_C is the maximum growth rate in the corresponding Ni-unamended, Ca^{2+}-sufficient control and it is a single value within each experiment; $pRER_{(Ni)}$ and similar terms used later (i.e. $pRER_{(Ca)}$, $pRER_{(Osm)}$), denoted by subscripts, are partial RER and independently quantify the relative effects of Ni, Ca and osmolarity on root growth, respectively. They are dimensionless and have values from 0 to 1; a_1 (μM^{-1}) is a strength coefficient that increases with the strength of the metal toxicity, and b_1 (dimensionless) is a shape coefficient (Taylor *et al.*, 1991; Kinraide, 1999; Kopittke *et al.*, 2011). It is noteworthy that sometimes large differences in tolerance are observed among plant species. The differences in the a_1 and b_1 coefficients for Eqn 1 may denote differences in sensitivity (Kinraide *et al.*, 2004).

If growth is only limited by osmotic stress, the $\{Ni^{2+}\}_0{}^{o}$ in Eqn 1 can be replaced by osmolarity (mOsM). If Ca^{2+} deficiency limits growth, then the addition of Ca^{2+} may enhance growth and plots of growth vs $\{Ca^{2+}\}_0{}^{o}$ may be sigmoidal. If growth is limited only by deficient levels of $\{Ca^{2+}\}_0{}^{o}$ (mM), then

where a_{12} (mV^{-1}) is a curve-fitting parameter (see Wang *et al.* (2011) for a detailed description of this equation); a_3 ($mOsM^{-1}$) is a strength coefficient and b_3 (dimensionless) is a shape coefficient. It was found that the term $pRER_{(Osm)}$ (i.e. $1/\exp[(a_3\mathrm{Osmolarity})^{b3}]$) could often be omitted from the equation for the hydroponic culture studies because osmolarity was low enough for the term to be equal to 1. The term $pRER_{(Ca)}$ (i.e. $[1 - 1/\exp(a_2\{Ca^{2+}\}_0{}^{o})]$) trends upwards as $\{Ca^{2+}\}_0{}^{o}$ increases. Equations incorporating the Ca term can be evaluated only for situations where $\{Ca^{2+}\}_0{}^{o}$ was low enough to limit root elongation in some of the treatments, otherwise the term is consistently equal to 1.

Although the addition of cations to the solution causes a nonspecific reduction in $\{Ni^{2+}\}_0{}^{o}$ because of a reduction in the negativity of $\psi_0{}^{o}$, it is also possible that one or more factors (often Ca^{2+}, Mg^{2+} or H^+) interact (specifically) with Ni^{2+} at the PM surface (for example, by influencing transport, ion channels or by competition). A way to express these specific interactions would be to incorporate these factors into the coefficient for the toxicant (Kinraide, 1998, 1999; Kopittke *et al.*, 2010; Wang *et al.*, 2010, 2011). Thus, if Mg had a specific effect (e.g. competition) on Ni^{2+} toxicity, a_1 $(1 + a_{12}\psi_0{}^{o})$ could be expanded as

$$a_1 = a_{11}(1 + a_{12}\psi_0{}^{o})/(1 + a_{13}\{\mathrm{Mg^{2+}}\}_0{}^{o}) \quad \text{Eqn 4}$$

where a_{13} is again a curve-fitting parameter (mM^{-1}). The second part of Eqn 4 $(1 + a_{13}\{Mg^{2+}\}_0{}^{o})$ denotes a quantitative expression of specific ameliorative effectiveness by Mg^{2+} (e.g. by competition for membrane transport) so that a_1 (i.e. the toxicity of Ni) decreases as $\{Mg^{2+}\}_0{}^{o}$ increases.

Analysis of the relative root length

In the literature, relative root elongation (rRE) is often plotted against the Ni^{2+} bulk-phase activities in solution or Ni

concentrations in soil to derive the effective activity or concentration yielding a 50% inhibition on growth (denoted as EC50$\{Ni^{2+}\}_b$ or EC50$[Ni]_{soil}$). Therefore, the rRE was also assessed in the current study. The rRE was calculated using the formula rRE, % = $100(RL_T - RL_S)/(RL_C - RL_S)$, in which RL_T represents the mean root length (RL) in the presence of Ni^{2+}, RL_C represents RL in the corresponding Ni-unamended control, and RL_S represents RL at the time of seedling transfer to the test media. In each particular experiment, RL_S is a single value, but each RL_T has its corresponding RL_C. The rRE implies that the difference between RL_T and RL_C is attributable solely to Ni^{2+}.

It is problematic to use rRE to explore the mechanisms of toxic effect on root growth, especially in the situation of Ca deficiency (Kinraide, 2003; Kopittke *et al.*, 2011). For example, consider two Ni-free solutions (control) in a Ni^{2+} toxicity assay using hydroponic cultures. The first test solution has low Ca^{2+} and low Mg^{2+} while the second has low Ca^{2+} but high Mg^{2+}. It is not correct that the two solutions impose similar stress to root elongation even although the rRE values in both solutions are equal to 100. The stress of root growth is greater in high Mg^{2+} solution because Mg^{2+} displaces Ca^{2+} from the PM surface inducing Ca deficiency. In hydroponic culture the RER in 'control (Ni-free media)' of study by Lock *et al.* (2007) is well expressed with Eqn 2 ($R^2 > 0.78$). Therefore rRE in solution with Ni is solely attributable to Ni^{2+} and is equal to $100 \times RER/RER_{control}$(=$100 \times RER/(RER_C \times pRER_{(Ca)})$). Therefore, each rRE can be calculated from the two predicted RERs in a Ni treatment and in its corresponding Ni-free control.

In soil culture, the rRE is attributable to both Ni toxicity and osmotic stress induced by added Ni (i.e. high osmolarity in soil solution consists of soluble Ni and released Ca and other ions from the soil solid phase); Ca is often not a growth-limiting factor in soils with metal contamination (the results of the current study). Therefore, for soils, the equation can be written as $100 \times RER/RER_C$ (=$100 \times pRER_{(Ni)} \times pRER_{(Osm)}$). Given the influencing factors on root growth other than Ni toxicity (soil structure, nutrients, etc.), and the other factors that influence these among soils, it may be more reasonable to explore mechanistic information in soils through fitting models to rRE.

Statistics

All coefficients in equations were evaluated by multiple, nonlinear regression analysis using SYSTAT 12 (Cranes Software International Ltd., Bangalore, India). No coefficients are reported whose 95% confidence interval encompassed zero. Root mean square error (RMSE) is given to estimate how close the predictions are to the observations by the formula $RMSE = \sqrt{\frac{1}{n}\sum (R_{predicted} - R_{observed})^2}$, where n is the number of data points, $R_{predicted}$ and $R_{observed}$ are the predicted and the observed RERs (or rREs), respectively.

Results

Hydroponic culture – root growth

Lock *et al.* (2007) and Li *et al.* (2009) investigated Ni^{2+} toxicity to barley seedlings in hydroponic cultures in response to variable concentrations of Ni^{2+}, major cations (Ca^{2+}, Mg^{2+}, sodium (Na^+) and potassium (K^+)), and pH (H^+) in a factorial array. Fig. 1(a) illustrates some of the experimental results by Lock *et al.* (2007) in which Ca^{2+} or Mg^{2+} was factorially arrayed with Ni^{2+}, and demonstrates that root growth in solutions with elevated Ni is influenced by at least three factors. First, Ni is highly toxic and reduces root growth. Second, additions of low concentrations of Mg alleviate Ni toxicity substantially (compare the curve for 1.1 mM Mg with those for Ca, Fig. 1). Finally, the addition of Mg, particularly at higher concentrations, appears to cause a reduction in root growth by inducing Ca deficiency (Kinraide, 2003; Kopittke *et al.*, 2011). Indeed, in the Ni-free solutions RER decreased from 0.75 to 0.33 mm h^{-1} as Mg was increased from 1.1 to 5.2 mM. For these solutions (1.1 to 5.2 mM Mg), $\{Ca^{2+}\}_0{}^o$ was calculated to decrease from 2.01 to 0.57 mM; reanalysis of the data of Carter *et al.* (1979) demonstrates that growth of barley is reduced by 50% at < *c.* 2.0 mM $\{Ca^{2+}\}_0{}^o$. Based upon these three observations above, values of RER were assessed with Eqn 5 (derived from Eqn 3, without the term for osmolarity, which was not limiting to growth in these solutions) to account for Ni toxicity, Mg alleviation of Ni toxicity and Ca deficiency.

