Soil Biology & Biochemistry 57 (2013) 720–730

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# An electrostatic model predicting Cu and Ni toxicity to microbial processes in soils

Peng Wang [a,b], Thomas B. Kinraide [c], Erik Smolders [d], Dong-Mei Zhou [a,*], Neal W. Menzies [b], Sagar Thakali [e], Wei-Wei Xia [a], Xiu-Zhen Hao [a], Willie J.G.M. Peijnenburg [f,g], Peter M. Kopittke [b]

[a] Key Laboratory of Soil Environment & Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] The University of Queensland, School of Agriculture and Food Sciences, St. Lucia, Queensland 4072, Australia
[c] Appalachian Farming Systems Research Center, Agricultural Research Service, United States Department of Agriculture, Beaver, WV 25813-9423, USA
[d] Division of Soil and Water Management, K. U. Leuven, 3001 Heverlee, Belgium
[e] Gradient, Cambridge, MA 02138, USA
[f] Laboratory for Ecological Risk Assessment RIVM – National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands
[g] Leiden University, Institute of Environmental Sciences (CML), Department of Conservation Biology, 2300RA Leiden, The Netherlands

ARTICLE INFO

*Article history:*
Received 2 April 2012
Received in revised form
1 September 2012
Accepted 2 September 2012
Available online 16 September 2012

*Keywords:*
Nickel
Copper
Potential nitrification rate
Glucose-induced respiration
Osmotic effect
Electrostatic toxicity model
Surface electrical potential
Zeta potential

ABSTRACT

Toxicity data for microorganism in soil or in soil less cultures have been described with ion competition models, however these models disregard electrostatic and osmotic effects which are known to affect ion sorption and toxicity. Using European soils with diverse characteristics, the factors that influence the toxicity of soil Cu or Ni to potential nitrification rate (PNR) and glucose-induced respiration (GIR) were evaluated based on the electrical potential ($\psi_0$) and ion activities ($\{M^{2+}\}_0$) at the outer surfaces of bacterial cell membranes (CMs). The zeta potentials ($\zeta$) of bacterial (*Escherichia coli*) protoplasts, as affected by the ionic composition of the solution, were measured and used to estimate the parameters of a Gouy–Chapman–Stern (GCS) model which was then used to compute $\psi_0$ values. The $\psi_0$ values varied widely with soil type and increased markedly (became less negative) as metal salts were added. Computed $\psi_0$ was then used to predict the surface ion activities from the soil solution composition. The toxicity data (both PNR and GIR) were statistically related to (i) surface activities of free metal ions ($\{M^{2+}\}_0$), (ii) the ameliorative effect of surface $H^+$ activity ($\{H^+\}_0$), (iii) the $\psi_0$-influenced electrical driving force for cation uptake across CMs, and (iv) osmotic effects. This electrostatic model predicted the observed GIR and PNR with $R^2_{adj} > 0.816$ for observed vs. predicted PNR and $R^2_{adj} > 0.861$ for observed vs. predicted GIR. These predictions were generally better than those by previous models. The suggestion that metal toxicity in spiked soils is partly related to a spike-induced osmotic increase is corroborated by fitting the model to spiked soils that were or were not leached and aged to reduce the osmotic increase. The predicted soil EC50 values (in mg metal/kg soil) were within a factor of 2.5 for up to nineteen European soils with a wide range of properties.



## 1. Introduction

Soil microbial processes are vital functions in soil ecosystems (*e.g.* C and N cycles) and their sensitivity to metal contamination suggests that these processes need to be included in risk assessments for metals (Giller et al., 1998, 2009; Smolders et al., 2001). Numerous assays have shown that the total concentration of metals in soils required to exert an inhibitory effect vary widely and depend upon metal speciation and soil characteristics (*e.g.* pH, organic carbon, cation exchange capacity (CEC), and the ionic composition of the soil solution) (Oorts et al., 2006a,b; Smolders et al., 2004). For example, the effective concentrations of spiked Cu in soil causing 50% inhibition of potential nitrification rate (PNR; see Table 1 for a list of symbols and abbreviations) ranged from 42 to 2350 mg $kg^{-1}$ in 19 European soils, and for glucose-induced respiration (GIR) concentrations ranged from 186 to 3660 mg $kg^{-1}$ (Oorts et al., 2006b). Risk assessments and regulations therefore need to consider the factors that influence the metal bioavailability. Empirical relationships that relate toxicity thresholds of metals to a limited number of bulk soil properties such as CEC (Oorts et al., 2006b) and organic matter (Lighthart et al., 1983; Oorts et al., 2006b) have been reported already, although the mechanisms by which soil properties influence the toxicity of metals are not well understood.

* Corresponding author. Tel.: +86 25 86881180; fax: +86 25 86881000.
*E-mail address:* dmzhou@issas.ac.cn (D.-M. Zhou).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2012.09.002

**Table 1**
Principal symbols and abbreviations.

| Symbol or abbreviation | Description | Units |
|---|---|---|
| BR | Biological response | % of control |
| CM | Cell membrane | |
| EC50 (or EA50) | Concentration (or activity) of an ion causing a 50% reduction in the rate of a process (*e.g.* PNR or GIR) | mM or μM |
| ETM | Electrostatic toxicity model | |
| FIAM | Free ion activity model | |
| GCS | Gouy–Chapman–Stern | |
| GIR | Glucose-induced respiration | % of control |
| PNR | Potential nitrification rate | % of control |
| TBLM | Terrestrial biotic ligand model | |
| $[M]_{soil}$ | Total concentration of metal M in soil solution | M, mM, or μM |
| $[M^{2+}]_b$ | Concentration of metal ion $M^{2+}$ in soil solution | M, mM, or μM |
| $\{M^{2+}\}_b$ | Activity of metal ion $M^{2+}$ in soil solution | M, mM, or μM |
| $\{M^{2+}\}_0$ | Activity of metal ion $M^{2+}$ at the CM surface | M, mM, or μM |
| $K_{P,I}$ | Equilibrium constant for the binding of ion $I$ at site $P^0$ | $M^{-1}$ |
| $K_{R,I}$ | Equilibrium constant for the binding of ion $I$ at site $R^-$ | $M^{-1}$ |
| $P_T$ | Total density of binding sites $P^0$ at the CM surface | μmol $m^{-2}$ |
| $R_T$ | Total density of negative charges (and binding sites $R^-$) at the CM surface (*i.e.*, negative charges in the absence of solute binding) | μmol $m^{-2}$ |
| $\psi_0$ | Electrical potential at the CM outer surface | mV |
| $\sigma$ ($\sigma_0$) | Surface charge density (intrinsic $\sigma$, *i.e.*, $\sigma$ in the absence of solute binding) at the CM outer surface | C $m^{-2}$ |
| ζ potential | Zeta potential; near-surface (plane of shear) electrical potential measured by electrophoresis | mV |

Cell surfaces of bacteria are usually negatively charged (Baygents et al., 1998; Soni et al., 2008). Like plant roots, the outer surfaces of bacterial cells become less negative as pH decreases and the ionic strength of the bathing solution increases (Boonaert and Rouxhet, 2000; Bushby, 1990; Butt, 1992; Kinraide and Sweeney, 2003; Morisaki et al., 1999; Van der mei et al., 1993). For example, Kinraide and Sweeney (2003) demonstrated decreased negativity of the rhizobium (*Rhizobium leguminosarum* bv. *trifolii*) cell surface as pH declined from 6.0 to 3.5.

Because of the electrical potential at the outer surfaces of bacterial cell outer membranes ($\psi_0$), the concentrations or activities of ions at the cell membrane (CM) surface differ significantly from those in the cell-bathing medium. The $\psi_0$ is often sufficiently negative to enrich cations and deplete anions at the CM surface by more than 10-fold relative to the bulk-phase medium. Cations in the bulk medium, such as $Al^{3+}$, $Ni^{2+}$, $Ca^{2+}$, $Mg^{2+}$, and $H^+$, reduce the negativity of $\psi_0$ by charge screening and ionic binding, thereby reducing the surface activity of cations such as $Cu^{2+}$ and $Ni^{2+}$. It is also likely that the values for $\psi_0$ of microorganisms in the soil range widely, given large variations in soil pH and solution concentrations of cations (Wolt, 1994). $\psi_0$ is difficult to measure directly, but the zeta (ζ) potential, the electrical potential at the hydrodynamic plane of shear (located at a small distance from the CM surface), can be determined by electrophoretic mobility. In addition, a Gouy–Chapman–Stern (GCS) model is available to calculate $\psi_0$ in response to the solution ionic composition (Kinraide et al., 1998; Kinraide and Wang, 2010; Yermiyahu and Kinraide, 2005).

The quantitative description, and prediction, of adverse effects on microbial processes in soils is less advanced than those for plants or invertebrates, due to a number of limitations. First of all, variations in soil properties affect the composition and metal sensitivity of the indigenous microbial community (Mertens et al., 2010). Consequently, different toxicity responses among soil microbial communities result not only from differences in metal bioavailability but also from differences in the sensitivity of the microbial community tested. The latter may be, in part, attributable to the variation in cell-surface negativity for bacteria which, in plants, has been reported to link the variation in salinity and Al sensitivity (Jozefaciuk and Szatanik-Kloc, 2004; Wagatsuma and Akiba, 1989; Yermiyahu et al., 1999). Secondly, the surface charge density ($\sigma$) of bacteria, as influenced by the ionic composition of the soil solution, has an important impact on the adhesion and mobility of bacteria (Baygents et al., 1998; Morisaki et al., 1999) and, consequently, influences the exposure and the sensitivity to soluble metals.

Spiking soils with soluble metal salts increases the ionic strength (*i.e.*, increases the soluble Ca, Mg, and the corresponding osmotic stress) and decreases the pH. All of these factors affect metal bioavailability to microbial processes. An increase in the osmotic potential results in inhibition of microbial processes, especially for nitrification (Li and Huang, 2008; Rysgaard et al., 1999). Indeed, the apparent decrease in toxicity with the leaching and aging of metal salt-amended soils may be partially attributed to the leaching of ions and the concomitant reduction in osmolarity. The effects of leaching and aging have been analyzed in terms of the changes in speciation and osmotic effects (Oorts et al., 2007), but have not yet been modeled (Buekers et al., 2010). Separation of these multiple toxic effects in soils should help to increase the ecological relevance of laboratory toxicological tests.

