Running Title: Dual Effects of Cell Membrane Surface Potential

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Research Category: Environmental Stress and Adaptation
Plasma Membrane Surface Potential: Dual Effects upon Ion Uptake and Toxicity

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1 This work was supported financially by the National Natural Science Foundation (Grant No. 40871115), the Natural Science Foundation of Jiangsu Province (Grant No. BK 2009339) and the Graduate Innovative Program of the Graduate School of the Chinese Academy of Sciences.

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Abbreviations: BLM, biotic ligand model; EA50{M}b or EA50{M}0, the activity of metal ion in the bulk-phase medium or at the external PM surface causing a 50% reduction in growth; E_m, electrical potential difference from bulk medium to cell interior; E_{m,surf}, electrical potential difference through the PM from surface to surface; EUM, electrostatic uptake model; ETM, electrostatic toxicity model; FIAM, free ion activity model; GCS, Gouy-Chapman-Stern; {I}_0^0 (or {M}_0^0), chemical activity of ion I with charge Z (or ion M) at the exterior PM surface; {I}_b or {M}_b, chemical activity of ion in the bulk-phase medium; PM, plasma membrane; RRE, relative root elongation; \psi_0 and \psi_i, electrical potentials at the exterior and interior PM surfaces; \sigma_0, intrinsic surface charge density on the exterior PM surface.
Abstract

Electrical properties of plasma membranes (PMs), partially controlled by the ionic composition of the exposure medium, play significant roles in the distribution of ions at the exterior surface of PMs and in the transport of ions across PMs. The effects of coexisting cations (commonly Al³⁺, Ca²⁺, Mg²⁺, H⁺, and Na⁺) on the uptake and toxicity of these and other ions (such as Cu²⁺, Zn²⁺, Ni²⁺, Cd²⁺, and H₂AsO₄⁻) to plants were studied in terms of the electrical properties of PMs. Increased concentrations of cations or decreased pH in rooting media, whether in solution culture or in soils, reduced the negativity of the electrical potential at the PM exterior surface ($\psi_0^o$). This reduction decreased the activities of metal cations at the PM surface and increased the activities of anions such as H₂AsO₄⁻. Further, the reduced $\psi_0^o$ negativity increased the surface-to-surface transmembrane potential difference ($E_{m, surf}$), thus increasing the electrical driving force for cation uptake and decreasing the driving force for anion uptake across PMs. Analysis of measured uptake and toxicity of ions using electrostatic models provides evidence that uptake and toxicity are functions of the dual effects of $\psi_0^o$ (i.e., altered PM-surface ion activity and $E_{m, surf}$ gradient). This study provides novel insights into the mechanisms of plant-ion interactions and extends current theory to evaluate ion bioavailability and toxicity, indicating its potential utility in risk assessment of metal(loid)s in natural waters and soils.
Introduction

Some solutes in growth media, such as cations and organic matter, influence the bioavailability and toxicity of metals in natural waters and soils (Peijnenburg et al., 1997; Weng et al., 2004; Kopittke et al., 2010). Novel insights into the bioavailability and toxicity of metals have inspired the development of models in order to allow accurate impact assessments of metals emitted into the environment. The Biotic Ligand Model (BLM) (Di Toro et al., 2001), as an extension of the Free Ion Activity Model (FIAM), incorporates site-specific competitions among cations (commonly Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, and H\textsuperscript{+}) and ionic toxicants (commonly heavy metals) for binding to a biotic ligand at the cell surface. The scientific and regulatory communities have become interested in the BLM, and have incorporated it into regulations. However, the BLM as the main determinant of toxicant bioavailability does not deserve uncritical acceptance, and the mechanism of the ameliorative effectiveness of cations must be considered carefully, especially in light of cation enhancement of anion toxicity (Kinraide, 2006). Previous studies (Kinraide, 2006; Wang et al., 2008) showed that global electrostatic interactions at the plasma membrane (PM) exterior surfaces, rather than site-specific mechanisms, may play the dominant role in the phytotoxicity of metals.

The process of metal uptake typically encompasses diffusion of the ion to the cell surface, speciation reactions, electrostatic interactions, and subsequent transport across PMs (Hille, 2001; Kinraide, 2001). The PM electrical properties play key roles in the distribution of ions at the exterior surface of PMs, in ion transport across PMs, and, hence, in ion intoxication. Three global electrical features of PMs have been recognized (Fig. 1, top part, and see Kinraide (2001)). The first includes the negative electrical potentials at the PM exterior and interior surfaces ($\psi_0^o$ and $\psi_0^i$, respectively). The second is the electrical potential difference through the PM from surface to surface ($E_{m,surf}$). The last is the transmembrane electrical potential ($E_m$) from bulk medium to cell interior. $E_m$ is composed of three potential differences ($E_m = E_{m,surf} + \psi_0^o - \psi_0^i$) and can be measured comparatively easily by insertion of microelectrodes into cells (Nobel, 1991). The cell wall appears to have comparatively little influence on $\psi_0^o$ and ion concentrations at the PM surface (Gage et al., 1985; Shomer et al., 2003; Kinraide, 2004). Hence $\psi_0^o$ is controlled by the composition of the soil solution and little, or not at all, by soil solid matter lying external to the cell walls except as that solid matter influences the soil solution.