$$\begin{aligned} RER &= RER_C \times pRER_{(Ca)} \times pRER_{(Ni)} \\ &= RER_C[1 - 1/\exp(a_2\{Ca^{2+}\}_0{}^o)]/\exp\{[a_{11}(1 + a_{12}\psi_0{}^o)\{Ni^{2+}\}_0{}^o/(1 + a_{13}\{Mg^{2+}\}_0{}^o)]^{b1}\} \end{aligned} \quad \text{Eqn 5}$$

Eqn 5 provides a term [1-1/exp($a_2\{Ca^{2+}\}_0{}^o$)] to allow for an increase of root growth as $\{Ca^{2+}\}_0{}^o$ increases (i.e. as Ca deficiency is overcome), while in the second part of the equation an increase in $\{Mg^{2+}\}_0{}^o$ alleviates intrinsic Ni^{2+} toxicity. Fitting Eqn 5 to the data of Lock *et al.* (2007) resulted in $R^2 = 0.781$, $P < 0.001$, RMSE = 0.159 ($n = 107$) (cf. the R^2 value of 0.688 if the term Ca deficiency was not included, or the value of 0.658 when $\{I^Z\}_b$ was used instead of $\{I^Z\}_0{}^o$ in equation RER = $RER_C[1 - 1/\exp(a_2\{Ca^{2+}\}_b)]/\exp\{[a_{11}\{Ni^{2+}\}_b/(1 + a_{14}$

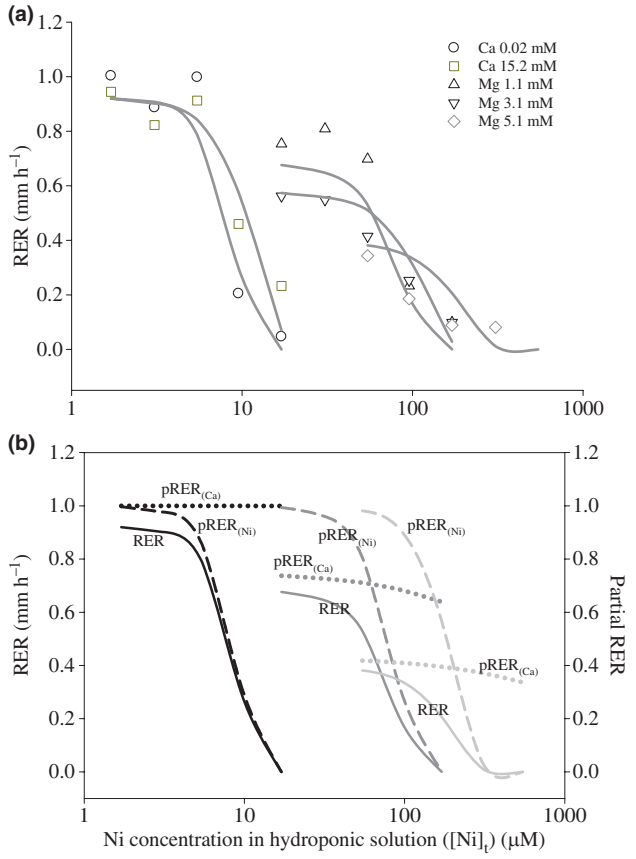

Fig. 1 Root elongation rate (RER) in hydroponic culture at pH 6.9 in responses to nickel (Ni^{2+}) and variable calcium (Ca) (0.02 mM and 15.0 mM), variable magnesium (Mg) (1.1, 3.1 and 5.2 mM) (a). Separating the components of toxic effectiveness and alleviation for RER (b) based on Eqn 5 and parameters presented in Table 1. In (b), only data of 0.02 mM Ca (black line), 1.1 mM Mg (dark tinted line) and 5.1 mM Mg (lighter tinted line) treatments are presented for clarity. The solid curves indicate overall RER conforming to Eqn 5 ($RER = RER_C \times pRER_{(Ca)} \times pRER_{(Ni)}$); dashed lines indicate the $pRER_{(Ni)}$ (Ni^{2+} intoxicant and alleviation) and dotted lines indicate the $pRER_{(Ca)}$. The Ni toxicity test data are from Lock *et al.* (2007).

$\{Mg^{2+}\}_b)]^{b1}\}$). Interestingly, when the term for $\{Mg^{2+}\}_0{}^o$ in the second part of the equation was excluded, coefficients for all other variables became insignificant, suggesting that both Ca deficiency and the specific alleviation of Ni toxicity by Mg^{2+} play important roles in root growth in the experiment of Lock *et al.* (2007). Importantly, no significant coefficients ($P > 0.05$) were obtained when RER was regressed with the Ni^{2+} activities in the bulk-phase solution with the equation $RER = RER_C/\exp[(a_1\{Ni^{2+}\}_b)^{b1}]$. Fitting Eqn 5 with the data of Li *et al.* (2009) resulted in $R^2 = 0.832$, $P < 0.001$, RMSE = 0.110 ($n = 189$), while regression analysis of the data of these two pooled studies yielded highly significant coefficients ($R^2 = 0.751$, $P < 0.001$, RMSE = 0.149). Based on Eqn 5 and parameters presented in Table 1, the rREs can be calculated from the two predicted RERs in a Ni treatment and in its corresponding Ni-free control. The calculated

Table 1 Summary of statistics from regression analyses according to Eqn 5 for root elongation rate (RER) in two studies conducted in hydroponic culture

Response	RER_C	a_2	a_{11}	a_{12}	a_{13}	b_1	n	R^2	RMSE	Data source
RER	0.890 ± 0.033	1.18 ± 0.17	0.029 ± 0.007	0.016 ± 0.001	0.973 ± 0.322	1.79 ± 0.28	107	0.781	0.154	Lock *et al.* (2007)
RER	0.862 ± 0.024	2.10 ± 0.33	0.010 ± 0.001	0.016 ± 0.000	0.802 ± 0.179	0.78 ± 0.06	189	0.832	0.110	Li *et al.* (2009)
RER	0.881 ± 0.024	1.52 ± 0.17	0.011 ± 0.001	0.016 ± 0.001	0.467 ± 0.096	0.86 ± 0.08	296	0.751	0.149	Above pooled data

Activities of ions are in mM except for the nickel (Ni^{2+}) activities, which are in μM; all reported values are significant from zero at the 5% level.

rREs agreed closely with the measured values (R^2 = 0.774, RMSE = 18.0 for the study by Lock *et al.*; R^2 = 0.923, RMSE = 9.10 for the study by Li *et al.*).

Table 1 provides much information about the differences in sensitivity. First, the toxic strength coefficient 'a_{11}' for the study by Lock *et al.* (0.029) is larger than that in the study by Li *et al.* (0.010), indicating that the same level of $\{Ni^{2+}\}_0^o$ is more toxic to the barley cultivar used by Lock *et al.* Second, the coefficients for the secondary effect for both studies are generally a_{12} = 0.016 and different studies do not appear to affect their values. The coefficients obtained in this study are consistent with that for phytotoxicity in eight other studies with six metals, including Ni (0.010–0.016, median 0.013; Table 2 in Wang *et al.*, 2011). Third, a_2 reflects the differences in sensitivity to Ca deficiency. The coefficient for the study by Li *et al.* (2.10) is larger than that in study by Lock *et al.* (1.18), indicating that the barley used by Li *et al.* is slightly more sensitive to Ca deficiency. Based on the coefficients, critical values for Ca deficiency corresponding to a 10% reduction in root growth were calculated as *c.* 1.10 mM $\{Ca^{2+}\}_0^o$ for the study by Li *et al.* and 1.95 mM $\{Ca^{2+}\}_0^o$ for the study by Lock *et al.* Finally, the coefficients of a_{13} are similar for both studies (0.973 for studies by Lock *et al.* and 0.802. for Li *et al.*), suggesting that the Ni toxicity was equally alleviated by Mg^{2+}.