The objectives of this study therefore are to 1) verify that a GCS model computes reasonable $\psi_0$ values for bacterial protoplasts that are at least proportional to measured ζ potentials, 2) estimate metal (Cu and Ni) toxicity toward microbial processes (PNR and GIR) in order to elucidate mechanisms by which soil properties affect the toxicity of metals, giving particular consideration to membrane electrical characteristics (both surface potential and transmembrane potential differences) and ion activities at the membrane surface, 3) isolate osmotic effects from other effects that influence microbial processes, and 4) develop electrostatic toxicity models (ETMs) for metal toxicity to soil microbes to predict the critical soil metal concentrations for soils with a wide range of properties.

## 2. Materials and methods

### 2.1. Determination of ζ potentials of bacterial protoplasts

*Escherichia coli* (DH-5α) is an engineered strain of this gram-negative, rod-shaped bacteria and its cell wall contains a thin peptidoglycan layer adjacent to the cytoplasmic membrane (Ishii et al., 2006). *E. coli* was cultured in 500-mL Erlenmeyer flasks containing 200 mL LB medium (Tryptone 2.0 g $L^{-1}$, Yeast Extract 1.0 g $L^{-1}$, NaCl 1.0 g $L^{-1}$). The pH was adjusted to 7.2 with 1 M NaOH or HCl, and the medium was autoclaved at 121 °C for 20 min. The culture was grown at 25 °C and continuously shaken at 200 rpm in the dark. After 16 h of growth, and during the logarithmic growth phase, cells were harvested by centrifugation at 3500 *g* for 20 min at 4 °C. The pellet was suspended in 0.01 M Tris buffer (pH 7.0) and centrifugally washed three times. The pellet was re-suspended in 0.01 M hyper-osmotic Tris buffer (pH 7.0) containing 0.5 M sucrose and then heated at 37 °C for 5 min. A lysozyme (Sigma-Aldrich) solution at 37 °C was added with a final concentration of 0.5 g $L^{-1}$. The cells were incubated at 150 rpm for 20 min at 37 °C. An EDTA solution was added with a final concentration of 0.01 M and incubated for an additional 15 min. The rate of protoplast formation

was more than 95% as determined by microscopy. Finally, the protoplasts were centrifuged at 2500 *g* for 20 min, then re-suspended and washed by two successive additions of 20 mL of 0.01 M hyper-osmotic Tris buffer.

In order to investigate the independent effect of different cations on the surface electrical potential, only one cation concentration varied at a time while all other cation concentrations were kept as nearly constant as possible. The $H^+$ ion is particularly effective in reducing the negativity of the electrical potential at cell membrane surface, and the pH-buffer was used to ensure constant $H^+$ (except for pH tests). Eight sets of ζ potential measurements were obtained, one each for $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, pH, $Cu^{2+}$, $Ni^{2+}$ and $Al^{3+}$, with three to six concentrations for each cation, giving a total of 34 treatments (Supplemental Table S1). Chloride-salts were used for all treatments. Media were buffered with 2.0 mM MES (2-[N-morpholino] ethane sulfonic acid) adjusted for pH with NaOH or HCl. The selected cation concentrations and pH reflected the variability occurring in the natural soil solutions (Wolt, 1994). The protoplasts (at least $10^5$ $mL^{-1}$) were suspended in the test media and incubated for 1 h at 25 ± 1 °C. The ζ potentials of the outer membrane of protoplasts were measured using a JS94H micro-electrophoresis meter (Powereach Instruments, Shanghai, China) (Wang et al., 2008).

### 2.2. Determination of surface potential using Gouy–Chapman–Stern model

The GCS model combines classic electrostatic Gouy–Chapman theory with ion binding to CMs (Stern model). The GCS model incorporates the intrinsic surface charge density ($\sigma_0$; in C $m^{-2}$) of a membrane, the ion composition of the bathing medium, ion binding to the membrane, and $\psi_0$. The intrinsic surface charge density ($\sigma_0$) is the surface charge density in the absence of ion binding to the membrane; the actual, or contingent, surface charge density ($\sigma$) combines the charges of $\sigma_0$ and the charges of the bound ions. The Gouy–Chapman portion of the model is expressed in this equation:

$$\sigma^2 = 2\varepsilon_r\varepsilon_0 RT \sum_i [I^Z]_b(\exp[-Z_i F\psi_0/(RT)] - 1) \quad (1)$$

where $2\varepsilon_r\varepsilon_0 RT = 0.00345$ at 25 °C for bulk-phase concentrations of $I^Z$ expressed in M and $\sigma$ expressed in (C $m^{-2}$)($\varepsilon_r$ is the dielectric constant for water, $\varepsilon_0$ is the permittivity of a vacuum, $R$ is the gas constant and $T$ is temperature). $[I^Z]_b$ is the concentration of ion $I^Z$ (the ith ion) in the bulk-phase medium; $Z_i$ is the charge on ion $I^Z$; $F$ is the Faraday constant (Yermiyahu and Kinraide, 2005).

For the Stern portion of the GCS model, the CM surfaces were assumed to comprise two classes of binding sites: one negatively charged ($R^-$) and one neutral ($P^0$). Ions may bind according to the reactions $R^- + I^Z \Leftrightarrow RI^{Z-1}$ and $P^0 + I^Z \Leftrightarrow PI^Z$ for which the binding constants $K_{R,I}$ (=$[RI^{Z-1}]/([R^-][I^Z]_0)$) and $K_{P,I}$(=$[PI^Z]/([P^0][I^Z]_0)$) are needed. $[R^-]$, $[P^0]$, $[RI^{Z-1}]$, and $[PI^Z]$ denote CM surface densities in mol $m^{-2}$, and $[I^Z]_0$ (=$[I^Z]_b\exp[-Z_iF\psi_0/(RT)]$, a Boltzmann equation) denotes the concentration in units M of an unbound ion at the CM surface. $F/(RT) = 1/25.7$ at 25 °C for $\psi_0$ expressed in mV. $\sigma$ can be expressed as the sum of the products of surface density and charge of each species, all times $F$:

$$\sigma = \left( -[R^-] + \sum_{RI}\left(Z-1\right)[RI^{Z-1}] + \sum_{PI} Z[PI^Z] \right) F \quad (2)$$

The computation of $\sigma$ by Eq. (2) requires values for the several species $RI^{Z-1}$ and $PI^Z$. To compute them, values for $K_{R,I}$, $K_{P,I}$, $R_T$ (total $R^-$ binding sites) and $P_T$ (total $P^0$ binding sites) must be known, and values for them have been computed by several alternative methods with substantial agreement among them. To compute $\psi_0$, trial values were assigned to it, and $\sigma$ in Eqs. (1) and (2) were computed until the values for $\sigma$ from the two equations converged. More detailed descriptions of the GCS model and model parameterization may be found in Yermiyahu and Kinraide (2005) and Kinraide and Wang (2010). For the present study, we estimated anew the parameters of the GCS model using the measured values of ζ potentials and the methods described in detail by Kinraide and Wang (2010). Calculations of $\psi_0$ and ion activities at the CM surface $\{I^Z\}_0$ were performed using the GCS computer program (available from the authors), details of which are provided in the Supporting Information. Although the cell wall is negatively charged, it has only a small effect on ion activities at the CM surface (Kinraide, 2004; Shomer et al., 2003).

### 2.3. Tested soils

Data for microbial processes (PNR and GIR) in response to Cu or Ni were compiled from soil culture experiments with nineteen (for Cu) and sixteen (for Ni) surface-soils (Oorts et al., 2006b). In brief, for PNR measurement, subsamples of each incubated soil spiked with $CuCl_2$ or $NiCl_2$ solution were amended with $(NH_4)_2SO_4$ solution (resulting in 100 mg $NH_4$–N $kg^{-1}$ fresh soil). The PNR (mg $NO_3$–N (kg fresh soil)$^{-1}$ $d^{-1}$) was calculated as the slope of the regression of soil nitrate concentration against time after substrate addition. For GIR measurement, subsamples of each soil at each metal dose were put into separate glass jars, amended with $^{14}C$-labeled glucose solution, mixed thoroughly, and immediately placed and sealed inside a preserving pot containing 1.0 M NaOH. Each sample was then incubated in the dark at 20 °C for 24 h, after which 1 mL of the NaOH was removed and added to 10 mL of scintillation cocktail (XT Gold) for activity determination by beta counting. The percentage of added [$^{14}C$] glucose respired was calculated from the ratio of the radioactivities. The PNR and GIR at each treatment were expressed as the percent of the control (without addition of metal salt). See Oorts et al. (2006b) for more details.

### 2.4. Ion activities in soil solution

Soil solutions were isolated by centrifugation from spiked soils and the compositions were measured, *i.e.*, elemental composition, dissolved organic carbon (DOC), pH, and major anions. Ion species activities in soil solutions were calculated by the speciation program WHAM 6.5 (Windermere Humic Aqueous Model; Tipping et al., 2003), with its default parameters. The activities were consistent with those of other studies performing metal speciation in soil solutions (Ponizovsky et al., 2006; Thakali et al., 2006a,b; Tye et al., 2004; Vulkan et al., 2000). Inputs to the speciation program included solution pH, concentrations of colloidal fulvic acid (FA), dissolved Cu or Ni, and major cation and anion concentrations (*e.g.* Ca, Mg, Na, K, $Cl^-$, $SO_4^{2-}$). The input concentration of fulvic acid was calculated from DOC by assuming that the dissolved organic matter (DOM) contained 50%C (DOM = 2 × DOC), and that 65% of the dissolved fulvic acid was available for metal binding (Tipping et al., 2003). Free Fe and Al ion activities were also included in the input data set because $Al^{3+}$ and $Fe^{3+}$ compete strongly with other cations to bind on FA. Fe and Al activities were calculated from the solubility products of $Fe(OH)_3$ (log $K_{so} = 2.7$) and $Al(OH)_3$ (log $K_{so} = 8.5$) (Tipping et al., 2003). Additionally, $Cl^-$ and $SO_4^{2-}$ in the molar ratio 6:1 were assumed to maintain electroneutrality (Ponizovsky et al., 2006). A temperature of 293 K and the atmospheric partial pressure of $CO_2$ ($P_{CO_2}$) of $10^{-3.5}$ atm were used in accordance with the experimental conditions. The $P_{CO_2}$ could be higher in soil systems, but the WHAM calculations have shown that metal activities are

insensitive to $P_{CO_2}$ for Cu speciation (Ponizovsky et al., 2006; Vulkan et al., 2000).