Both $\psi_0^o$ and $E_{m,surf}$ are physiologically important because electrical potential gradients influence the distribution and transport and, hence, the biotic effects of ions. The $\psi_0^o$ is often sufficiently negative to concentrate cations and deplete anions at the PM surface by more than 10-fold relative to the bulk-phase medium. The ionic composition of the bulk-phase medium influences $\psi_0^o$, and cations, commonly Al\textsuperscript{3+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Na\textsuperscript{+}, and H\textsuperscript{+}, reduce the negativity of $\psi_0^o$ by charge screening and ion
binding. Values for $\psi^0_o$ in soil-grown plants range widely because of large variations in soil solution concentrations of cations (Wolt, 1994; Kinraide, 2003b).

$E_{\text{m,surf}}$ influences ion-channel gating and the driving force for ion fluxes across the PM (Hille, 2001; Kinraide, 2001). A consideration of physiological effects (such as ion uptake and intoxication) upon roots only in terms of activities in the bulk-phase medium can be misleading. The common neglect of $\psi^0_o$ is inconsistent with its importance, and may reflect the difficulty of measuring $\psi^0_o$. Nevertheless, several studies have considered physiological phenomena in terms of $\psi^0_o$ (Barber, 1980; McLaughlin, 1989; Zhang et al., 2001; Kinraide, 2003a; Yermiyahu and Kinraide, 2005; Kinraide, 2006; Wang et al., 2008; Kinraide and Wang, 2010; Kopittke et al., 2010), and a fully parameterized Gouy-Chapman-Stern model is now available for calculating $\psi^0_o$ (Wang et al., 2008; Kinraide and Wang, 2010).

The aims of this study were 1) to emphasize the importance of the PM electrical properties for transport and toxicity of ions while developing electrostatic models for uptake and toxicity, 2) to determine possible multiple effects of $\psi^0_o$ upon ion uptake and toxicity, and 3) to apply electrostatic models, incorporating membrane surface-to-surface activities and electrical gradients, to predict the uptake and toxicity of metal ions for use in risk assessment in natural waters and soils.

**Theory**

**Calculation of $\psi^0_o$ and Ion Activities at the PM Exterior Surface**

The Gouy-Chapman-Stern (GCS) model combines electrostatic theory (Gouy-Chapman theory) with ion binding (Stern model) so that $\psi^0_o$ can be computed (Tatulian, 1999; Yermiyahu and Kinraide, 2005; Kinraide and Wang, 2010). For a clear introduction to electrostatic theory presented without the restraints upon space required here, we recommend the book chapter by Yermiyahu and Kinraide (2005); and we recommend Barber (1980) and Tatulian (1999) for clear, more in-depth, theoretical treatments. This model incorporates the intrinsic surface charge density ($\sigma_0$) of a membrane, the ion composition of the bulk-phase medium, and ion binding to the membrane. A detailed analysis (Kinraide and Wang, 2010) indicates the suitability of $\sigma_0 = -30$ mC m$^{-2}$ whilst also noting small variability among membranes. A computer program for the GCS model may be obtained from the authors. Knowledge of $\psi^0_o$ enables the calculation of ion activities at the PM exterior surface. The activity of ion $I^Z$ at the PM exterior surface ($\{I^Z\}_0^o$) is computed from the activity of $I^Z$ in the bulk-phase medium ($\{I^Z\}_b$) according to the Nernst equation $\{I^Z\}_0^o = \{I^Z\}_b \exp[-ZF\psi^0_o/(RT)]$ where $Z$ is the charge on ion $I$, $F$ is the Faraday constant, $R$ is the gas constant, and $T$ is temperature. $Fi/(RT) = 25.7$ when $\psi^0_o$ is expressed in mV and $T = 25^\circ C$.

**Development of Electrostatic Models of Ion Uptake and Toxicity**

Electrostatic Uptake Model (EUM)
To evoke a biotic effect (e.g., transport, toxicity, alleviation of toxicity, enzyme activity), a metal ion must first react with physiologically active sites $R_{cell}$ (e.g., transporter, ion channel, enzyme) on the PM surface, often but not necessarily followed by uptake (Fig. 1, bottom part).

$$M + R_{cell} \xrightarrow{k_a} M-R_{cell} \xrightarrow{k_{int}} M_{int} + R_{cell} \quad \text{Evoke biotic effects}$$  \hspace{1cm} (1)

$$J_{uptake} = k_{int} [M-R_{cell}] = k_{int} [R_{cell}]_t \{M\}_b/(K_M + \{M\}_b), \quad \text{where } K_M = (k_d + k_{int})/k_a$$  \hspace{1cm} (2)

where $J_{uptake}$ is the ion influx; $M_{int}$ represents the metal taken up with concurrent recycling of $R_{cell}$; $\{M\}_b$ is the activity of metal in the bulk-phase medium; $[M-R_{cell}]$ is the surface density (mol m$^{-2}$) of metal bound to $R_{cell}$ (all charges omitted for simplicity), and $[R_{cell}]_t$ is the metal binding capacity of $R_{cell}$; $k_a$, $k_d$, and $k_{int}$ are the association, dissociation and uptake rate constants. The conditional stability constant $K_{M-R_{cell}}$ for the binding of the metal to $R_{cell}$ at the external PM surface can be denoted as $K_{M-R_{cell}} (= k_d/k_a)$. In most cases, $k_{int} \ll k_d$, so that $K_M \approx k_d/k_a = 1/K_{M-R_{cell}}$. Equation 2 resembles the Michaelis-Menten equation that is often used to describe steady-state uptake in models such as the Free Ion Activity Model (FIAM).