Soil culture – soil solution properties and Ni^{2+} activities

As expected, the addition of Ni to soil resulted in an increase in the Ni concentration in the soil solution, with concomitant increases in other major cations, especially Ca and Mg, and decreases in soil solution pH (increases in H^+) (Table S1). Consequently, the osmolarity of the soil solution also increased upon the addition of Ni. In the Guadalajara soil, for example, soil solution Ni concentrations increased linearly with additions of Ni. Similarly, the concentrations of Ca and Mg in soil solution increased > 40-fold (from 2.68 to 125 mM for Ca and from 0.30 to 12.5 mM for Mg) in this Guadalajara soil as soil Ni increased from 0 to 1600 mg kg^{-1}. Correspondingly, the calculated osmolarity increased from 9.9 to 367 mOsM, which was largely dependent on the solution Ca and Ni salt concentrations.

According to a solid–solution partitioning model for metals (Sauvé *et al.*, 2000; Lofts *et al.*, 2004), the free Ni^{2+} activities for all soil solutions conform to Eqn 6, as obtained by multivariate linear regression.

$$\log\{Ni^{2+}\}_b = 1.730\log([Ni]_{soil}) - 0.467pH - 0.262\log(OC) - 1.266\log(CEC) + 0.172\log(I) - 3.629 \quad \text{Eqn 6}$$

(R^2 = 0.950, P < 0.001, RMSE = 0.32, n = 105; $[Ni]_{soil}$ in mg kg^{-1}, OC in mg kg^{-1}, CEC in cmol kg^{-1} and the ionic

Table 2 Equations for relative root elongation (rRE) in soil culture were evaluated in response to ion activities at the surfaces of root-cell plasma membranes or/and osmolarity (OsM) of soil solution

$rRE = 100/\exp[(a_1\{Ni^{2+}\}_0^{o})^{b1}]$ a_1 = 0.0035 ± 0.0003, b_1 = 1.23 ± 0.12	R^2 = 0.884	RMSE = 13.6	Eqn 7
$rRE = 100/\exp[(a_1\{Ni^{2+}\}_0^{o})^{b1} + (a_3\text{Osmolarity})^{b3}]$ a_1 = 0.0029 ± 0.0002, b_1 = 1.28 ± 0.13, a_3 = 0.0051 ± 0.0005, b_3 = 3.09 ± 0.70	R^2 = 0.917	RMSE = 11.5	Eqn 8
$rRE = 100/\exp\{[a_1(1 + a_{12}\psi_0^{o})\{Ni^{2+}\}_0^{o}]^{b1} + (a_3\text{Osmolarity})^{b3}\}$ a_1 = 0.0036 ± 0.0006, a_{12} = 0.032 ± 0.005, b_1 = 1.18 ± 0.12, a_3 = 0.0047 ± 0.0005, b_3 = 3.82 ± 1.08	R^2 = 0.937	RMSE = 10.0	Eqn 9
$rRE = 100/\exp\{[a_1(1 + a_{12}\psi_0^{o})\{Ni^{2+}\}_0^{o}/(1 + a_{13}\{Mg^{2+}\}_0^{o})]^{b1} + (a_3\text{Osmolarity})^{b3}\}$ a_1 = 0.0046 ± 0.0006, a_{12} = 0.030 ± 0.005, a_{13} = 0.151 ± 0.089, b_1 = 1.21 ± 0.12, a_3 = 0.0046 ± 0.0005, b_3 = 3.55 ± 1.02	R^2 = 0.940	RMSE = 9.74	Eqn 10

Activities of ions are in mM except for the nickel (Ni^{2+}) activities, which are in μM; beneath each equation are the evaluated coefficients ± SE. The Ni toxicity test data are taken from Rooney *et al.* (2007).

strength, I, of soil solution in mM). The free Ni^{2+} activity predicted with Eqn 6 agreed well with the values calculated using Visual MINTEQ 3.0.

Soil culture – ψ_0^o and ion activities at the PM surface

The values calculated for ψ_0^o based upon soil solutions from the control soils varied from −35.6 to −2.0 mV because of variations in pH and concentrations of cations, specifically Ca and Mg (Fig. 2). The negativity of ψ_0^o decreased markedly as Ni was added because of increases in Ni^{2+} but also because of increases of Ca^{2+} and Mg^{2+} resulting from desorption from the solid soil matrix (Fig. 2 and Table S1). In the Houthalen soil (low pH and low OC), for example, addition of 160 mg Ni kg^{-1} increased $\{Ni^{2+}\}_b$ to 3.0 mM, which (together with high Ca, Mg and H) induced a high positive surface potential (+23.1 mV) resulting in a Ni^{2+} surface activity (0.5 mM) that was depleted by sixfold relative to that in the bulk soil solution.

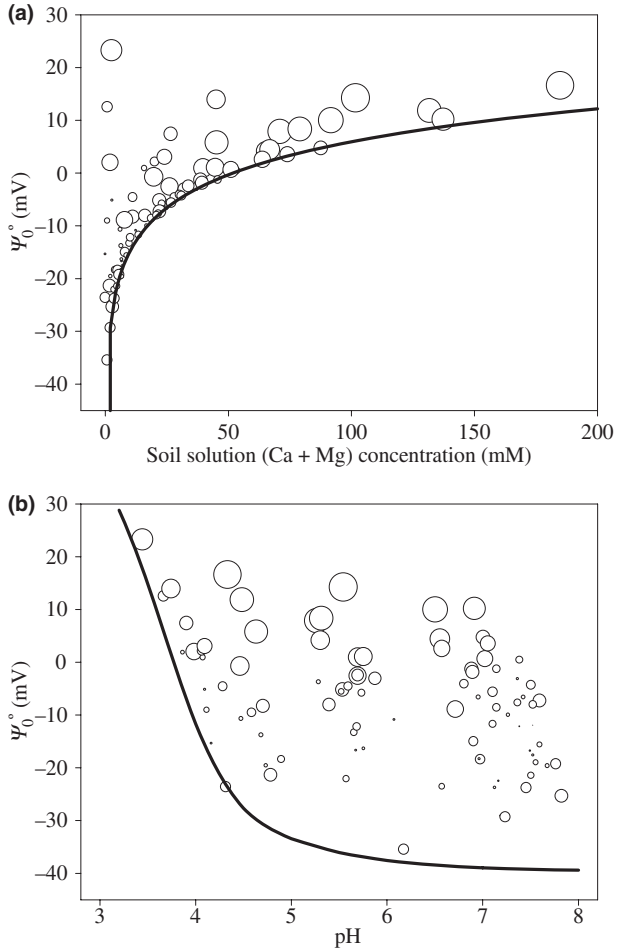

Fig. 2 Electrical potential of root-cell plasma membrane (PM) surface (ψ_0^o) as functions of soil solution (calcium (Ca) + magnesium (Mg)) concentrations (a) and soil solution pH (b). Circle areas are proportional to the free nickel (Ni^{2+}) activities in soil solution. The curves in (a) and (b) indicate the changes in ψ_0^o with increasing Ca + Mg concentration and increasing pH in solution without Ni^{2+}, respectively. Data of ionic compositions in soil solutions are from Rooney *et al.* (2007).

Soil culture – root growth

As shown earlier for the solution culture experiments of Lock *et al.* (2007) and Li *et al.* (2009), root growth in Ni-amended solutions may be influenced by Ni toxicity, Ca deficiency and Mg alleviation. However, it was apparent from the soil solution data of Rooney *et al.* (2007) that, given that soil solution Ca concentrations tended to increase substantially as Ni was added (Table S1), Ca was not present at growth-limiting concentrations except for the highly acidic Houthalen soil; with the exception of this soil, the $\{Ca^{2+}\}_0^o$ was sufficiently high for the Ca deficiency term $[1 - 1/\exp(a_2\{Ca^{2+}\}_0^o)]$ to be consistently equal to 1. Although the growth was not limited by Ca deficiency, it was limited by high osmolarity, with calculated values of up to 660 mOsM in some treatments (Table S1). Although barley is relatively tolerant to salinity (Rooney *et al.*, 2007), it is likely that additions of large amounts of soluble Ni salt could cause a direct toxic effect of salinity, especially in treatments above the EC50 dose of Ni addition (Stevens *et al.*, 2003). Therefore, root growth in these toxic Ni soil solutions could be affected by Ni^{2+} toxicity and osmotic effects (but not Ca deficiency). Indeed, rRE was related negatively to $\{Ni^{2+}\}_0^o$ (R^2=0.884, Eqn 7 in Table 2), with $\{Ni^{2+}\}_0^o$ in combination with osmolarity (R = 0.917, Eqn 8 in Table 2). Incorporating the dual effects of ψ_0^o into Eqn 8 improved R^2 by a further 0.020 (R^2 = 0.937, Eqn 9 in Table 2). In order to test whether Mg specifically alleviated Ni toxicity, the toxic strength coefficient 'a_1' in Eqn 4 was expanded, and a significant coefficient was obtained for specific alleviation of $\{Mg^{2+}\}_0^o$, but not for $\{Ca^{2+}\}_0^o$ (Eqn 10 in Table 2). It was also noted that some plant Ca^{2+} channels also transport Mg^{2+} and Ni^{2+} (White *et al.*, 2000). However, when a term for specific alleviation of Ni toxicity by Ca^{2+} was added, the corresponding coefficient was not significant (not shown). Therefore, this analysis suggests that for the soil culture data of Rooney *et al.* (2007), root growth was influenced by Ni toxicity, osmolarity (salinity) and Mg-alleviation of Ni toxicity. As for the solution culture experiments, it was also noted that root growth was more closely correlated with $\{I^Z\}_0^o$ than with $\{I^Z\}_b$; an R^2 value of 0.832 ($P < 0.001$, n = 105) was obtained with $\{I^Z\}_b$ compared with a value of 0.917 ($P < 0.001$, n = 105) with $\{I^Z\}_0^o$ in Eqn 8.