### 2.5. Osmolarity

Osmolarity (mOsM) was calculated using the formula: osmolarity $= \sum \varphi_i C_i$, where $\varphi_i$ is the osmotic coefficient of salt $i$ (being 1.86 for NaCl, 1.85 for KCl, 2.58 for $MgCl_2$, 2.56 for $CaCl_2$, 2.54 for $CuCl_2$, and 2.57 for $NiCl_2$ (Robinson and Stokes, 2002)) and $C_i$ is the concentration of salt $i$ (mM).

### 2.6. Electrostatic toxicity model (ETM)

Here we shall formulate an equation that incorporates factors that influence biological responses (BR) to toxicants. BR values for PNR, GIR, growth, survival, etc. may be expressed as a percentage with values ranging from 0 (complete inhibition) to 100 (no inhibition). A simple, but versatile, equation for responses to a toxicant, such as a divalent metal ion, is this:

$$\mathrm{BR} = 100/\exp\left\{\left[a_1\left\{\mathrm{M}^{2+}\right\}_0\right]^{\beta 1}\right\} \quad (3)$$

$\{M^{2+}\}_0$ is the ion activity of the metal toxicant at the CM surface ($\{M^{2+}\}_b$, instead of $\{M^{2+}\}_0$, denotes activity in the bulk-phase medium). $a_1$ is a coefficient that increases with the strength of the toxicant (BR = 36.8 when $\{M^{2+}\}_0 = 1/a_1$, irrespective of the value of coefficient $\beta_1$). $\beta_1$ is a shape coefficient that confers sigmoidality when its value is >1. Coefficient $a_1$ in Eq. (3) may be expanded, as described next, to incorporate factors that influence the apparent toxicity of $\{M^{2+}\}_0$.

Wang et al. (2011) proposed that $\psi_0$ has dual effects on the uptake and toxicity of metal ions. Firstly, it influences the activity of metal ions at the CM surface ($\{M^{2+}\}_0$). Secondly, it influences the surface-to-surface transmembrane potential difference, $E_{m,\mathrm{surf}}$ (*i.e.*, the difference in potential between the inner and outer surfaces of the CM), which is the electrical driving force for the transport of ions across the membrane (Hille, 2001; Kinraide, 2001). Therefore decreases in the negativity of $\psi_0$, induced by the addition of cations, result in decreases in the surface activities of other cations not added and a simultaneous increase in the negativity of $E_{m,\mathrm{surf}}$ (see diagrams in Kinraide, 2001 and Wang et al., 2011). This increase in the electrical driving force (increase in the negativity of $E_{m,\mathrm{surf}}$) is linearly related to the reduction of negativity in $\psi_0$ (Kinraide, 2001; Wang et al., 2011). This increased driving force for the uptake of $M^{2+}$ enhances the toxic response to $\{M^{2+}\}_0$, and this increased toxic response may be expressed by the expansion of the strength coefficient $a_1$ to $a_1(1 + a_{11}E_{m,\mathrm{surf}})$. Because $E_{m,\mathrm{surf}}$ is linearly related to $\psi_0$, we may write $a_1(1 + a_{11}\psi_0)$. For positive values of $a_{11}$ the value of the expanded strength coefficient increases with increasing (less negative) values of $\psi_0$. Eq. (3) may now be written

$$\mathrm{BR} = 100/\exp\left\{\left[a_1(1 + a_{11}\psi_0)\left\{\mathrm{M}^{2+}\right\}_0\right]^{\beta 1}\right\} \quad (4)$$

For this Weibull equation, the strength coefficient may be further expanded from $a_1(1 + a_{11}\psi_0)$ to $a_1(1 + a_{11}\psi_0)(1 + a_{12}/\{H^+\}_0)$ to incorporate the ameliorative effect of $H^+$ (by competition or other effect) beyond its effect upon $\psi_0$.

Finally, a second toxic effect – osmolarity (expressed in units mOsM) – may be added

$$\mathrm{BR} = 100/\exp\left\{\left[a_1(1 + a_{11}\psi_0)\left(1 + a_{12}/\left\{\mathrm{H}^+\right\}_0\right)\left\{\mathrm{M}^{2+}\right\}_0\right]^{\beta 1} + (a_2\mathrm{Osmolarity})^{\beta 2}\right\} \quad (5)$$

Because $(a_2\mathrm{Osmolarity})^{\beta 2}$ appears as a term added to the exponent, the effect is to multiply the toxic effect of $M^{2+}$ by the toxic effect of osmolarity – that is, the effects are noninteractive (*i.e.*, independent and multiplicative). For example, if $M^{2+}$ alone reduces BR to 60 and osmolarity alone reduces BR to 70, then both toxic effects acting together will reduce BR to 42 (=100 × 0.60 × 0.70). See Kopittke et al. (2011) and Wang et al. (2011) for further descriptions and references regarding the use of nonlinear equations to express multiple toxic and ameliorative effects.

### 2.7. Data treatment and statistics

The EC50-values expressed as total metal concentration or EA50-values expressed as free metal ion activity in soil solution (denoted $EC50[M]_{soil}$ or $EA50\{M^{2+}\}_b$) were calculated from the observed PNR or GIR expressed as either $[M]_{soil}$ or as the WHAM-calculated $\{M^{2+}\}_b$. The soil solution properties at $EC50[M]_{soil}$ for each soil were interpolated from the measured values (Supplemental Tables S2–S5). The osmolarity, $\psi_0$, and ion bulk and surface activities were then calculated using the approaches described above.

All coefficients in the equations were evaluated by regression analysis so that $R^2_{adj}$ and the statistical significance of the coefficients could be evaluated using SYSTAT 12 (Cranes Software International Ltd, India). In regression analyses, unless otherwise noted, concentrations and activities are expressed in μM units, and strength coefficients are expressed in $\mu M^{-1}$ units. In all instances, no coefficients are reported where the 95% confidence interval encompassed zero.

Four models based on extensions of the Weibull equation $\mathrm{BR} = 100/\exp[(aT)^{\beta}]$ (*T*: toxicant intensity; see Eq. (3)) were each fitted to the same data for comparison among models (Table 2). The first model is the total metal in soil. The second model is the free ion activity model (FIAM) and uses the WHAM-calculated $\{M^{2+}\}_b$ as *T*. The third model is the terrestrial biotic ligand model (TBLM) that uses the faction ($f_{\mathrm{TBLM}}$) of the total "biotic ligand sites" bound by $M^{2+}$ as *T*. The model parameters of the TBLM for Cu and Ni toxicity to PNR and GIR were from Thakali et al. (2006b), which were parameterized for only the non-calcareous soils. The fourth model, ETM, developed in this study, uses the term $[a_1(1 + a_{11}\psi_0)(1 + a_{12}/\{H^+\}_0)\{M^{2+}\}_0]^{\beta 1} + (a_2\mathrm{Osmolarity})^{\beta 2}$ in Eq. (5) as *T*. In addition, for prediction of EC50s, empirical models (EM) with the highest prediction probability (*i.e.*, the equations with the highest $R^2$) reported by Oorts et al. (2006b) were also included in comparison with the four models above. The differences between values predicted by a model and the values actually observed were estimated with root mean square error (RMSE). We chose the Weibull equation instead of a logistic equation because the former can be easily extended. There are no differences in conclusions drawn from the two equations.

## 3. Results and discussion

### 3.1. Effects of cations on ζ potentials of bacterial surfaces

Measured ζ potentials of *E. coli* protoplasts exposed to different cations at environmentally relevant activities are summarized in Supplemental Table S1. The basal medium contained 0.25 mM $CaCl_2$, 0.25 mM $MgCl_2$, 1.0 mM NaCl, and 0.5 mM KCl at ca. pH 5.73 for all but the Al treatments, where pH = 4.36, and the pH treatments where pH ranged from 4.40 to 7.21. As shown in Fig. 1, the negativity of the ζ potentials decreased with increases in concentrations of the cations in the bacterial bathing media. The order of effectiveness for reducing the negativity of the ζ potential was $Al^{3+} > H^+ > Cu^{2+} > Ni^{2+} > Ca^{2+} \approx Mg^{2+} > Na^+ \approx K^+$. This order is

**Table 2**
Summary of the four models used in current study.

| Model | Equation | Parameter | Number of parameters |
|---|---|---|---|
| Total metal model | BR, % = $100/\exp[(a[M]_{soil})^{\beta}]$ | $a$ and $\beta$ | 2 |
| Free ion activity model (FIAM) | BR, % = $100/\exp[(a\{M^{2+}\}_b)^{\beta}]$ | $a$ and $\beta$ | 2 |
| Terrestrial biotic ligand model (TBLM)[a] | BR, % = $100/\exp[(af_{TBLM})^{\beta}]$ where $f_{TBLM} = K_{MBL}\{M^{2+}\}_b/(1 + K_{MBL}\{M^{2+}\}_b + K_{HBL}\{H^+\}_b + K_{CaBL}\{Ca^{2+}\}_b + K_{MgBL}\{Mg^{2+}\}_b)$ | $a$ and $\beta$<br>For Cu, additional $K_{CuBL}$, $K_{HBL}$ and $K_{MgBL}$<br>For Ni, additional $K_{NiBL}$, $K_{HBL}$ and $K_{MgBL}$ | 5 |
| Electrostatic toxicity model (ETM)[b] | BR, % = $100/\exp\{[a_1(1 + a_{11}\psi_0)\ (1 + a_{12}/\{H^+\}_0)\ \{M^{2+}\}_0]^{\beta 1} + (a_2\text{Osmolarity})^{\beta 2}\}$ | $a_1$, $a_{11}$, $a_{12}$, $\beta_1$, $a_2$, and $\beta_2$ | 6 |

[a] The TBLM parameters for Cu and Ni used in current study were from Tables 1 and 2 in Thakali et al. (2006b), which were parameterized for only the non-calcareous soils.
[b] Apart from these parameters in ETM, nother independent parameters (the third column in Table 3) were used to compute the $\psi_0$ and ion activities at the cell membrane surface.

a function of ion charge and binding strength at the CM surface, and is consistent with previous reports (Kinraide et al., 1998; Wang et al., 2008).