However, other uptake models, expressing saturation by substrate, have been proposed (Hille, 2000; Kinraide, 2001), and other models may be equally suitable for our analyses (see Equation 9). Specifically, the precise nature of the $M-R_{cell}$ complex is not essential. $M-R_{cell}$ may also be envisioned as restricted flow through a channel rather than an actual metal-ligand binding. The important feature of our analysis is the impact of $\psi^0_o$ upon $k_{int}$ as developed next.

Commonly, metal ion transport across PMs is expressed in terms of $k_{int}$ and its activity in the bulk-phase medium ($\{M\}_b$), rather than the activity at the PM exterior surface ($\{M\}_0^o$), but root responses to ions often correlate poorly with $\{M\}_b$ and often correlate well with $\{M\}_0^o$ (Zhang et al., 2001; Yermiyahu and Kinraide, 2005; Wang et al., 2008; Kinraide and Wang, 2010; Kopittke et al., 2010). Furthermore, the $k_{int}$ is influenced by the electrical driving force on the ion, i.e. $E_{m,surf}$. Therefore, the objective of the present study was to ascertain the dual effects of changes in $\psi^0_o$ (changes in PM-surface activity of ions and changes in $E_{m,surf}$), independent of changes in $E_m$, upon ion uptake and intoxication. To do so, Equation 2 was modified.

Initially, Equation 2 was modified to take into account the effect of $\psi^0_o$ on the enrichment of cations and depletion of anions at the PM surface. This was accomplished by replacing $\{M\}_b$ with $\{M\}_0^o$.

$$J_{uptake} = k_{int} [R_{cell}]_t \{M\}_0^o/(K_M + \{M\}_0^o)$$  \hspace{1cm} (3)

The internalization constant $k_{int}$ resembles in some ways $P_{ZFE}E_{m,surf}/(RT)$ in a modified
Goldman-Hodgkin-Katz flux equation (Kinraide, 2001), where $P_j$ is a permeability coefficient. Thus $k_{int}$ is proportional to $E_{m,surf}$, which may be computed from $E_{m,surf} = E_m - \psi_0^o + \psi_0^i$. $E_m$ remained essentially constant under the conditions of our experiments, or changes were small or transient (Llamas et al. 2000; Kinraide, 2001). $\psi_0^i$ was small ($\approx -10$ mV) and changes were small relative to changes in $\psi_0^o$ because of high [Mg$^{2+}$], high ionic strength, and constant pH in the cytoplasm (Marschner, 1995). Therefore, $E_{m,surf} = A - \psi_0^o$, where A is a constant equal to $E_m + \psi_0^i$. Uptake, therefore, was driven by both the PM-surface activity of ions and $\psi_0^o$, the latter as a surrogate for $E_{m,surf}$. Accordingly, Equation 3 was modified, yielding the EUM:

$$J_{uptake} = k_{int} [R_{cell}] \frac{[M]_0^o}{(K_M + [M]_0^o)} = kE_{m,surf} [R_{cell}] \frac{[M]_0^o}{(K_M + [M]_0^o)}$$

$$= a(1 + b\psi_0^o)[M]_0^o/(K_M + [M]_0^o), \quad \text{where} \quad a = k[R_{cell}] A; \quad b = -1/A \quad (4)$$

Electrostatic Toxicity Model (ETM)

When growth responds to measures of toxicant intensity the resulting curves are often downwardly sigmoidal and can be expressed in the following equation where growth is limited by $[M]_b$ and is expressed as relative root elongation ($RRE$, as % of nonintoxicated growth).

$$RRE = 100/\exp[(\alpha[M]_b)^\beta] \quad (5)$$

where $\alpha$ is a strength coefficient that increases with the strength of the metal toxicity, and $\beta$ is a shape coefficient that confers sigmoidality when its value > 1. Using $[M]_0^o$ rather than $[M]_b$ takes into account the effect of $\psi_0^o$ on the enrichment of cations and depletion of anions. Thus Equation 5 becomes

$$RRE = 100/\exp[(\alpha[M]_0^o)^\beta] \quad (6)$$

This versatile equation adequately represents dose responses to most toxicants (see Figure 8 in Yermiyahu and Kinraide, 2005). The electrical component of the driving force may be incorporated into Equation 6 by expanding the coefficient $\alpha$ to include $\psi_0^o$, yielding the ETM.