It must also be noted, however, that intercorrelations were observed between some variables for the soil culture study. For example:

$$\text{Osmolarity} = 3.98\{Ca^{2+}\}_0^{o} + 0.127\{Ni^{2+}\}_0^{o} \quad (R^2 = 0.576, P < 0.001, n = 105) \qquad \text{Eqn 11}$$

$$\{Ni^{2+}\}_0^{o} = -149\{Ca^{2+}\}_0^{o} - 203\{Mg^{2+}\}_0^{o} - 11.1\{H^{+}\}_0^{o} + 2156 \quad (R^2 = 0.437, P < 0.001, n = 105) \qquad \text{Eqn 12}$$

Thus, osmolarity was correlated positively with $\{Ni^{2+}\}_0^{o}$ and $\{Ca^{2+}\}_0^{o}$, and $\{Ni^{2+}\}_0^{o}$ was correlated negatively with $\{Mg^{2+}\}_0^{o}$, $\{Ca^{2+}\}_0^{o}$ and $\{H^{+}\}_0^{o}$. Such intercorrelations often hinder the interpretation of data. For example, given that $\{Ca^{2+}\}_0^{o}$ was related to osmolarity (Eqn 11), removal of the term for osmolarity in Eqn 10 and the extension of the toxic strength coefficient 'a_1' to $a_1(1 + a_{12}\psi_0^{o} + a_{14}\{Ca^{2+}\}_0^{o})$ resulted in the highest value of R^2 (0.959) with a negative significant coefficient a_{14} (−0.058). We propose, however, that this negative value for the Ca coefficient reflects that growth at high osmolarity (corresponding also to high Ca) was reduced more than in solutions with low osmolarity (and hence low Ca). Indeed, as shown earlier, Ca had no specific effect on Ni toxicity when the effects of osmolarity were included. It is noteworthy that the detrimental effects of salinity in the field are lower as a result of the leaching and aging of Ni-contaminated soils.

Modelling rRE

The rRE values can be calculated from the two predicted RERs using an ETM developed for individual studies in hydroponic culture (Eqn 5, parameters presented in Table 1) and can be predicted with Eqn 10 (Table 2) in soils. Using these equations to compare measured and predicted values, linear regression analysis demonstrated a good relationship between measured and predicted root growth (R^2 = 0.898, Fig. 3a). However, it is noteworthy that the toxic strength coefficient 'a_1' for hydroponic culture (0.011) was 2.3 times greater than that for soil culture (0.0046), indicating that the same level of $\{Ni^{2+}\}_0^{o}$ was less toxic in soil than in solution culture. Indeed, when the parameters obtained from the two pooled studies in hydroponic culture (Table 1) were used to predict the rRE values for the soil culture (Fig. 3b), it was apparent that the rRE values calculated for the soils were an underestimate on the basis of the ETM equation developed in hydroponic cultures.

Prediction of EC50s

The electrostatic toxicity model from solution culture can be used to predict the Ni concentrations (or activities) that are ecologically protective. To accomplish this, data concerning physico-chemical properties can be compiled from solutions for which the biological response is constant (e.g. 50% inhibition). Thus, for variable combinations of coexistent ions, Ni^{2+} is adjusted to result in 50% inhibition of rRE. When rRE was assigned a value of 50%, a full electrostatic toxicity model equation based on Eqns 5 and 10 was rearranged and a corresponding $\{Ni^{2+}\}_{b(50)}$ (denoted as $EC50\{Ni^{2+}\}_b$, activities producing 50% inhibition of root elongation as often seen in literature) were obtained by

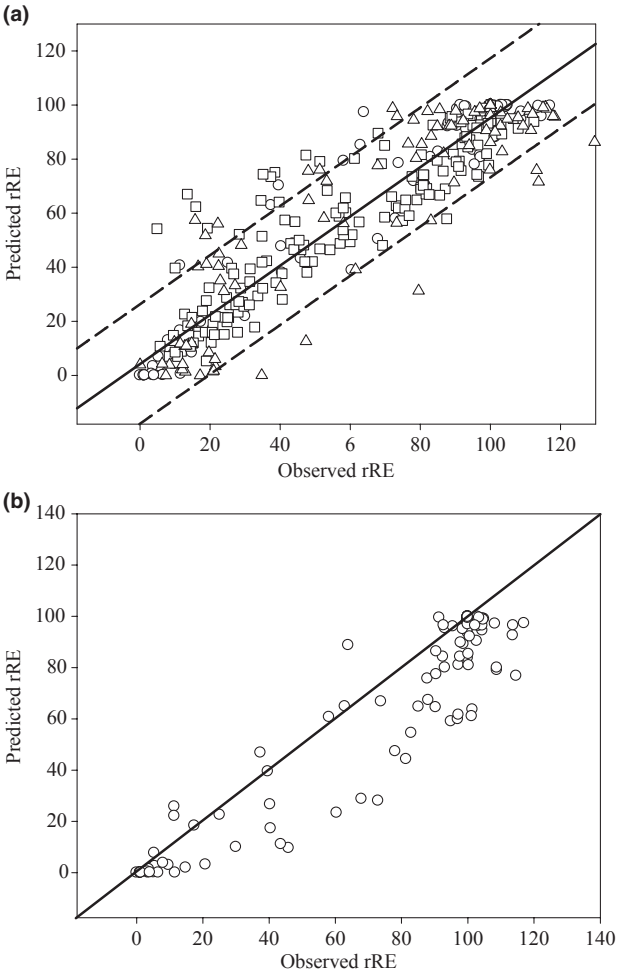

Fig. 3 Relationship between the observed and predicted relative root elongation (rRE). (a) The predicted rREs were calculated from the two predicted RERs using an electrostatic toxicity model developed for individual studies in hydroponic culture (Eqn 5, constants presented in Table 1) and using Eqn 10 (Table 2) in soils. Circles, soil culture (Rooney *et al*., 2007); squares, hydroponic culture (Li *et al*., 2009); triangles, hydroponic culture (Lock *et al*., 2007). The solid lines represent the linear regression relationship between the predicted and the observed values; the dashed lines are 95% prediction intervals. (b) Comparison between the observed rRE values in soils and the predicted values based on the electrostatic model with the constants developed in two pooled studies in hydroponic cultures. The solid line represents 1 : 1 line.

$$\{Ni^{2+}\}_{b(50)} = \exp(2\psi_0{}^{o}/25.7)[\log_e 2 - (a_3\text{Osmolarity})^{b3}]^{1/b1}[1 + a_{13}\{Mg^{2+}\}_b\exp(-2\psi_0{}^{o}/25.7)]/a_1(1 + a_{12}\psi_0{}^{o}) \quad \text{Eqn 13}$$

If the osmolarity in solution is < 115 mOsM, the term $(a_3\text{Osmolarity})^{b3}$ can be omitted from the equation. Using the solution concentrations of ions interpolated at EC50, the $\psi_0{}^{o}$ was calculated initially with the GCS model and then the $\{Ni^{2+}\}_{b(50)}$ can be predicted with Eqn 13. As shown in Fig. 4(a), almost all the predicted EC50s for Ni^{2+} activities in hydroponic solutions and soil solutions using the parameters developed in individual studies differed from the observed EC50s by a factor of less than two, except for one outlier in soil culture (Cordoba 1).