### 3.2. Estimation of GCS model parameters

Parameters for the GCS model have been estimated several times by several methods as summarized in Kinraide and Wang (2010; also see footnotes to Table 3). For the present study we have estimated the parameters again using the ζ potentials for the bacterial protoplasts. To do this, the parameter values of the GCS model were altered to optimize the correspondence between the measured ζ potentials and the computed potentials ($\psi_s$) at the hydrodynamic plane of shear at distance $s$ from the CM surface. The detailed procedures for systematically altering then fixing the parameter values are presented in Kinraide and Wang (2010). The parameters so derived are presented in Table 3.

Among the optimized parameters are the equilibrium constants for ion binding to negative binding sites ($R^-$) on the bacterial CM surface ($K_{R,K} = K_{R,Na}$, $K_{R,Ca} = K_{R,Mg}$, $K_{R,Cu}$, $K_{R,Ni}$, $K_{R,Al}$ and $K_{R,H}$) (Column 1 in Table 3). The $K_{R,Ni}$ and $K_{R,H}$ are somewhat lower than those estimated by the other two methods – adsorption (Column 3 in Table 3) and General Binding Strength (Column 5 in Table 3). The $K_{R,Al}$ was lower than that estimated by adsorption but near to that estimated from General Binding Strength. The binding constants in the Standard Model for plant CMs were measured from ion adsorption to CM vesicles of wheat root cells (Vulcan et al., 2004; Yermiyahu et al., 1997). The General Binding Strength values are derived from the relative strength of ion binding to hard ligands (*e.g.* ligands with $F^-$ or O donors) as opposed to soft ligands (*e.g.* ligands with $I^-$ or S donors) (Kinraide, 2009). Fig. 2 illustrates this close correspondence in plots of rescaled values (cube roots) between the binding constants in this study and those evaluated by other methods. A sensitivity analysis of equilibrium constants for ion binding to neutral binding sites ($P^0$) on the CM surface indicated the relative insensitivity to changes in $K_{P,I}$.

Based on the electrophoresis method (ζ potential measurement), $R_T$ was estimated at 0.0433 μmol $m^{-2}$ ($\sigma_0 = -3.99$ mC $m^{-2}$), which is in the mid range of values estimated from ζ potentials of plant CMs ($\sigma_0 = -2$ to $-8$ mC $m^{-2}$; Kinraide and Wang (2010)). Kinraide and Wang (2010), in a dedicated study of $\sigma_0$, noted that $\sigma_0$ values estimated by ζ potentials are generally ca. 6 fold lower than those estimated by five other methods. The two classes of methods (electrophoresis and all others) yield results that are highly correlated and proportional, and these authors concluded that the smaller magnitude values of the electrophoresis method were peculiar to that technique and that a value of $\sigma_0 = -30$ mC $m^{-2}$ is suitable for plant CMs irrespective of taxon or tissue type. Because the ζ potentials for bacterial CMs and plant CMs are similar, the value $R_T = 0.3074$ μmol $m^{-2}$ ($= -30$ mC $m^{-2}$) was assigned to bacteria for the computation of $\psi_0$. Butt (1992) measured a value of $\sigma_0 = -50$ mC $m^{-2}$ (=0.5182 μmol $m^{-2}$) for $\sigma$ of CMs isolated from *Halobacterium halobium* with a scanning atomic force microscope. Additionally, a single value for total neutral binding sites $P_T$ (=2.4 μmol $m^{-2}$) was assigned on the basis of the number of phosphatidic acids per $m^2$ in a typical CM (Kinraide et al., 1998). For the regression of ζ potentials *vs.* computed $\psi_0$ with the GCS model using the assigned parameters above (Column 2 in Table 3), $R^2_{adj} = 0.961$ (Fig. 3).

### 3.3. Soil solution properties and $\psi_0$

The addition of metal salts to soils resulted in an increase in the Cu or Ni concentration in the soil solution, with concomitant increases in other major cations, especially Ca and Mg, and decreases in soil solution pH. Consequently, the osmolarity of the soil solution also increased upon the addition of metal salts. In the Barcelona (Spain) soil, for instance, addition of $CuCl_2$ from 0 to 2400 mg $kg^{-1}$ resulted in substantial increases in the dissolved Cu (from 1.10 to 77.9 μM), Ca (from 7.1 to 131 mM) and Mg (from 1.6 to 25.1 mM) and decreases of soil solution pH from 7.66 to 5.96. Correspondingly, the calculated osmolarity was increased from 28.4 to 418.7 mOsM.

The values calculated for bacterial $\psi_0$ based upon soil solutions from unamended soils varied from −40.1 to +26.4 mV due to large variations in pH and cation concentrations, specifically Ca and Mg. The lowest (most negative) $\psi_0$ occurred in Aluminusa (Italy) soil due to its high pH (6.84) and low Ca and Mg. The highest $\psi_0$ occurred at low pH, for instance the Gudow (Germany) soil has the highest value due to its very low pH (3.34). The negativity of $\psi_0$ decreased markedly as metal salts were added due to increases in dissolved concentrations of $Cu^{2+}$ or $Ni^{2+}$ and increases of $Ca^{2+}$ and $Mg^{2+}$ concentrations resulting from desorption from the solid soil matrix. In the Aluminusa soil, for example, addition of 688 mg Ni $kg^{-1}$ increased $\{Ni^{2+}\}_b$ to 1.41 mM which (together with higher solution concentrations of Ca, Mg, and H) caused an increase of $\psi_0$ from −40.1 mV to +2.14 mV. In turn, this resulted in the $Ni^{2+}$ enrichment factor ($\{Ni^{2+}\}_0/\{Ni^{2+}\}_b$) at the CM surface decreasing from 22.6 to 0.85.

### 3.4. Dose–response relationships

Microbial processes in metal-amended soils with a wide range of pH values were considered to be influenced by at least four factors. Firstly, $Cu^{2+}$ and $Ni^{2+}$ are highly toxic and inhibit microbial processes. Secondly, microbial processes may be reduced by osmotic effects, with osmolarity increasing to 621.7 mOsM in some treatments. Thirdly, for alkaline soils, an increased $H^+$ may also

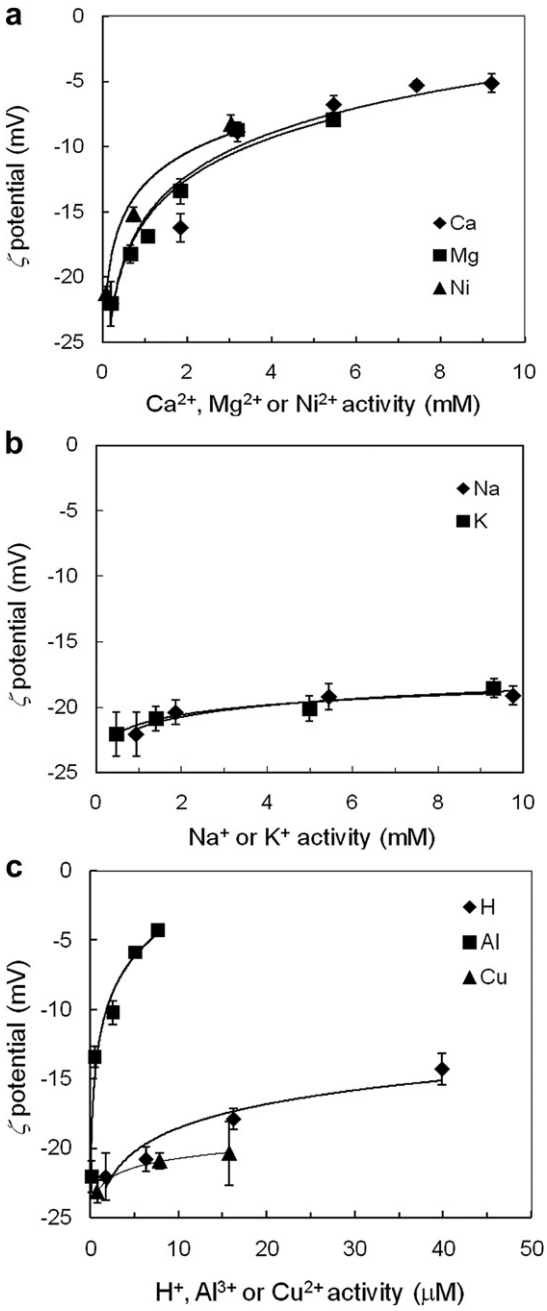


**Fig. 1.** Zeta (ζ) potentials of *E. coli* protoplasts as a function of the activity of (a) $Ca^{2+}$, $Mg^{2+}$ or $Ni^{2+}$, (b) $Na^{+}$ or $K^{+}$, or (c) activity of $H^{+}$, $Al^{3+}$ or $Cu^{2+}$ in the bacterial bathing medium. Note change from mM to μM in (c). Vertical bars represent the standard deviations.

exert an ameliorative effect on metal toxicity. Fourthly, reductions in the negativity of $\psi_0$ increase the negativity of the surface-to-surface potential difference ($E_{m,surf}$). With regard to osmotic effects, many investigators have reported that the nitrification potential decreased with increasing salt concentration. Rysgaard et al. (1999) showed that 1% salinity in sediment caused a reduction of approximately 50% in nitrification. It is therefore likely that additions of large amounts of soluble metal salts could cause a toxic effect through salinity (osmotic stress).

These factors were apparent in the current study, and can be demonstrated by using Cu as an example. Firstly, PNR was related negatively to $\{Cu^{2+}\}_0$ (PNR, % = $100/\exp[(0.107\{Cu^{2+}\}_0)^{0.47}]$, $n = 112$;

**Table 3**
Parameter values for a Gouy–Chapman–Stern model for cell membranes.

| Model parameters | ζ potential-derived model | Model for bacteria[a] | Standard model for plants[b] | General binding strength[c] |
|---|---|---|---|---|
| $R_T$ μmol $m^{-2}$ | 0.0433 | 0.3074 | 0.3074 | |
| $P_T$ μmol $m^{-2}$ | 2.4 | 2.4 | 2.4 | |
| $K_{R,K}$ $M^{-1}$ | 0 | 1 | 1 | 0.9 |
| $K_{R,Na}$ | 0 | 1 | 1 | 1.0 |
| $K_{R,Ca}$ | 31.3 | 31.3 | 30 | 27.5 |
| $K_{R,Mg}$ | 31.3 | 31.3 | 30 | 29.0 |
| $K_{R,Cu}$ | 1070 | 1070 | 400 | 697 |
| $K_{R,Ni}$ | 52.9 | 52.9 | | 192 |
| $K_{R,Al}$ | 15,550 | 15,550 | 20,000 | 14,800 |
| $K_{R,H}$ | 12,300 | 12,300 | 21,500 | |
| $K_{P,I}$ | $K_{R,I}/3455$[d] | $K_{R,I}/3455$[d] | $K_{R,I}/180$[d] | |

[a] Model parameters estimated for calculating $\psi_0$ of bacterial CMs in this study.
[b] The binding constants for ions were computed on the basis of measured ion adsorption to CM vesicles of wheat root cells (Yermiyahu et al., 1997; Vulcan et al., 2004).
[c] Based upon a scale presented in Kinraide (2009) for ion binding to hard ligands. That scale may be converted to CM binding constants by the formula $K_{R,I} = 10^{(3\ +\ 1.75(\text{HL Scale}))}$.
[d] These ratios refer to cations.