$$RRE = 100/\exp\{[\alpha(1 + b\psi_0^o)[M]_0^o]^\beta\} \quad (7)$$

Equations 4 and 7 now incorporate the dual effects of $\psi_0^o$ on ion uptake and toxicity, namely, the
effects upon the enrichment of cations or depletion of anions at the PM surface and upon the driving force across the PM. It is noteworthy that sometimes large differences in uptake and tolerance are observed among plant species. The differences in the $\alpha$ and $\beta$ coefficients for Equations 4 and 7 may denote differences in uptake and sensitivity. In the case of cationic uptake and toxicity, we would expect coefficient $b$ to have a positive value so that increasing values of $\psi_0$ (i.e., decreasing negativity of $\psi_0$ resulting in increasing negativity of $E_{m,\text{surf}}$) will increase $k_{\text{int}}$, causing $J_{\text{uptake}}$ to increase and $R_{\text{RE}}$ to decrease. In the case of anions, we would expect a negative value for $b$. All coefficients in Equations 2 through 7 were evaluated by regression analysis so that $R^2$ and the statistical significance of the coefficients could be evaluated. No coefficients are reported whose 95% confidence intervals encompassed zero.

Results and Discussion

Effects of Coexistent Cations on Electrical Potentials and the Uptake and Toxicity of Copper

As expected, increases in Ca$^{2+}$, Mg$^{2+}$, or H$^+$ reduced Cu$^{2+}$ uptake and toxicity (Expts. 3 and 11, Table I). Increases in Ca$^{2+}$ concentrations from 0.25 to 4.0 mM significantly decreased the 48-h uptake of Cu$^{2+}$ by wheat (Triticum aestivum L. cv. Yangmai 14) roots (Fig. 2A). In addition, the 48-h EA50{$Cu^{2+}$}$_b$ (i.e., the activity of Cu$^{2+}$ in the bulk-phase medium causing a 50% reduction in growth in 48 h) increased from 0.68 to 1.6 $\mu$M (Fig. 2B). Similar results were observed with increasing {Mg$^{2+}$}$_b$ and decreasing pH (data not shown). To elucidate $\psi_0$ effects we computed the 48-h EA50{$Cu^{2+}$}$_0$ (i.e. the activity of Cu$^{2+}$ at the external surface of the PM causing a 50% reduction). These values for 48-h EA50{$Cu^{2+}$}$_0$ reflect the intrinsic sensitivity of roots to metals, but note that a decrease in EA50{$Cu^{2+}$}$_0$ reflects an increase in sensitivity. Interestingly, the intrinsic sensitivity of Cu$^{2+}$ was increased significantly with increasing Ca$^{2+}$, with the 48-h EA50{$Cu^{2+}$}$_0$ decreasing from 21.1 to 7.4 $\mu$M (Fig. 2C). This was consistent with our previous studies that also demonstrated that the extrinsic sensitivity to Cu$^{2+}$ (sensitivity to {Cu$^{2+}$}$_b$) decreased as $\psi_0$ became less negative and that the intrinsic sensitivity to Cu$^{2+}$ (sensitivity to {Cu$^{2+}$}$_0$) increased (see Fig. 7a in Wang et al. (2008)), but those effects were not explained. Reanalysis of previous studies demonstrates that decreased $\psi_0$ negativity enhances the intrinsic toxicity of Cu$^{2+}$ (Lock et al., 2007a), Co$^{2+}$ (Lock et al., 2007b), and Ni$^{2+}$ (Lock et al., 2007c) to barley (Hordeum vulgare L.).

We propose that this increase in intrinsic sensitivity caused by reductions in $\psi_0$ negativity results from increases in the $E_{m,\text{surf}}$ negativity – this would increase the electrical driving force for cationic influxes (Fig. 3). Changes in $\psi_0$ have little impact on $E_m$ (Llamas et al., 2000; Hille, 2001; Kinraide, 2001), in contrast to effects upon $E_{m,\text{surf}}$. Consequently, changes in $\psi_0$ are offset almost entirely by

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changes in $E_{m,surf}$ (compare solid and dashed lines in Fig. 3 where $E_{m,surf} = E_m - \psi_0^o + \psi_0^i$). Thus, the decreased negativity of $\psi_0^o$ resulted in reduced attraction of Cu$^{2+}$ to the PM surface (the $\{\text{Cu}^{2+}\}_0^o$ decreased from 38.3 to 1.1 µM, Fig. 3B) hence a decreased extrinsic sensitivity, but the decreased negativity of $\psi_0^o$ caused an increase in the negativity of $E_{m,surf}$ hence an increased intrinsic sensitivity.