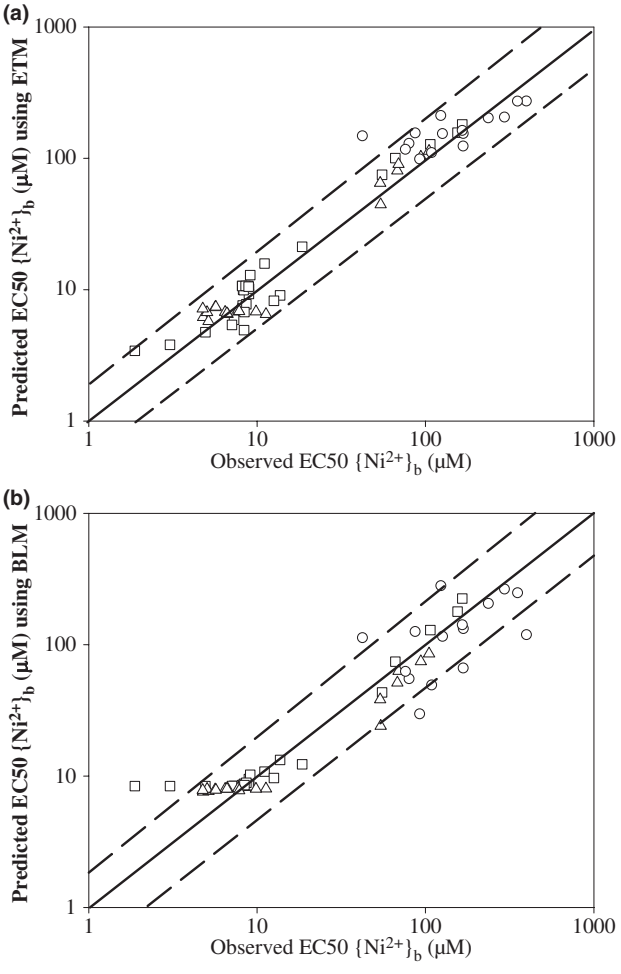

Fig. 4 Relationship between the observed and predicted EC50s expressed as free nickel (Ni^{2+}) activity in the solution based on (a) the electrostatic toxicity model (ETM) and (b) the biotic ligand model (BLM). The solid lines indicate a 1 : 1 fit and dashed lines are a factor of 2 above and below the 1 : 1 line. Circles, soil culture (Rooney *et al*., 2007); squares, hydroponic culture (Li *et al*., 2009); triangles, hydroponic culture (Lock *et al*., 2007).

For the soil data, the predicted $\{Ni^{2+}\}_{b(50)}$ is then fixed in Eqn 6 to calculate the EC50 for total soil Ni concentration (mg kg^{-1} soil) using the specific soil properties OC, CEC and interpolated ionic strength. The predicted EC50 $[Ni]_{soil}$ for 16 soils fitted well with the observed EC50s by a factor of less than two, except for two calcareous soils, but all within a factor of less than three (Fig. 5).The RMSE of the predicted EC50 soil Ni concentrations (log transformed) was 0.252. When the parameters in the ETM developed in pooled studies in hydroponic solution (Eqn 5, parameters in Table 1) were used to predict the EC50s for soils, an overprediction of Ni toxicity in soils was observed (data not shown).

Discussion

Effects of Ca, Mg and pH on root growth in toxic Ni solutions

Cations reduce the negativity of $\psi_0{}^{o}$. For ions commonly of environmental and agricultural importance, the order of effectiveness for reducing the negativity is $H^+ > Ni^{2+} > Ca^{2+} \approx Mg^{2+} > Na^+ \approx K^+$ (Kinraide & Yermiyahu, 2007; Kinraide & Wang, 2010). A decrease in pH from 6.83 to 4.09 (rows 1 and 2 in Table 3), for example, reduced the negativity of $\psi_0{}^{o}$ from −50.3 to −18.6 mV, which would lower $\{Ca^{2+}\}_0{}^{o}$ from 8.16 μM to 0.74 mM, leading to Ca^{2+} deficiency ($pRER_{(Ca)}$ declined from 1.00 to 0.57). The reduced $\psi_0{}^{o}$ also decreased the Mg^{2+}($\{Mg^{2+}\}_0{}^{o}$ from

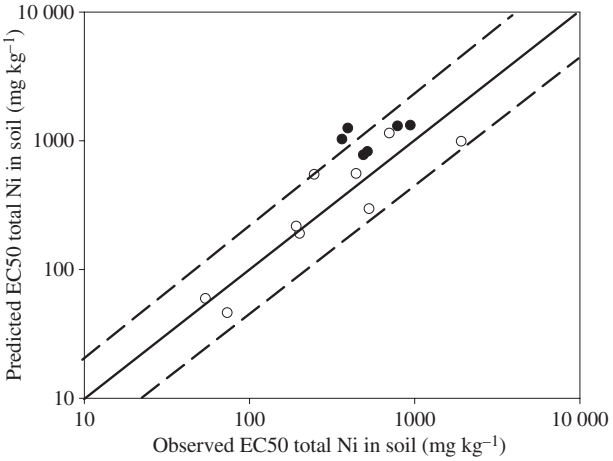

Fig. 5 Relationship between the observed and predicted EC50s expressed as total nickel (Ni) concentration in soil based on the electrostatic toxicity model. Open circles, soil pH < 7.0; closed circles, pH > 7.0. The solid lines indicate a 1 : 1 fit and dashed lines are a factor of 2 above and below the 1 : 1 line.

Table 3 Components of toxic effectiveness on root elongation rate in barley (*Hordeum vulgare*) seedlings

No.	pH	$[Ca^{2+}]_t$ (mM)	$[Mg^{2+}]_t$ (mM)	$[Na]_t$ (mM)	$[Ni^{2+}]_b$ (μM)	OsM (mM)	$\psi_0{}^o$ (mV)	$\{Ca^{2+}\}_0{}^o$ (mM)	$\{Mg^{2+}\}_0{}^o$ (mM)	$\{Ni^{2+}\}_0{}^o$ (μM)	$pRER_{(Ca)}$ / partial RER	$pRER_{(Ni)}$ / partial RER	$pRER_{(Osm)}$ / partial RER	RER_{Cal} (mm h^{-1})	RER_{Obs} (mm h^{-1})
1	4.09	0.20	0.05	0.08	4.75	0.95	−18.6	0.74	0.19	20.3	0.58	0.85	1.00	0.445	0.476
2	6.83	0.20	0.05	2.58	3.07	4.60	−50.3	8.16	2.03	124	1.00	0.92	1.00	0.825	0.887
3	6.80	15.2	0.05	2.58	2.51	43.9	−10.0	14.9	0.05	5.5	1.00	0.95	1.00	0.867	0.912
4	6.92	0.20	0.05	24.3	5.22	46.1	−36.9	1.90	0.47	92.4	0.89	0.54	1.00	0.435	0.474
5	6.86	0.20	1.08	2.58	70.6	8.50	−29.4	1.46	7.89	695	0.82	0.23	1.00	0.171	0.153
6	6.89	0.20	5.19	2.58	59.0	19.1	−18.5	0.52	13.6	249	0.46	0.85	1.00	0.348	0.281
7	6.86	0.20	0.05	2.58	0	4.30	−51.2	8.75	2.19	0	1.00	1.00	1.00	0.890	0.879
8	6.81	15.2	0.05	2.58	0	43.8	−10.0	14.9	0.05	0	1.00	1.00	1.00	0.890	0.896
9	4.29	8.36	2.91	0.64	204	32.4	−4.7	6.46	2.21	136	1.00	0.71	1.00	0.650	0.582
10	5.76	38.1	6.72	0.77	2598	134	0.9	6.64	1.69	696	1.00	0.05	0.84	0.041	0.037
11	7.39	38.7	14.8	1.93	0.85	147	0.4	9.43	3.60	1.52	1.00	1.00	0.78	0.719	0.794
12	7.15	44.0	1.84	0.33	141	121	−1.4	13.8	0.59	42.9	1.00	0.91	0.89	0.752	0.737
13	7.06	66.8	7.46	0.29	195	195	3.4	12.1	1.33	228	1.00	0.45	0.51	0.179	0.178

Data from part of experiments testing Ni toxicity to barley root elongation in hydroponic and soil cultures. The partial root elongation rates (RERs) were computed according to the Eqn 5. Numbers 1–8 are from root growth experiments in hydroponic culture (Lock *et al.*, 2007), and numbers 9–13 are from soil culture (Rooney *et al.*, 2007).