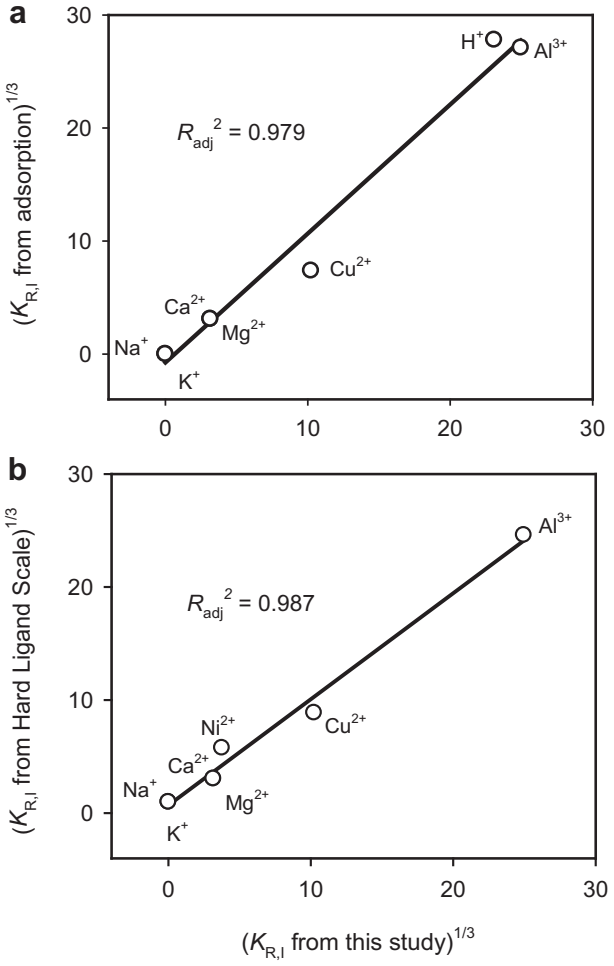


**Fig. 2.** Comparison of equilibrium constants for ion binding to negative sites on the CM surfaces of *E. coli* determined by electrophoresis in this study and the binding constants determined by ion adsorption and relative binding strength to hard ligands (see Table 3). Cube roots of the constants were taken to compress the scales.

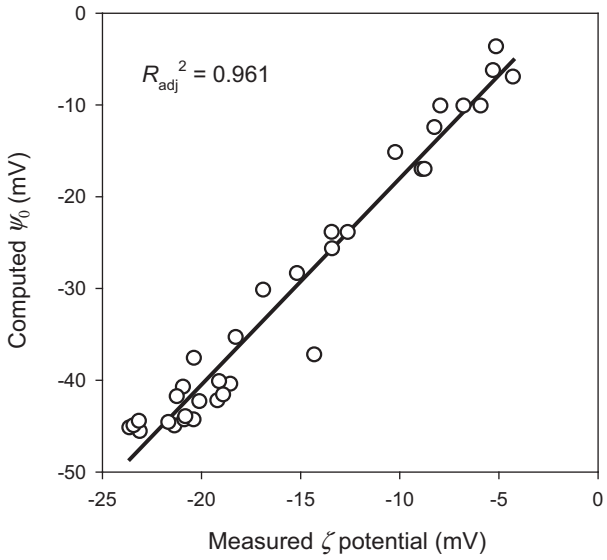


**Fig. 3.** Measured ζ potentials plotted against computed $\psi_0$. $\psi_0$ are the bacterial CM surface potentials computed with a GCS model with parameters optimized for best correspondence between ζ potentials and $\psi_s$ (second number column Table 3).

$R^2_{adj} = 0.592$, RMSE = 25.2, $p < 0.001$), demonstrating the direct toxic effect of $Cu^{2+}$. Secondly, the $R^2_{adj}$ value improved substantially when a term for osmolarity was included in the equation (PNR, % = $100/\exp[(0.100\{Cu^{2+}\}_0)^{1.05} + (0.0051\text{Osmolarity})^{2.24}]$; $R^2_{adj} = 0.789$, RMSE = 18.5, $p < 0.001$). Thirdly, incorporating the secondary effect of $\psi_0$ (*i.e.*, the effect of $\psi_0$ on the driving force for ion transport across the CM) improved $R^2_{adj}$ further (PNR, % = $100/\exp\{[0.106\ (1 + 0.033\psi_0)\ \{Cu^{2+}\}_0]^{0.90} + (0.0050\text{Osmolarity})^{2.33}\}$; $R^2_{adj} = 0.804$, RMSE = 17.7, $p < 0.001$). Finally, in order to test the effect of $\{H^+\}_0$, the equation was expanded as in Eq. (5), and a significant coefficient was obtained for the effect of $\{H^+\}_0$; the $R^2_{adj}$ value increased to 0.816, and RMSE = 17.2 (Table 4). Similar results were obtained for the toxic effect of Cu on GIR and those of Ni on PNR and GIR (Table 4). On average, the $R^2_{adj}$ value increased by 0.150 when the osmotic effect was included. From the changes in $R^2_{adj}$ value, it appears that the surface activities of metal ions and osmolarity play important roles in influencing microbial processes. Soil pH influences metal toxicity in at least two different ways by considering the changes in surface activities of metal ions which have a pH dependency in terms of $\psi_0$. Firstly, $H^+$ can depolarize the CM and hence decrease the negativity of $\psi_0$, thereby decreasing the attraction of metal ions to the CM surface. On the other hand, $H^+$ exerts an intrinsic (specific) amelioration by reducing "$a_1$" with increasing $\{H^+\}_0$ (see Eq. (5)), but this effect is minor compared to other effects. This specific effect of $H^+$ may reflect a transfer of community utilization (*e.g.* fungus dominance at lower pH) and the extrapolation of model dealing with different communities (*i.e.*, bacteria and fungus) needs further investigation. Finally, it is noteworthy that the value for $R^2_{adj}$ improves with increasing number of adjustable parameters.

The role of osmotic effects on toxicity, as statistically confirmed above, is also confirmed by the observation that metal toxicity decreases in spiked soils when spiked soils are leached (Oorts et al., 2007; Stevens et al., 2003). However, caution must be exerted since metal contamination in the freshly spiked soils is correlated with osmolarity. Soil solution data of three Ni- and Cu-spiked soils that were leached and aged (Oorts et al., 2006a, 2007) were analyzed along with the changes in toxicity. Leaching and aging reduced the increase in osmolarity, but the metal contamination remained. For example, in the Jyndevad soil, solution osmolarity increased from 20.0 to 322.1 mM in freshly spiked soils dosed up to 690 mg $kg^{-1}$ $NiCl_2$ whereas 15 months outdoor aging of the same set of soils with free drainage yielded a solution osmolarity ranging only 4.3–23.6 mM, yet Ni toxicity was still detectable. Eq. (5) was fitted to these data (three soils and five treatments: spiked, leached, and aged 5, 10 or 15 months for Ni-spiked soils and aged 6, 12, or 18 months for Cu-spiked soils) – all yielded significant values similar to those reported for freshly spiked soils (Table 4). The high $R^2_{adj}$ values indicate that the four influencing factors listed above are still relevant under field conditions (*i.e.*, leached and aged soils). In addition, the $R^2_{adj}$ values increased for all data by including the osmotic effect, the increment ranging between 0.056 and 0.22 (average of 0.14) depending on metal and assay (details not shown). Such analyses, hence, underpin the role of osmotic stress.

To examine the ability of the various models to predict metal toxicity, PNR and GIR were fitted with the equation BR = $100/\exp[(aT)^\beta]$, with toxicant intensity ($T$) variously expressed and incorporated into various models. Thus $T$ may be expressed as (i) $[M]_{soil}$, (ii) $\{M^{2+}\}_b$ (incorporated into FIAM), (iii) the fraction ($f_{TBLM}$) of the total biotic ligand sites bound by $M^{2+}$ (incorporated into the TBLM; Thakali et al., 2006b), or (iv) the term of $T_{ETM}$ from Eq. (5) (incorporated into ETM). Generally, in the case of Cu toxicity, the correlation between PNR and the term $T_{ETM}$ (Fig. 4d) is superior to the other correlations (Fig. 4a–c). The Cu toxicity to GIR follows a similar trend for correlation with an exception that GIR was related more closely with $[Cu]_{soil}$ (Fig. 4e) than with $\{Cu^{2+}\}_b$ (Fig. 4f). For the Ni toxicity to PNR and GIR, similar results were observed (Fig. 5).