Modeling Ion Uptake and Toxicity with Electrostatic Models (EUM and ETM)

1. Cu$^{2+}$ Uptake and Toxicity Affected by Ca$^{2+}$, Mg$^{2+}$, or pH (Expts. 3 and 11)

Figure 4 illustrates Cu$^{2+}$ uptake and toxicity as functions of $\{\text{Cu}^{2+}\}_b$ (Figs. 4A and 4E), $\{\text{Cu}^{2+}\}_0^o$ (Figs. 4B and 4F), and calculated Cu$^{2+}$ uptake and toxicity based on the EUM (Eq. 4) and ETM (Eq. 7) (Figs. 4C and 4G). The Cu$^{2+}$ uptake corresponds to the calculated Cu$^{2+}$ uptake ($R^2 = 0.949$; Fig. 4C) more closely than to $\{\text{Cu}^{2+}\}_0^o$ ($R^2 = 0.867$; Fig. 4B) or $\{\text{Cu}^{2+}\}_b$ ($R^2 = 0.840$; Fig. 4A). For toxicity, $RRE$ correlates better with calculated $RRE$ ($R^2 = 0.956$; Fig. 4G) than with $\{\text{Cu}^{2+}\}_0^o$ ($R^2 = 0.794$; Fig. 4E) or $\{\text{Cu}^{2+}\}_b$ ($R^2 = 0.843$; Fig. 4F). Also, Figure 4 illustrates the uptake (Fig. 4D) and the $RRE$ (Fig. 4H) as functions of $\psi_0^o$ and $\{\text{Cu}^{2+}\}_0^o$. The curved surfaces are based on the electrostatic uptake and toxicity models using the parameters in Tables II and III (Expts. 3 and 11).

2. Ca$^{2+}$ Uptake Affected by Al$^{3+}$, La$^{3+}$, Mg$^{2+}$, or Sr$^{2+}$ (Expts. 1 and 2)

Huang et al. (1994, 1996) measured radiotracer Ca$^{2+}$ influx into right-side-out PM vesicles from wheat roots (cv. Scout 66) in response to variable external Ca$^{2+}$, Al$^{3+}$, La$^{3+}$, Mg$^{2+}$, Sr$^{2+}$, and pH. $E_m$ was controlled by the adjustment of internal and external K$^+$, which, with valinomycin, clamped $E_m$ at –100 mV. Their data demonstrate the well-known inhibitory effect of Al$^{3+}$, La$^{3+}$, Mg$^{2+}$, and Sr$^{2+}$ on short-term (5 min) Ca$^{2+}$ uptake, which is commonly attributed to blockade or competition at a Ca$^{2+}$ channel. However, we question whether the inhibition occurs as a consequence of channel blockade (or competition), or as a consequence of electrostatic reduction of $\{\text{Ca}^{2+}\}_0^o$. An analysis of published patch-clamp studies (Kinraide, 2001) revealed that inhibition of Ca$^{2+}$ uptake usually entails reduced unitary channel conductance rather than reduced probably of channel openness (i.e., true blockade).

In order to investigate the possible dual effects of $\psi_0^o$, measured Ca$^{2+}$ uptake was plotted against $\{\text{Ca}^{2+}\}_b$, $\{\text{Ca}^{2+}\}_0^o$ and against calculated Ca$^{2+}$ uptake based on the EUM (Eq. 4). Figure 5 indicates that Ca$^{2+}$ uptake corresponds to this calculated Ca$^{2+}$ uptake ($R^2 = 0.938$) more closely than to $\{\text{Ca}^{2+}\}_0^o$ ($R^2 = 0.775$) or to $\{\text{Ca}^{2+}\}_b$ ($R^2 = 0.458$) (Fig. 5 and Table II). Figure 5D illustrates that the Ca$^{2+}$ uptake depended on $\psi_0^o$ and $\{\text{Ca}^{2+}\}_0^o$, indicating that the effects of Al$^{3+}$, La$^{3+}$, Mg$^{2+}$, and Sr$^{2+}$ on Ca$^{2+}$ uptake may be attributable to the dual effects of $\psi_0^o$ rather than to a channel blockade or competition. The curved surface (Fig. 5D) is based on the EUM with the parameter values in Table II (Expts. 1 and 2).
3. Na\(^+\) Uptake Affected by Ca\(^{2+}\) and Ca\(^{2+}\) Uptake Affected by Na\(^+\) (Expts. 5 and 6)

Davenport et al. (1997) measured short-term (20 min) and long-term (7 d) Na\(^+\) unidirectional uptake into roots of two species of wheat (T. aestivum L. cv. Kharchia and T. turgidum L. cv. Modoc) cultured in variable concentrations of NaCl and \{Ca\(^{2+}\)\}_b. It is clear from the authors’ Figures 3, 4, and 5 that Ca\(^{2+}\) effectively inhibited Na\(^+\) uptake and vice versa. It was assumed that Na\(^+\) uptake occurred passively via Ca\(^{2+}\) channels and that the decreased Na\(^+\) uptake was attributable to Ca\(^{2+}\) binding to a specific site on such channels, thereby altering the gating and selectivity of the channels. However, correlation between measured and calculated uptake for both Na\(^+\) and Ca\(^{2+}\) was superior when using the EUM (Eq. 4) (Table II, Expts. 5 and 6). These results indicate that cation effects may be attributed to the dual effects of \(\psi_0\) rather than to specific blocking effects.