2.03 μM to 0.19 mM) and surface Ni^{2+} activities ($\{Ni^{2+}\}_0{}^o$ from 124 μM to 20.3 μM). Although $\{Ni^{2+}\}_0{}^o$ was lowered, the alleviation effect of intrinsic Ni^{2+} toxicity by $\{Mg^{2+}\}_0{}^o$ also decreased substantially owing to a reduction in $\{Mg^{2+}\}_0{}^o$. Meanwhile, the secondary effect of $\psi_0{}^o$ was increased (i.e. increase in the PM electrical driving force in facilitating Ni^{2+} transport into the cell), all of which resulted in an increase in intrinsic Ni^{2+} toxicity ($pRER_{(Ni)}$ declined from 0.92 to 0.85). As a consequence, the net effect of a decrease in pH in this case is a decrease of the RER from 0.887 mm h^{-1} to 0.476 mm h^{-1} (the calculated RER ranged from 0.825 mm h^{-1} to 0.445 mm h^{-1}). An increase in Ca^{2+} (rows 2 and 3 in Table 3), reduced the $\psi_0{}^o$ negativity, resulting in an increase in $\{Ca^{2+}\}_0{}^o$ (> 1.9 mM; $pRER_{(Ca)}$ = 1.00) but decreases in $\{Mg^{2+}\}_0{}^o$ and $\{Ni^{2+}\}_0{}^o$. The toxic effectiveness of a reduction in $\{Ni^{2+}\}_0{}^o$ was almost offset by the reduced alleviation of intrinsic Ni^{2+} toxicity by $\{Mg^{2+}\}_0{}^o$ and, consequently, the $pRER_{(Ni)}$ changed only marginally (from 0.91 to 0.95). Thus, there was also little change in RER in this case (the observed RER ranged from 0.887 to 0.912 mm h^{-1}). Addition of Mg^{2+} (rows 5 and 6 in Table 3) caused decreases in $\{Ca^{2+}\}_0{}^o$ ($pRER_{(Ca)}$ from 0.82 to 0.46) and $\{Ni^{2+}\}_0{}^o$, but an increase in $\{Mg^{2+}\}_0{}^o$. The decreased $\{Ni^{2+}\}_0{}^o$ and greatly increased $\{Mg^{2+}\}_0{}^o$ alleviated the intrinsic Ni^{2+} toxicity ($pRER_{(Ni)}$ from 0.23 to 0.85). The net effectiveness stimulated RER from 0.153 to 0.281 mm h^{-1} (the predicted RER increased from 0.171 to 0.348 mm h^{-1}).

Comparison between soil and solution culture

Hydroponic culture systems have frequently been applied to evaluate ion uptake and interactions in plants; results from such studies have been used to derive model parameters. In the present study, root growth was more sensitive to excess Ni in hydroponic culture than in soil culture, with Ni toxicity in soil overestimated when using the parameters derived from hydroponic culture (Fig. 3b). The observation is consistent some previous studies, which have reported that the plants grown in hydroponic culture have an enhanced sensitivity to Al^{3+}, Ni^{2+} and salinity (Horst *et al.*, 1990; Zaiter & Mahfouz, 1993; Allen *et al.*, 2008). There are some possible reasons for this. First, roots in soils are not in contact with a homogeneous soil solution because of the presence of soil particles or air spaces (Kinraide, 2003). Second, stirred solutions are more toxic than unstirred solutions, which illustrates that the specific conditions in soils such as diffusion limitations, more restricted mass flow and the presence of other organisms (e.g. mycorrhizae) may decrease uptake. Third, given the relatively high metal concentration encountered in the toxicity data, it is possible that the saturation status would lead to nonlinearity in this relationship (Lofts *et al.*, 2004). The theoretical calculations should be treated with some cautions because the free metal

ion might be controlled by precipitation of metal salts at high metal loadings, which are not currently simulated by MINTEQ. Finally, MINTEQ overpredicts the free Ni^{2+} activity in soils with OC < 1% (Thakali *et al.*, 2006).

Soil pH is the most important soil characteristic affecting bioavailability and toxicity of metals. It can influence toxic effectiveness of ions in at least two different ways in soil culture. On the biotic side, for example a plant root, Ni–root interactions can be understood by considering the changes in PM surface activities of Ni^{2+} and other cations such as Ca^{2+} and Mg^{2+} (e.g. rows 1 and 2 in Table 3), which have a pH dependency in terms of $\psi_0{}^o$. Hydrogen ions can depolarize the negativity of $\psi_0{}^o$ and hence decrease the attraction of Ni^{2+} to the PM surface. Therefore the bioavailability and toxicity of Ni^{2+} may be reduced by a decrease in pH in the soil solution if $\{Ni^{2+}\}_b$ remains constant. On the soil side, pH affects Ni^{2+} activities in the soil solution (Eqn 6). Soil pH is connected closely to the chemical processes of precipitation, sorption and complexation, which determine to a large extent the metal partitioning and speciation in solution (Weng *et al.*, 2004). An increase in soil pH will shift Ni^{2+} partitioning toward the soil solid phase and hence decrease the $\{Ni^{2+}\}_b$. Therefore, Ni^{2+} phytotoxicity is often increased when plants are grown in solutions in which pH increased, while alleviation of Ni toxicity is observed for plants growing in soils (Weng *et al.*, 2003, 2004). The balance, therefore, depends on the relative magnitude of the two effects on the soil and plant root systems.

The problem of intercorrelation

Variables relating to ion activities and osmolarity (e.g. Eqns 11 and 12) were intercorrelated in soil cultures. The issue of colinearity is a major problem for studies investigating ion–plant interactions in soil cultures. It is difficult to discern intrinsic Ni^{2+} toxicity, extrinsic osmolarity and intrinsic effects of other ions under conditions of simultaneous Ni^{2+} and osmolarity intoxication. However, the osmolarity in both hydroponic cultures (Lock *et al.*, 2007; Li *et al.*, 2009) was always < 50 mOsM and the intercorrelations among ion activities in the bulk solution or at the PM surface were very weak (R^2 < 0.10). These criteria enable the investigation of Ni^{2+} intrinsic toxicity and possible intrinsic interactions.

The supposed mechanisms behind ion interactions

Our analyses suggest that several factors influence the toxic effects of excessive Ni^{2+} and the interactions with Ca^{2+}, Mg^{2+} and H^+ upon root growth.

The dual effects of $\psi_0{}^o$ First, the negativity of $\psi_0{}^o$ influences ion activities at the PM surface, increasing the activity of cations but decreasing anion activities. However, the addition of cations such as Ca^{2+} and H^+ nonspecifically alleviate Ni^{2+} toxicity by reducing the negativity of $\psi_0{}^o$ and hence reducing the activity of Ni^{2+} at the PM surface (this effect has been demonstrated and reviewed elsewhere; Kinraide, 2006; Wang *et al.*, 2008; Kopittke *et al.*, 2011). However, the second possible role of $\psi_0{}^o$ on the toxicity of Ni^{2+}, the influence of $\psi_0{}^o$ upon surface-to-surface transmembrane potential difference ($E_{m,surf}$, a component of the electrical driving force for ion uptake) has not been adequately demonstrated previously, especially for soil culture (Kinraide, 2001; Wang *et al.*, 2011). The results of the current study provide support for this second possible effect of $\psi_0{}^o$ in both soil culture as and in solution culture (see the term $(1 + a_{12}\psi_0{}^o)$ in Eqns 5, 9 and 10). Therefore, additions of Ca^{2+}, Mg^{2+} and H^+ (decrease in pH) to the rooting medium cause a reduction in the negativity of $\psi_0{}^o$ which decreases the electrostatic attraction of Ni^{2+} to the PM surface, but increases $E_{m,surf}$, thus increasing the electrical driving force for Ni^{2+} uptake across PMs.