Table 4 provides information on the differences in sensitivity. Firstly, the toxic strength coefficient '$a_1$' for PNR (0.0075 for Ni and 0.110 for Cu) is larger than that for GIR (0.00083 for Ni and 0.00075 for Cu), indicating that $\{M^{2+}\}_0$ is substantially less toxic to GIR than to PNR. The data also indicate that both metals appear to have similar toxicities to GIR with their similar values for $a_1$ (0.00083 and 0.00075). Secondly, the coefficients for the secondary effect of

**Table 4**
Summary of dose–response parameters for the electrostatic toxicity model (Eq. (5); BR, % = $100/\exp\{[a_1(1 + a_{11}\psi_0)(1 + a_{12}/\{H^+\}_0)\{M^{2+}\}_0]^{\beta_1} + (a_2\text{Osmolarity})^{\beta_2}\}$). The values in parentheses are the standard errors. $\{M^{2+}\}_0$ and $\{H^+\}_0$ refer ion surface activity (μM) of metal and $H^+$, respectively. Osmolarity is expressed in mOsM.

| Endpoint | Metal | $a_1$ | $a_{11}$ | $a_{12}$ | $\beta_1$ | $a_2$ | $\beta_2$ | $R^2_{adj}$ | RMSE |
|---|---|---|---|---|---|---|---|---|---|
| *Freshly spiked soils* | | | | | | | | | |
| PNR | Ni | 0.0075 (0.0008) | 0.044 (0.004) | 0.039 (0.010) | 0.72 (0.05) | 0.0069 (0.0008) | 3.44 (0.96) | 0.961 | 8.35 |
| PNR | Cu | 0.110 (0.018) | 0.036 (0.009) | 0.40 (0.17) | 0.82 (0.11) | 0.0047 (0.0005) | 2.81 (0.75) | 0.816 | 17.2 |
| GIR | Ni | 0.00083 (0.00002) | 0.052 (0.003) | 0.21 (0.06) | 0.55 (0.05) | 0.0036 (0.0008) | 2.43 (0.84) | 0.883 | 12.1 |
| GIR | Cu | 0.00075 (0.00003) | 0.054 (0.002) | 110.1 (40.5) | 0.49 (0.05) | 0.0035 (0.0003) | 2.15 (0.43) | 0.861 | 12.0 |
| *Freshly spiked, leached, and aged soils* (data from Oorts et al., 2006a, 2007) | | | | | | | | | |
| PNR | Ni | 0.0045 (0.0007) | 0.033 (0.011) | 0.353 (0.122) | 0.65 (0.07) | 0.0090 (0.0011) | 2.61 (0.99) | 0.855 | 14.2 |
| PNR | Cu | 0.186 (0.033) | 0.030 (0.011) | 1.10 (0.30) | 0.97 (0.16) | 0.0060 (0.0010) | 2.35 (0.87) | 0.776 | 17.9 |
| GIR | Ni | 0.00056 (0.00002) | 0.035 (0.006) | 0.37 (0.16) | 0.48 (0.10) | 0.0040 (0.0010) | 2.37 (0.84) | 0.690 | 19.2 |
| GIR | Cu | 0.00064 (0.00017) | 0.020 (0.007) | 16.54 (6.28) | 0.47 (0.06) | 0.0031 (0.0006) | 2.15 (0.97) | 0.729 | 18.4 |

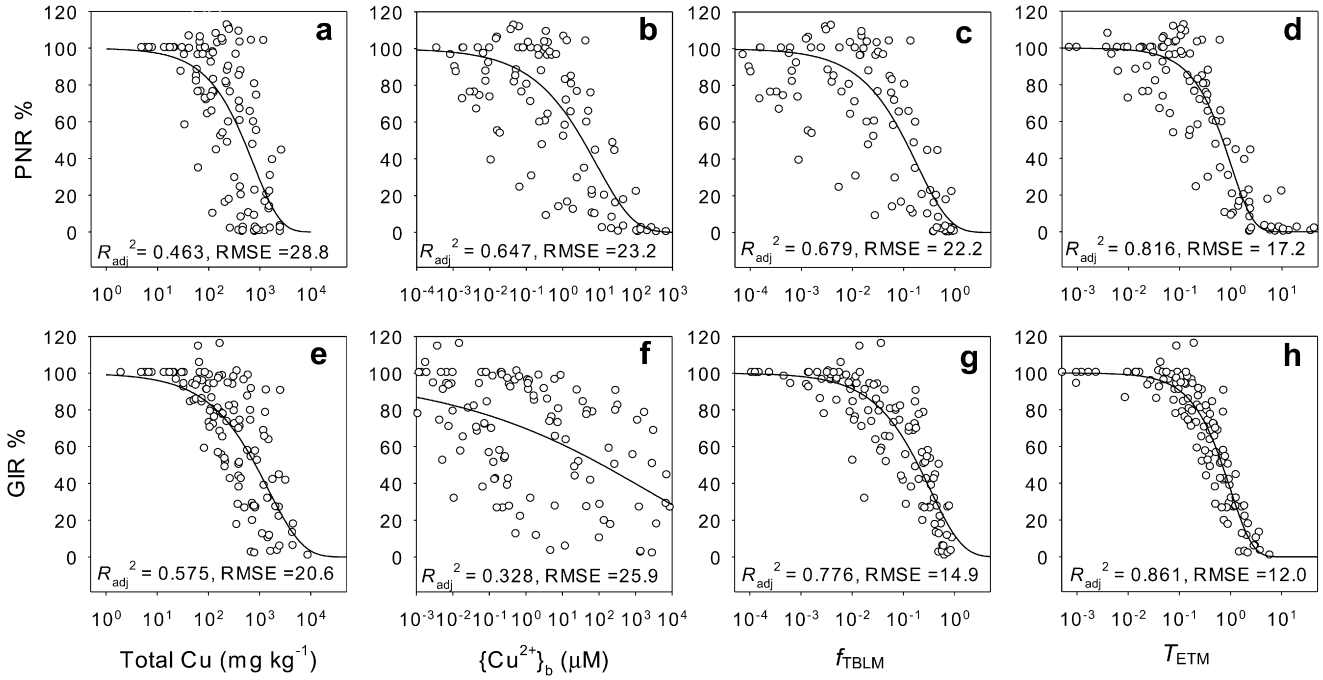


**Fig. 4.** Cu toxicity to PNR (a–d) and GIR (e–h) as a function of total Cu concentration in soils, free $Cu^{2+}$ activity ($\{Cu^{2+}\}_b$) in soil solution, the fraction of total biotic ligand bound by $Cu^{2+}$ based on the TBLM ($f_{TBLM}$), and toxicity based on the ETM ($T_{ETM}$). $T_{ETM}$ combines the surface ion activity, interactions between $H^+$ and $Cu^{2+}$, and osmolarity. Refer to Eq. (5) and Table 2.

$\psi_0$ (electrical driving force for ion transport across the CM) for PNR of the two metals are similar ($a_{11} = 0.036–0.044$), but are slightly greater ($a_{11} = 0.052–0.054$) for GIR, suggesting that the two metals affect the values similarly, and that the secondary effect exerts more impact on metal toxicity to GIR than to PNR. Thirdly, $a_2$ and $\beta_2$ reflect the differences in sensitivity to osmolarity. The coefficients for PNR are larger than for GIR, indicating that PNR is slightly more sensitive to osmotic stress. Based on the coefficients (Table 4), critical values for osmolarity corresponding to a 50% reduction in microbial processes were calculated as ca. 203 mOsM

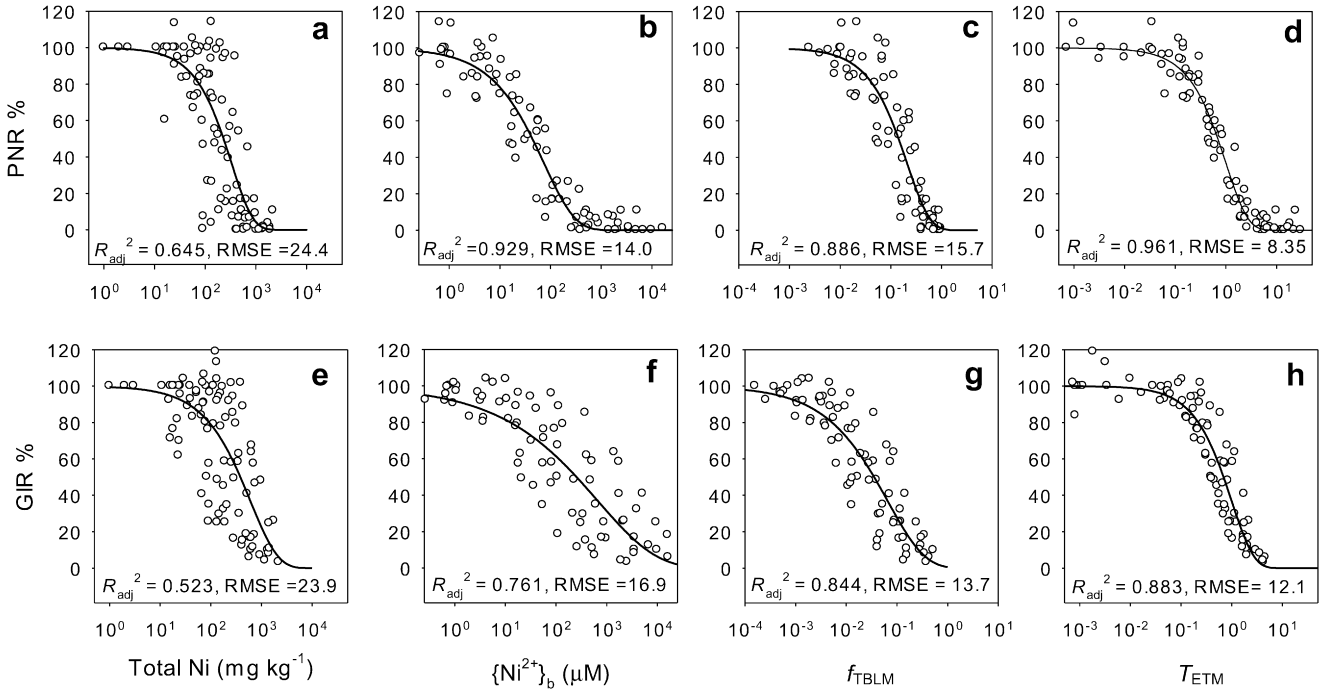


**Fig. 5.** Ni toxicity to PNR (a–d) and GIR (e–h) as a function of total Ni concentration in soils, free $Ni^{2+}$ activity ($\{Ni^{2+}\}_b$) in soil solution, the fraction of total biotic ligand bound by $Ni^{2+}$ based on the TBLM ($f_{TBLM}$), and the toxicity based on the ETM ($T_{ETM}$). $T_{ETM}$ combines the surface ion activity, interactions between $H^+$ and $Ni^{2+}$, and osmolarity. Refer to Eq. (5) and Table 2.