4. Zn\(^{2+}\), Cu\(^{2+}\), Cd\(^{2+}\), and Ni\(^{2+}\) Uptake Affected by Ca\(^{2+}\) or pH (Expts. 4, 7, 8, 9)

In a study with pea (Pisum sativum L. cv. Lincoln), Wu (Ph. D. thesis, 2007) measured changes in uptake of Zn\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), and Cd\(^{2+}\) in response to added Ca\(^{2+}\) and H\(^+\). In nearly all cases, increases in Ca\(^{2+}\) or decreases in pH reduced metal uptake. The study measured some characteristics of pea roots and considered theoretical aspects of the FIAM and the BLM. For our reanalysis, uptake data were taken from the author’s tables. For the Zn\(^{2+}\) uptake experiments, increases in Ca\(^{2+}\) concentration or decreases in pH resulted in decreases in the negativity of \(\psi_0\) and reductions in Zn\(^{2+}\) uptake (data not shown). The measured Zn\(^{2+}\) uptake was better correlated with the Zn\(^{2+}\) uptake calculated by the EUM \((R^2 = 0.967)\) than with \(\{\text{Zn}^{2+}\}_0\) \((R^2 = 0.851)\) or \(\{\text{Zn}^{2+}\}_b\) \((R^2 = 0.821)\) (Table II, Expt. 4). Similar results were observed for Cu\(^{2+}\), Ni\(^{2+}\) or Cd\(^{2+}\) uptake (Table II, Expts. 7, 8, and 9).

5. Cu\(^{2+}\) Toxicity Affected by Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\) or pH (Expts. 12 and 13)

Parker et al. (1998) investigated Cu\(^{2+}\) rhizotoxicity to roots of wheat (cv. Yecora Rojo) in response to variable concentrations of CuCl\(_2\), CaCl\(_2\), MgCl\(_2\), and pH in a factorial array. Increase in Ca\(^{2+}\), Mg\(^{2+}\), or H\(^+\) alleviated Cu\(^{2+}\) stress. For our reanalysis, data were taken from the authors’ Figures 2 to 5 and Tables 1 and 2. For each datum point in the figures and tables, Cu\(^{2+}\) and Ca\(^{2+}\) concentrations, pH, and relative net elongation were recorded, and \(\psi_0\) and \(\{\text{Cu}^{2+}\}_0\) were calculated. Measured RRE correlated more strongly with the calculated RRE based on the ETM (Eq. 7) \((R^2 = 0.921)\) than with \(\{\text{Cu}^{2+}\}_0\) \((R^2 = 0.878)\) or \(\{\text{Cu}^{2+}\}_b\) \((R^2 = 0.745)\) (Table III, Expt. 12). This indicates a dual effect of \(\psi_0\).

Kinraide (2006) presented results from experiments assessing wheat seedling (cv. Atlas 66) root elongation in solutions containing variable concentrations of CuSO\(_4\), CaCl\(_2\), NaCl, and pH. Increases in Ca\(^{2+}\), Na\(^+\), or H\(^+\) in the rooting media alleviated the Cu\(^{2+}\) rhizotoxicity. This study considered the first effect of \(\psi_0\) upon \(\{\text{Cu}^{2+}\}_0\) and the possibility of competition at a binding site at the PM surface (BLM).
In the present reanalysis, $RRE$ correlated more strongly with the calculated $RRE$ based on the ETM (Table III, Expt. 13) than with $\{Cu^{2+}\}_0$ or $\{Cu^{2+}\}_b$, suggesting that the ameliorative effectiveness of $Ca^{2+}$, $Na^+$, and $H^+$ results from the dual effects of $\psi_0^o$.

6. Al$^{3+}$ Toxicity Affected by $Ca^{2+}$, Mg$^{2+}$, or pH (Expt. 14)

We re-evaluated the $Ca^{2+}$ and Mg$^{2+}$ alleviation of Al$^{3+}$ rhizotoxicity in wheat roots (cv. Tyler) from data archived from, but not presented in, a study by Kinraide and Parker (1987). Two-day-old seedlings were transferred to solutions containing 0.4 mM CaCl$_2$ at pH 4.3 variously supplemented with AlCl$_3$ and MgCl$_2$ or additional CaCl$_2$. Addition of $Ca^{2+}$ or Mg$^{2+}$ alleviated Al$^{3+}$ toxicity. Kinraide et al. (1992) reassessed the interactive effects of $Ca^{2+}$ and Mg$^{2+}$ on Al$^{3+}$ toxicity in terms of $\psi_0^o$ and demonstrated that the $\psi_0^o$ plays an important role in Al$^{3+}$-Ca$^{2+}$ and Al$^{3+}$-Mg$^{2+}$ interactions by the first effect of $\psi_0^o$ – the enhancement of cations at the PM surface. For the present reanalysis, the dual effects were considered simultaneously in the ETM. Again, the correlation between measured and calculated Al$^{3+}$ toxicity was significantly improved (Fig. 6 and Table III, Expt. 14), indicating that the $Ca^{2+}$ and Mg$^{2+}$ alleviation of Al$^{3+}$ toxicity may be attributed to effects upon both $E_{m,surf}$ and $\{Al^{3+}\}_0$, i.e., the dual effects of $\psi_0^o$.