The roles of Ca^{2+} The results suggest that Ca^{2+} may have at least three roles regarding root growth other than causing a reduction in the negativity of $\psi_0{}^o$ (thus causing a nonspecific reduction in $\{Ni^{2+}\}_0{}^o$). First, Ca^{2+} is essential for root elongation as an intrinsic requirement (illustrated by inclusion of the term $(1 - 1/\exp[a_2\{Ca^{2+}\}_0{}^o])$ in Eqn 5), but the addition of a PM-depolarizing solute may reduce $\{Ca^{2+}\}_0{}^o$ to growth-limiting activities (i.e. induce a Ca deficiency). Second, Ca^{2+} contributes to the reduction of the water potential (i.e. increase in osmolarity) in soil cultures and thereby contributes to toxicity. This effect is independent of ionic toxicity and is expressed using the term $[(a_3\text{Osmolarity})^{b3}]$ in Eqns 8 and 10. Finally, Ca^{2+} (and other cations also) may exert an extrinsic intoxicating effect by decreasing $\{Mg^{2+}\}_0{}^o$ (by decreasing the negativity of $\psi_0{}^o$) and thereby reducing the magnitude of the specific alleviation of intrinsic Ni^{2+} toxicity by $\{Mg^{2+}\}_0{}^o$.

The roles of Mg^{2+} The Mg^{2+} ion resembles Ca^{2+} with respect to decreasing $\{Ni^{2+}\}_0{}^o$ as a result of a decrease in the negativity of $\psi_0{}^o$. However, Mg^{2+} also exerts an intrinsic (specific) amelioration of intrinsic Ni^{2+} toxicity by reducing 'a_1' with increasing $\{Mg^{2+}\}_0{}^o$. This effect is expressed using the term $[a_{11}/(1 + a_{14}\{Mg^{2+}\}_0{}^o)]$ in Eqns 5 and 10. It is likely that Mg^{2+} alleviates intrinsic Ni^{2+} toxicity by specific competition for membrane transporters, given that the radius of Mg^{2+} (0.72 pm) is similar to that of Ni^{2+} (0.69 pm). Snavely *et al.* (1991) reported that Ni^{2+} was transported into the cell by all three Mg^{2+} transport systems, and thus Mg^{2+} will compete with Ni^{2+} for binding sites on the Mg^{2+} transporters and, as a result, less Ni^{2+} will be taken up. In addition, high Mg^{2+} concentrations can downregulate the expression of Mg^{2+} transporters and reduce Ni^{2+} uptake (Snavely *et al.*, 1991). In its final role, Mg^{2+} may express itself as an extrinsic intoxicant by inducing Ca deficiency.

Table 4 Model fit summary with the biotic ligand model (BLM) and electrostatic toxicity model (ETM) for nickel (Ni) toxicity to barley (*Hordeum vulgare*) root elongation

Root response	Model	Hydroponic culture 1[a] RMSE	Hydroponic culture 1[a] R^2	Hydroponic culture 2[b] RMSE	Hydroponic culture 2[b] R^2	Soil culture[c] RMSE	Soil culture[c] R^2
RER	BLM	0.213	0.556	0.105	0.842	0.158	0.830
	ETM	0.154	0.781	0.110	0.832	0.156	0.830
rRE	BLM	25.3	0.655	9.70	0.914	12.8	0.898
	ETM	18.0	0.774	9.10	0.923	9.74	0.940

The root elongation rate (RER) and relative root elongation (Rre) in hydroponic culture were predicted with the BLM using constants reported in the studies (Li *et al*., 2009; Lock *et al*., 2007), and in soil culture the constants used to predict the RER and rRE were derived from Thakali *et al.* (2006), who developed the terrestrial BLM to predict Ni toxicity to barley root elongation in eight noncalcareous soils (pH < 7.0) of the current study.
[a]Root growth data from Lock *et al.* (2007).
[b]Root growth data from Li *et al.* (2009).
[c]Root growth data from Rooney *et al.* (2007).

Comparison of the modelling of the BLM and the ETM

Lock *et al.* (2007) and Li *et al.* (2009) conducted similar growth experiments using hydroponic cultures to develop a Ni-BLM to predict Ni toxicity to barley root elongation. In a similar manner, Thakali *et al.* (2006) developed a terrestrial BLM to predict Ni toxicity for the same endpoint in eight noncalcareous soils. However, the analyses reported here raises a question regarding whether the addition of cations (H^+, Ca^{2+} and Mg^{2+}) alleviates Ni^{2+} toxicity as a result of specific competition for biotic ligands, as suggested by the BLM. Rather, we contend that observed alleviation of Ni toxicity often results from nonspecific electrostatic effects arising from an effect of the cations on $\psi_0{}^o$ and the subsequent influence on $\{Ni^{2+}\}_0{}^o$ and the driving force of Ni^{2+} transport across the PM (E_{msurf}). Our analyses also account for specific interactions between Ni^{2+} and Mg^{2+}. Table 4 and Fig. 4 present a comparison between the model fit for RER and rRE for the BLM and for the ETM. The ETM predictions show a better correlation with the observed RER and rRE than the BLM predictions based on the RMSE and R^2. The RMSEs of the predicted EC50 expressed as Ni^{2+} activity in soil solution (log transformed) was 0.196 for the ETM and 0.287 for the BLM (Fig. 4b). It also should be noted that the comparison of the two models should acknowledge the difference in the number of adjustable parameters (five parameters for BLM and six parameters for ETM).

Some uncertainties

Some of the forgoing discussion is based on the assumptions that the metal speciation model and the GCS model used in this study are valid. However, there are still some uncertainties. First, the metal speciation in soil solution was modelled based on bulk soil solution and bulk soil properties. The bulk soil may be different from the soil in the rhizosphere, which is influenced by processes such as exudation of proton and metal-complexing compounds, and these may have effects on metal bioavailability to plants (McLaughlin *et al.*, 1998). Also, ion concentrations in the rhizosphere may be different from concentration in the bulk soil owing to soil transpirational flow. Another uncertainty is the validity of applying the GCS model for calculating the $\psi_0{}^o$ of plant roots in contact with solution to plant roots in soil. In reality, roots do not have homogeneous contact with the soil solution and a thin, variable layer of soil solution will exist because of close contact with the soil air or with soil particles (Kinraide, 2003). These uncertainties suggest that ion activities at the PM surface may be somewhat different in roots grown in soil culture than in roots taken from hydroponic cultures, but this appears not to reduce the trends for changes in ion surface activities in response to the ionic composition of root-bathing media. Despite these uncertainties, there is evidence from the present study for electrostatic effects and specific interactions, given that they can account for > 75.1% of the variance (see Tables 1, 2) in root growth in both hydroponic cultures and soils. Given that the Ni^{2+} concentrations in soil solution are not even relevant for most natural highly contaminated soils, the effects of Ni^{2+} on the surface potential and $\{Ca^{2+}\}_0{}^o$ may be minor and extrapolation of some results also requires some caution.

Conclusions

The study set out to evaluate the factors influencing Ni^{2+} toxicity and the interactions with Ca^{2+}, Mg^{2+} and H^+ upon barley root elongation in both soil and hydroponic cultures. Electrostatic toxicity models were developed to relate RER and rRE to $\{Ni^{2+}\}_0{}^o$, the dual effects of $\psi_0{}^o$, osmolarity effects and the Ni^{2+} toxicity alleviated by Mg^{2+}. Fitting electrostatic toxicity models to observational data suggests different roles of Ca^{2+} and Mg^{2+} in Ni toxicity. For example a specific alleviation of intrinsic Ni^{2+} toxicity by Mg^{2+} was observed. This study also suggests that the electrostatic toxicity model provides a robust mechanistic framework to assess metal ecotoxicity and predict critical metal concentrations linked to plant root growth.

Acknowledgements

This work was supported financially by the Natural Science Foundation of Jiangsu Province (Grant No. BK 2009339). Rothamsted Research is an institute of the Biotechnology and Biological Sciences Research Council of the UK. Funds for the soil studies were provided by the Nickel Producers Environmental Research Association.

References

Allen HE, Lin YQ, Di Toro DM. 2008. Ecotoxicity of Ni in soil. *Mineralogical Magazine* **72**: 367–371.

Carter MR, Webster GR, Cairns RR. 1979. Calcium deficiency in some Solonetzic soils of Alberta. *Journal of Soil Science* **30**: 161–174.

ECB. 2009. *European union risk assessment report on nickel and nickel compounds*. Ispra, Italy: European Chemicals Bureau.

Hanson JB. 1984. The functions of calcium in plant nutrition. In: Tinker PB, Läuchli A, eds. *Advances in plant nutrition, vol 1*. New York, NY, USA: Praeger Scientific, 149–208.