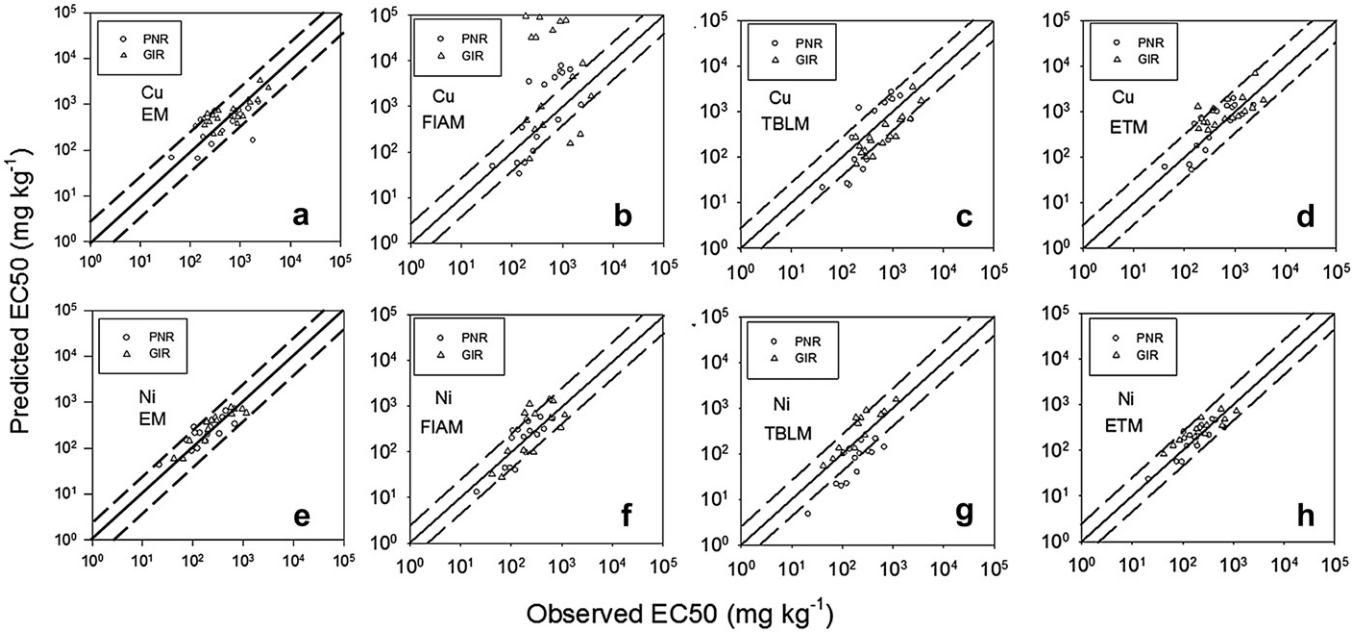


**Fig. 6.** Predicted vs. observed EC50 soil total concentrations of Cu (a–d) and Ni (e–h) for PNR and GIR in soils. The solid lines represent 1:1 ratio and the dashed lines represent a factor 2.5 above and below the 1:1 lines. The predictions are based on the empirical model (EM, reported by Oorts et al., 2006b) (a and e), FIAM (b and f), the TBLM (c and g), and the ETM (d and h).

for PNR and 257 mOsM for GIR. The values are lower than the value of ca. 264 mOsM (142 mM NaCl) for nitrification reported by Rysgaard et al. (1999). Finally, the coefficients of $a_{12}$ for Cu toxicity are much larger than those for Ni, suggesting that changes in $\{H^+\}_0$ are of more importance for Cu toxicity than for Ni; this difference in sensitivity to $\{H^+\}_0$ reflects either an intrinsic effect of $\{H^+\}_0$ or the formation of metal toxic species (for which $\{H^+\}_0$ is acting as a surrogate).

### 3.5. Predictions of EC50s

The ETM (Eq. (5)) can be used to predict free metal ion activities in soil solution that cause inhibition on microbial process. To accomplish this, data concerning soil solution properties can be compiled for which the biological response is constant (*e.g.* 50% inhibition). To compute $\{M^{2+}\}_{b(50)}$ (also denoted EA50$\{M^{2+}\}_b$), set BR (PNR or GIR) = 50 in Eq. (5). Next set $\{M^{2+}\}_0 = \{M^{2+}\}_b \exp(-2\psi_0/25.7)$ and $\{H^+\}_0 = \{H^+\}_b \exp(-\psi_0/25.7)$. Next, rearrange Eq. (5) to obtain Eq. (6).

$$\left\{M^{2+}\right\}_{b(50)} = \exp(2\psi_0/25.7)\left[\ln 2 - (a_2\,\text{Osmolarity})^{\beta 2}\right]^{(1/\beta 1)} / \left\{a_1(1+b\psi_0)\left[1+a_{12}/\left\{H^+\right\}_b \exp(-\psi_0/25.7)\right]\right\} \quad (6)$$

Using the solution concentrations of ions interpolated at EC50 $[M]_{soil}$ (Supplemental Tables S2–S5), the $\psi_0$ was calculated initially with the GCS model using parameters in Table 3 (second number column), and then the $\{M^{2+}\}_{b(50)}$ can be predicted with Eq. (6). In addition, this predicted $\{M^{2+}\}_{b(50)}$ can subsequently be used to estimate total soil metal concentration yielding the 50% inhibition. The free $Cu^{2+}$ and $Ni^{2+}$ activities in the bulk soil solution can be computed by multivariate linear regression equations (Eq. (7) in Supplemental Material) from bulk soil properties such as pH, total metal content, OC, and CEC (Lofts et al., 2004). Eq. (6) can then be combined with Eq. (7) to compute the EC50$[M]_{soil}$ (mg metal $kg^{-1}$ soil) with the specific soil properties (OC, CEC, solution pH). Similarly the FIAM- and TBLM- based EC50$[M]_{soil}$ were also computed using the FIAM- and TBLM- based EC50$\{M^{2+}\}_b$.

A comparison of the predicted EC50$[M]_{soil}$ for PNR and GIR to the observed values shows marked differences for the three models (Fig. 6). Interestingly, the empirical models (EM) which relate toxicity thresholds to soil properties (Oorts et al., 2006b) predicted EC50$[M]_{soil}$ well (Fig. 6a,e). These models only require 2 adjustable parameters and one soil variable (*e.g.* the soil CEC, organic carbon, or clay content, Oorts et al., 2006b) which is well below that for the FIAM, t-BLM or ETM (2–6 parameters and 1–10 soil variables). Thirty out of 34 values for Cu toxicity and 26 out of 27 values for Ni toxicity are within a factor of 2.5 of the observed values with the empirical model. Given the Eqs. (6) (electrostatic model) and 7 (speciation model), it is no surprising that the toxicity thresholds are related to soil properties. The drawback of the empirical models is that the leaching and aging effect cannot be explained, in contrast with the ETM. The FIAM predictions were based on Eq. (7) (Supplemental Material) using constant EC50 $\{Cu^{2+}\}_b$ (3.12 μM for PNR and 101.6 μM for GIR) and EC50$\{Ni^{2+}\}_b$ (47.8 μM for PNR and 204.3 μM for GIR) for all soils irrespective of their properties. These predictions (Fig. 6b,f) correlated poorly with the observed values for Cu but are well correlated for Ni. By accounting for the "competitive" effects of coexistent cations in solution, the TBLM predictions (Fig. 6c,g) correlate better than the FIAM with the observed values, except for Ni toxicity to PNR. The ETM accounts for the dual effects of $\psi_0$, the specific ameliorative effect by surface $H^+$ activity, and the osmotic effects. The ETM predictions (Fig. 6d,h) achieved the best relationship between the predicted and observed EC50$[M]_{soil}$. The majority of the ETM-predicted EC50$[M]_{soil}$ values are within a factor of 2.5 of the observed values (31 out of 34 values for Cu toxicity, Fig. 6d; 25 out of 27 values for Ni toxicity, Fig. 6h). Additionally, all the predicted EC50$[M]_{soil}$ values are within a factor of 3 of the observed values except for one value for Cu toxicity to GIR (Marknesse soil). It is remarkable that the ETM approach is able to predict EC50 generally within a factor of 2.5 for the two metals (Cu and Ni) and the two microbial processes (PNR and GIR) in such diverse soils. It

should be noted that the TBLM that Thakali et al. (2006b) developed do not consider the osmotic effects and did not include calcareous soils. Additionally, caution should be taken in comparing model performances because of the different number of adjustable parameters used, *i.e.*, the model explaining most of the variation here was also that model using the largest number of adjustable parameters (Table 2).

### 3.6. Some uncertainties

For protoplasts or vesicles of plant CMs, the GCS model has been largely confirmed, that is, the model accurately estimates values for $\psi_0$ that are at least proportional to $\zeta$ potentials (Kinraide et al., 1998; Kinraide, 2001; Kinraide and Wang, 2010; Wang et al., 2008; Yermiyahu et al., 1997). The model is robust for parameters relating to ion binding strength at the CM surface in light of several lines of evidence, including $\zeta$ potential measurements, adsorption measurements (Yermiyahu and Kinraide, 2005), and the relative strength of ion binding to hard ligands (Kinraide, 2009; Kinraide and Wang, 2010). An uncertainty exists for another critical parameter, the surface density of negative charges of bacteria CMs. A dedicated study established $R_T = 0.3074$ μmol $m^{-2}$ as an appropriate value for plants, and based upon agreement between $\zeta$ potentials for both plant and bacterial protoplasts, we assigned the same value to bacterial $R_T$. An error in this value means that the calculated $\psi_0$ and ion surface activities may be only proportional to the actual values. However, sensitivity analysis demonstrates that a factor of two in the variation of $R_T$ (0.5 $R_T$ and 2 $R_T$) does not substantially reduce the values for RMSE and the $R^2_{adj}$. Another uncertainty that remains is that the GCS model assumes that microbial communities are exposed to soil solution. Soil microorganisms are usually strongly attached to soil particles, implying that the local composition of the bacterial bathing solution may differ from the pore water (Allison and Prosser, 1993). Finally, it should be noted that the changes in microbial processes (*i.e.*, PNR and GIR) may be may be related to altered soil microbial communities rather than toxic effects. Despite these uncertainties, the present study suggests the importance of electrostatic and osmotic effects, given that they significantly enhance the power to predict the toxicity of Cu and Ni to PNR and GIR in soils with wide ranging properties and including the effects of leaching and aging.