7. Anion H$^2$AsO$_4^-$ Uptake and Toxicity Affected by $Ca^{2+}$, Mg$^{2+}$, Na$^+$, or pH (Expts. 10 and 15)

The addition of $Ca^{2+}$ or Mg$^{2+}$ or the reduction of pH will increase the surface activity of H$^2$AsO$_4^-$ and thus the uptake and toxicity of As. Arsenate (H$^2$AsO$_4^-$) may become transformed in the roots into the even more toxic arsenite (H$^2$AsO$_3^-$) (Pickering et al., 2000). Further transformation, translocation, and secretion from the roots may provide some relief of As toxicity. Even so, one would expect $\psi_0^o$ to have an effect upon As uptake, retention, and toxicity. Our hypothesis was that the effects of $\psi_0^o$ upon anionic As toxicants would be opposite to the effects upon cationic toxicants.

Indeed, an increase in $Ca^{2+}$ from 0.25 to 4.0 mM, decreased the 48-h EA50{$As(V)$}$_b$ from 2.9 to 1.7 μM, indicating an increased extrinsic sensitivity. In contrast, the corresponding EA50{$As(V)$}$_0$ increased from 0.48 to 1.49 μM, indicating a decreased intrinsic sensitivity. In contrast to Cu$^{2+}$, As(V) uptake was sigmoidal and thus did not comply with the Michaelis-Menten equation. This may be related to the concentrations used in our treatments and to hormesis by As(V) (Calabrese, 2008; Wang et al., 2008), which was always observed in low As treatments. A stimulation of root elongation would dilute the As(V) concentration in roots. Therefore the uptake of As(V) was expressed using a generalized sigmoidal equation:

$$J_{uptake} = J_{max}\{1 - 1/exp[\alpha (H^2AsO_4^-)_b]^\beta]\}$$  (8)

where $J_{max}$ is the maximum influx, which occurs when $\{As(V)\}_b$ is very large; $\alpha$ and $\beta$ resemble their...
counterparts in Equation 5. Regression analysis with Equation 8 for As(V) uptake yielded \( R^2 = 0.830 \). Subsequently, \( [\text{H}_2\text{AsO}_4^-]_b \) was replaced with \( [\text{H}_2\text{AsO}_4^-]_0 \) to include the first effect of \( \psi_0 \) upon the depletion of anions at the PM surface, and the coefficient \( \alpha \) was expanded to \( \alpha (1 + b \psi_0) \) to incorporate the electrical component of the driving force (the second effect of \( \psi_0 \)).

\[
J_{\text{uptake}} = J_{\text{max}} \{1 - 1/\exp[\alpha(1 + b \psi_0) [\text{H}_2\text{AsO}_4^-]_0 \psi_0^\beta]\} \tag{9}
\]

In the case of anionic toxicants, \( b \) is expected to be negative so that a decreasing negativity of \( \psi_0 \) will decrease the value of the expanded coefficient, causing \( J_{\text{uptake}} \) to decrease. Regression analysis with Equation 9 indeed produced a negative value for \( b (-0.0649) \) and an increase in \( R^2 \) to 0.910 (Table II, Expt. 10).

To assess As(V) toxicity, the measured \( RRE \) was plotted against \( RRE \) calculated on the basis of the ETM (Eq. 7) to estimate the dual effects of \( \psi_0 \). The correlation between \( RRE \) and the model-calculated \( RRE (R^2 = 0.937) \) was superior to the correlation between \( RRE \) and \( [\text{H}_2\text{AsO}_4^-]_0 \) \( (R^2 = 0.919) \) or \( [\text{H}_2\text{AsO}_4^-]_b \) \( (R^2 = 0.903) \) (Table III, Expt. 15). As expected, the regression analysis also yielded a negative value for \( b \) in Equation 7 \( (b = -0.0607, \text{close to the value of } -0.0649 \text{ reported for the EUM, i.e., } b \text{ in Eq. 9).} \)