Horst WJ, Klotz F, Szulkiewicz P. 1990. Mechanical impedance increases aluminum torerant of soybean (*Glycine max*) roots. In: van Beusichem ML, ed. *Plant nutrition – physiology and application*. Dordrecht, the Netherlands: Kluwer Academic Publications.

International Organisation for Standardisation (ISO). 1993. *Soil quality-determination of the effects of pollutants on soil flora. Part 1. Methods for the measurement of inhibition of root growth*. No. 11269-1. Geneva, Switzerland: ISO.

Kinniburgh DG, Van Riemsdijk WH, Koopal LK, Borkovec M, Benedetti MF, Avena MJ. 1999. Ion binding to nature organic matter: competition, heterogeneity, stoichiometry, and thermodynamic consistency. *Colloids and Surface A: Physicochemical and Engineering Aspects* **151**: 147–166.

Kinraide TB. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. *Plant Physiology* **118**: 513–520.

Kinraide TB. 1999. Interactions among Ca^{2+}, Na^{+} and K^{+} in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. *Journal of Experimental Botany* **50**: 1495–1505.

Kinraide TB. 2001. Ion fluxes considered in terms of membrane-surface electrical potentials. *Australian Journal of Plant Physiology* **18**: 605–616.

Kinraide TB. 2003. Toxicity factors in acidic forest soils: attempts to evaluate separately the toxic effects of excessive Al^{3+} and H^{+} and insufficient Ca^{2+} and Mg^{2+} upon root elongation. *European Journal of Soil Science* **54**: 323–333.

Kinraide TB. 2004. Possible influence of cell walls upon ion concentrations at plasma membrane surfaces. Toward a comprehensive view of cell-surface electrical effects upon ion uptake, intoxication, and amelioration. *Plant Physiology* **136**: 3804–3813.

Kinraide TB. 2006. Plasma membrane surface potential (PM) as a determinant of ion bioavailability: a critical analysis of new and published toxicological studies and a simplified method for the computation of plant PM. *Environmental Toxicology and Chemistry* **25**: 3188–3198.

Kinraide TB, Pedler JF, Parker DR. 2004. Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH. *Plant and Soil* **259**: 201–208.

Kinraide TB, Wang P. 2010. The surface charge density of plant cell membranes (σ): an attempt to resolve conflicting values for intrinsic σ. *Journal of Experimental Botany* **61**: 2507–2518.

Kinraide TB, Yermiyahu U. 2007. A scale of metal ion binding strengths correlating with ionic charge, Pauling electronegativity, toxicity, and other physiological effects. *Journal of Inorganic Biochemistry* **101**: 1201–1213.

Kinraide TB, Yermiyahu U, Rytwo G. 1998. Computation of surface electrical potentials of plant cell membranes. Correspondence to published Zeta potentials from diverse plant sources. *Plant Physiology* **118**: 505–512.

Kopittke PM, Blamey FPC, Asher CJ, Menzies NW. 2010. Trace metal phytotoxicity in solution culture: a review. *Journal of Experimental Botany* **61**: 945–954.

Kopittke PM, Blamey FPC, Kinraide TB, Wang P, Richman SM, Menzies NW. 2011. Separating multiple, short-term deleterious effects of saline solutions on the growth of cowpea seedlings. *New Phytologist* **189**: 1110–1121.

Li B, Zhang X, Wang XD, Ma YB. 2009. Refining a biotic ligand model for nickel toxicity to barley root elongation in solution culture. *Ecotoxicology and Environmental Safety* **72**: 1760–1766.

Lock K, Van Eeckhout H, De Schamphelaere KAC, Criel P, Janssen CR. 2007. Development of a biotic ligand model (BLM) predicting nickel toxicity to barley (*Hordeum vulgare*). *Chemosphere* **66**: 1346–1352.

Lofts S, Spurgeon DJ, Svendsen C, Tipping E. 2004. Deriving soil critical limits for Cu, Zn, Cd, and pH: a method based on free ion concentrations. *Environmental Science & Technology* **38**: 3623–3631.

McLaughlin MJ, Smolders E, Mercks R. 1998. Soil–root interface: physicochemical processes. In: Huang PM, Adriano DC, Logan TC, Chechai RT, eds. *Soil chemistry and ecosystem health*. Madison, WI, USA: Soil Sciences Society of America, 233–277.

Munns R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment* **25**: 239–250.

Peijnenburg WJGM, Posthuma L, Eijsackers HJP, Allen HE. 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicology and Environmental Safety* **37**: 163–172.

Robinson RA, Stokes RH. 2002. *Electrolyte solutions*. Minerola, NY, USA: Dover Publications.

Rooney CP, Zhao FJ, McGrath SP. 2007. Phytotoxicity of nickel in a range of European soils: influence of soil properties, Ni solubility and speciation. *Environmental Pollution* **145**: 596–605.

Sauvé S, Hendershot W, Allen HE. 2000. Solid–solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. *Environmental Science & Technology* **34**: 1125–1131.

Snavely MD, Gravina SA, Cheung TT, Miller CG, Maguire ME. 1991. Magnesium transport in *Salmonella typhimurium*. Regulation of mgtA and mgtB. *Journal of Biological Chemistry* **266**: 824–829.

Stevens DP, McLaughlin MJ, Heinrich T. 2003. Determining toxicity of lead and zinc runoff in soils: salinity effects on metal partitioning and on phytotoxicity. *Environmental Toxicology and Chemistry* **22**: 3017–3024.

Tatulian SA. 1999. *Surface electrostatics of biological membranes and ion binding*. New York, NY, USA: Marcel Dekker.

Taylor GJ, Stadt KJ, Dale MRT. 1991. Modeling the phytotoxicity of aluminum, cadmium, copper, manganese, nickel, and zinc using the Weibull frequency-distribution. *Canadian Journal of Botany* **69**: 359–367.

Thakali S, Allen HE, DiToro DM, Ponizovsky AA, Rooney CP, Zhao FJ, McGrath SP. 2006. A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. *Environmental Science & Technology* **40**: 7085–7093.

Wang P, Kinraide TB, Zhou DM, Kopittke PM, Peijnenburg WJGM. 2011. Cell membrane surface potential: dual effects upon ion uptake and toxicity. *Plant Physiology* **155**: 808–820.

Wang P, Zhou DM, Kinraide TB, Luo XS, Li LZ, Li DD, Zhang HL. 2008. Cell membrane surface potential (ψ_0) plays a dominant role in the phytotoxicity of copper and arsenate. *Plant Physiology* **148**: 2134–2143.

Wang P, Zhou DM, Peijnenburg WJGM, Li LZ. 2010. Evaluating mechanisms for plant–ion (Ca^{2+}, Cu^{2+}, Cd^{2+} or Ni^{2+}) interactions and their effectiveness on rhizotoxicity. *Plant and Soil* **344**: 277–288.

Weng LP, Lexmond TM, Wolthoorn A, Temminghoff EJM, Van Riemsdijk WH. 2003. Phytotoxicity and bioavailability of nickel: chemical speciation and bioaccumulation. *Environmental Toxicology and Chemistry* **22**: 2180–2187.

Weng LP, Temminghoff EJM, Lofts S, Tipping E, Van Riemsdijk WH. 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sand soil. *Environmental Science & Technology* **36**: 4804–4810.

Weng LP, Wolthoorn A, Lexmond TM, Temminghoff EJM, Van Riemsdijk WH. 2004. Understanding the effects of soil characteristics on phytotoxicity and bioavailability of nickel using speciation models. *Environmental Science & Technology* **38**: 156–162.

White PJ, Pineros M, Tester M, Ridout MS. 2000. Cation permeability and selectivity of a root plasma membrane calcium channel. *Journal of Membrane Biology* **174**: 71–83.

Yermiyahu U, Brauer DK, Kinraide TB. 1997. Sorption of aluminum to plasma membrane vesicles isolated from roots of Scout 66 and Atlas 66 cultivars of wheat. *Plant Physiology* **115**: 1119–1125.

Zaiter HZ, Mahfouz B. 1993. Salinity effect on root and shoot characteristics of common and tepary beans evaluated under hydroponic and sand culture. *Journal of Plant Nutrition* **16**: 1569–1592.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Soil solution properties of the nickel (Ni)-amended soils used in phytotoxicity tests

Notes S1 Gouy–Chapman–Stern (GCS) model.

Notes S2 Computation of ψ_0 by a fully parameterized Gouy–Chapman–Stern model.

Notes S3 The activity of free ions at charged membrane surfaces.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.