## 4. Conclusions

This study set out to evaluate the factors that influence Cu and Ni toxicity to microbial processes (PNR and GIR) in spiked soils with a wide range of properties based on the surface electrical potential of bacterial CMs. This approach produced a model (Eq. (5)) that related PNR and GIR to (i) metal ion CM surface activities, (ii) the electrical driving force for cation uptake across CMs, (iii) the specific ameliorative effect of $H^+$, and (iv) osmotic stress. The negativity of $\psi_0$ of bacterial CM surfaces decreased markedly as metal salts were added due to increases in $Cu^{2+}$ or $Ni^{2+}$ concentrations in solution and to increases in concentrations of $Ca^{2+}$, $Mg^{2+}$, and $H^+$ that result from desorption of these cations from the solid soil matrix. The reduced $\psi_0$ negativity affects the metal toxicity by (i) decreasing the enrichment factor ($\{M^{2+}\}_0/\{M^{2+}\}_b$) at the CM surfaces, and (ii) increasing the electrical driving force of $M^{2+}$ transport into cells. This study also suggests that the ETM provides a robust mechanistic framework to assess metal ecotoxicity and predicts critical metal concentrations linked to microbial effects.

## Acknowledgments

This work was supported financially by the National Natural Science Foundation of China (31172034) and the Knowledge Innovative Project of Chinese Academy of Sciences (KZCX2-YW-Q02-02). Statement of Equal Opportunity and Product Disclaimer: The USDA is an equal opportunity provider and employer. Mention of trade names or commercial products does not imply recommendation or endorsement by the USDA.

## Appendix A. Supplementary material

Supplementary materials related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2012.09.002.

## References

Allison, S.M., Prosser, J.I., 1993. Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biology & Biochemistry 25, 935–941.
Baygents, J.C., Glynn, J.R., Albinger, O., Biesemeyer, B.K., Ogden, K.L., Arnold, R.G., 1998. Variation of surface charge density in monoclonal bacterial populations: implications for transport through porous media. Environmental Science & Technology 32, 1596–1603.
Boonaert, C.J.P., Rouxhet, P.G., 2000. Surface of lactic acid bacteria: relationships between chemical composition and physicochemical properties. Applied and Environmental Microbiology 66, 2548–2554.
Buekers, J., Mertens, J., Smolders, E., 2010. Toxicity of the molybdate anion in soil is partially explained by effects of the accompanying cation or by soil pH. Environmental Toxicology and Chemistry 29, 1274–1278.
Bushby, H.V.A., 1990. The role of bacterial surface charge in the ecology of root nodule bacteria – an hypothesis. Soil Biology & Biochemistry 22, 1–9.
Butt, H.J., 1992. Measuring local surface charge densities in electrolyte solutions with a scanning force microscope. Biophysical Journal 63, 578–582.
Giller, K.E., Witter, E., McGrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biology & Biochemistry 30, 1389–1414.
Giller, K.E., Witter, E., McGrath, S.P., 2009. Heavy metals and soil microbes. Soil Biology & Biochemistry 41, 2031–2037.
Hille, B., 2001. Ion Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA.
Ishii, S., Ksoll, W.B., Hicks, R.E., Sadowshy, M.J., 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Applied and Environmental Microbiology 72, 612–621.
Jozefaciuk, G., Szatanik-Kloc, A., 2004. Decrease in variable charge and acidity of root surface under Al treatment are correlated with Al tolerance of cereal plants. Plant and Soil 260, 137–145.
Kinraide, T.B., 2001. Ion fluxes considered in terms of membrane-surface electrical potentials. Australian Journal of Plant Physiology 18, 605–616.
Kinraide, T.B., 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, T.B., 2009. Improved scales for metal ion softness and toxicity. Environmental Toxicology and Chemistry 28, 525–533.
Kinraide, T.B., Sweeney, B.K., 2003. Proton alleviation of growth inhibition by toxic metals (Al, La, Cu) in rhizobia. Soil Biology & Biochemistry 35, 199–205.
Kinraide, T.B., Wang, P., 2010. The surface charge density of plant cell membranes ($\sigma$): an attempt to resolve conflicting values for intrinsic $\sigma$. Journal of Experimental Botany 61, 2507–2518.
Kinraide, T.B., 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, P.M., Blamey, F.P.C., Kinraide, T.B., Wang, P., Reichman, S.M., Menzies, N.W., 2011. Separating multiple, short-term deleterious effects of saline solutions to the growth of cowpea seedlings. New Phytologist 189, 1110–1121.
Li, J., Huang, G., 2008. Pilot study of salinity (NaCl) affecting nitrogen transformation in silt loam soil. Research of Environmental Sciences 21, 98–103.
Lighthart, B., Baham, J., Volk, V.V., 1983. Microbial respiration and chemical speciation in metal–amended soils. Journal of Environmental Quality 12, 543–548.
Lofts, S., Spurgeon, D.J., Svendsen, C., Tipping, E., 2004. Deriving soil critical limits for Cu, Zn, Cd, and Pb: a method based on free ion concentrations. Environmental Science & Technology 38, 3623–3631.
Mertens, J., Wakelin, S.A., Broos, K., McLaughlin, M.J., Smolders, E., 2010. Extent of copper tolerance and consequences for functional stability of the ammonia-oxidizing community in long-term copper-contaminated soils. Environmental Toxicology and Chemistry 29, 27–37.
Morisaki, H., Nagai, S., Ohshima, H., Ikemoto, E., Kogure, K., 1999. The effect of motility and cell-surface polymers on bacterial attachment. Microbiology 145, 2797–2802.

Oorts, K., Bronckaers, H., Smolders, E., 2006a. Discrepancy of the microbial response to elevated copper between freshly spiked and long-term contaminated soils. Environmental Toxicology and Chemistry 25, 845–853.

Oorts, K., Ghesquiere, U., Swinnen, K., Smolders, E., 2006b. Soil properties affecting the toxicity of $CuCl_2$ and $NiCl_2$ for soil microbial processes in freshly spiked soils. Environmental Toxicology and Chemistry 25, 836–844.

Oorts, K., Ghesquiere, U., Smolders, E., 2007. Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. Environmental Toxicology and Chemistry 26, 1130–1138.

Ponizovsky, A.A., Thakali, S., Allen, H.E., Di Toro, D.M., Ackerman, A.J., 2006. Effect of soil properties on copper release in soil solutions at low moisture content. Environmental Toxicology and Chemistry 25, 671–682.

Robinson, R.A., Stokes, R.H., 2002. Electrolyte Solutions. Dover Publications, Minerola, NY, USA.

Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P.B., Sloth, N.P., 1999. Effects of salinity on $NH_4^+$ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 22, 21–30.

Shomer, I., Novacky, A.J., Pike, S.M., Yermiyahu, U., Kinraide, T.B., 2003. Electrical potentials of plant cell walls in response to the ionic environment. Plant Physiology 133, 411–422.

Smolders, E., Brans, K., Coppens, F., Merckx, R., 2001. Potential nitrification rate as a tool for screening toxicity in metal-contaminated soils. Environmental Toxicology and Chemistry 20, 2469–2474.

Smolders, E., Buekers, J., Oliver, I., McLaughlin, M.J., 2004. Soil properties affecting toxicity of zinc to soil microbial properties in laboratory-spiked and field-contaminated soils. Environmental Toxicology and Chemistry 23, 2633–2640.

Stevens, D.P., McLaughlin, M.J., 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.

Soni, K.A., Balasubramanian, A.K., Beskok, A., Pillai, S.D., 2008. Zeta potential of selected bacteria in drinking water when dead, starved, or exposed to minimal and rich culture media. Current Microbiology 56, 93–97.

Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., 2006a. 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.

Thakali, S., Allen, H.E., Di Toro, D.M., Ponizovsky, A.A., Rooney, C.P., Zhao, F.J., McGrath, S.P., Criel, P., Van Eeckhout, H., Janssen, C.R., Oorts, K., Smolders, E., 2006b. Terrestrial biotic ligand model. 2. Application to Ni and Cu toxicities to plants, invertebrates, and microbes in soil. Environmental Science & Technology 40, 7094–7100.

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M.R., Lofts, S., Hill, M.T.R., Farago, M.E., Thornton, I., 2003. The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. Environmental Pollution 125, 213–225.

Tye, A.M., Young, S., Crout, N.M.J., Zhang, H., Preston, S., Zhao, F.J., McGrath, S.P., 2004. Speciation and solubility of Cu, Ni and Pb in contaminated soils. European Journal of Soil Science 55, 579–682.

Van der mei, H.C., Devries, J., Busscher, H.J., 1993. Hydrophobic and electrostatic cell surface properties of thermophilic dairy streptococci. Applied and Environmental Microbiology 59, 4305–4312.

Vulcan, R., Yermiyahu, U., Mingelgrin, U., Rytwo, G., Kinraide, T.B., 2004. Sorption of copper and zinc to the plasma membrane of wheat root. Journal of Membrane Biology 202, 97–104.

Vulkan, R., Zhao, F.J., Barbosa-Jefferson, V., Preston, S., Paton, G.I., Tipping, E., McGrath, S.P., 2000. Copper speciation and impacts on bacterial biosensors in the pore water of copper-contaminated soils. Environmental Science & Technology 34, 5115–5121.

Wagatsuma, T., Akiba, R., 1989. Low surface negativity of root protoplasts from aluminum tolerant plant species. Soil Science and Plant Nutrition 35, 443–452.

Wang, P., Kinraide, T.B., Zhou, D.M., Kopittke, P.M., Peijnenburg, W.J.G.M., 2011. Cell membrane surface potential: dual effects upon ion uptake and toxicity. Plant Physiology 155, 808–820.

Wang, P., Zhou, D.M., Kinraide, T.B., Luo, X.S., Li, L.Z., Li, D.D., Zhang, H.L., 2008. Cell membrane surface potential ($\psi_0$) plays a dominant role in the phytotoxicity of copper and arsenate. Plant Physiology 148, 2134–2143.

Wolt, J., 1994. Soil Solution Chemistry: Applications to Environmental Science and Agriculture. Wiley, NY, USA, pp. 1–345.

Yermiyahu, U., Kinraide, T.B., 2005. Binding and electrostatic attraction of trace elements to plant root surfaces. In: Huang, P.M., Gobran, G.R. (Eds.), Biogeochemistry of Trace Elements in the Rhizosphere. Elsevier, Amsterdam, Netherlands, pp. 365–389.

Yermiyahu, U., Brauer, D.K., Kinraide, T.B., 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.

Yermiyahu, U., Nir, S., Ben-Hayyim, G., Kafkafi, U., Scherer, G.F.E., Kinraide, T.B., 1999. Surface properties of plasma membrane vesicles isolated from melon (*Cucumus melo* L.) root cells differing in salinity tolerance. Colloids and Surfaces B: Biointerfaces 14, 237–249.