8. A dual role for \( \psi_0 \) expressed in soil culture

A dual role for \( \psi_0 \) appears to be expressed in soil cultures as well as in solution cultures. Ninety-four soil samples were collected from various horizons and soil series from forests in Appalachia, the northeastern USA, the Netherlands, Sweden, and Germany (Kinraide, 2003b). Some of the soils were used in growth experiments and growth of wheat, switchgrass, and subterranean clover were assessed in terms of the extracted soil solutions. The plant growth in these soils was dependent on the \( \text{Al}^{3+} \) toxicity and \( \text{H}^+ \) toxicity and on \( \text{Ca}^{2+} \) deficiency. A reanalysis of those data demonstrates the dual influence of \( \psi_0 \) upon plant growth. When the \( \alpha \) coefficient was expanded to \( \alpha (1 + b \psi_0) \) in the growth equations \( (RRE_{\text{Al}} = 100/\exp[\alpha(\text{Al}^{3+})_0 \psi_0^\beta], RRE_{\text{H}} = 100/\exp[\alpha(\text{H}^+)_0 \psi_0^\beta], \text{and } RRE_{\text{Ca}} = 100(1 - 1/\exp[\alpha(\text{Ca}^{2+})_0 \psi_0^\beta])] \) the \( R^2 \) increased by 0.315 (from 0.340 to 0.655), 0.294 (from 0.151 to 0.445), and 0.072 (from 0.553 to 0.625), respectively; \( n = 52; \) and coefficients \( \alpha \) and \( b \) were significant \( (p < 0.05) \) in each case. These \( R^2 \) values are generally lower than those for solution culture studies, and this reflects the complexity of soils. Indeed, a better prediction was achieved when combined effects of two toxicants (\( \text{Al}^{3+} \) and \( \text{H}^+ \)) and an ameliorant (\( \text{Ca}^{2+} \)) were quantified by the equation \( RRL = 100 \times RRE_{\text{Al}} \times RRE_{\text{H}} \times RRE_{\text{Ca}} \) in terms of the dual effects of \( \psi_0 \) (not shown). Regardless, the improvements in the \( R^2 \) values demonstrate the importance of the dual effects of \( \psi_0 \) upon plant growth. For a reanalysis of published soils studies demonstrating the first effect of \( \psi_0 \) in soils, see Kinraide (2006; pp. 3195 and 3196).
Prediction of Ion Uptake and Toxicity with the FIAM, SIAM and Electrostatic Models

All observed values from Experiments 1 to 15 were plotted against the values predicted by each model (FIAM, the Surface Ion Activity Model (SIAM), the EUM, and the ETM) (data not shown). The SIAM considered the effect of $\psi_0^o$ on both surface ion activities. The SIAM predictions ($R^2 = 0.861$, n = 427 for uptake; $R^2 = 0.802$, n = 334 for toxicity) were superior to FIAM predictions ($R^2 = 0.716$ for uptake; $R^2 = 0.721$ for toxicity). The correlations for the electrostatic models ($R^2 = 0.940$ for the EUM and $R^2 = 0.901$ for the ETM), incorporating the effect of $\psi_0^o$ on both surface ion activities and $E_{m,surf}$, were superior to the SIAM predictions. These results indicate that the electrostatic models could form the basis for the prediction of uptake and toxicity of both metal cations and metalloid anions and for the interpretation of other plant-ion interactions. In most cases the treatment periods in this study ranged from a few minutes (some uptake studies) to a few days (toxicity studies), but studies of longer duration, especially in soils, would certainly be useful for more accurate predictions.

General Evaluation of the Electrostatic Approach to Ion Uptake and Toxicity

The Biotic Ligand Model attempts to explain toxicity and alleviation in terms of metal ion binding to cell-surface ligands as a key step leading to toxicity. It is proposed in the BLM that alleviation of toxicity by cations is caused by the competition of those ions with the toxic ions for binding to the same ligands. These assumptions may be true but are not verified in most cases. Also, the BLM fails to interpret the enhancement of uptake and toxicity of arsenate or selenate anions by the treatments that reduce the toxicity of toxic cations (Kinraide, 2003a; Wang et al., 2008). The present study shows that electrostatic mechanisms provide a unified interpretation of both phenomena – the cation reduction of cation uptake and toxicity and the cation enhancement of anion uptake and toxicity.

The alleviation of cation toxicity by site-specific mechanisms, such as competitive binding, may occur in some cases. For example, Weng et al. (2004), and others have noted significant reductions in metal-induced toxicity with reductions in pH in the 6 to 7 range. Such pH reductions would change $\psi_0^o$ by < 1 mV in most media – a change far too small to account for toxicity reductions. Instead, some key ligands, contributing little to $\psi_0^o$, such as sites on ion channels or enzymes, may bind H⁺ strongly enough for pH shifts from 7 to 6 to be effective. Despite clear evidence for site-specific mechanisms (such as competition), such mechanisms appear to be unimportant in many cases (Kinraide, 2006; Wang et al., 2008). In those cases, including those in the present study, the dual effects of $\psi_0^o$ are sufficient to explain the ion-toxicant interactions (Kinraide, 2006; Wang et al., 2008; results in the present study).

The GCS model, incorporating the parameter values used by us, appears to be quite robust (Yermiyahu et al., 1997; Zhang et al., 2001; Wang et al., 2008; Kinraide and Wang, 2010), and a fully parameterized GCS model is available from the authors. The model is suitable for the calculation of $\psi_0^o$. 
and for the interpretation of many plant responses to changes in the composition of the aqueous exposure medium.

Conclusions

The present study provides evidence that PM surface ion activities, as determined by $\psi_0^o$, are often superior to activities in the bulk-phase medium as indicators of plant-ion interactions. The study also indicates that $\psi_0^o$ plays an additional role: it is a determinant of $E_{m, surf}$, the electrical component of the driving force for ion uptake. Our findings indicate that these dual effects of $\psi_0^o$ play important roles in ion uptake and toxicity in both soil and solution cultures, and that the electrostatic uptake and toxicity models provide a novel approach for predicting the uptake and toxicity of both metal cations and metalloid anions. The electrostatic uptake and toxicity models do not negate entirely the BLM. Site-specific competition surely explains some features of ion toxicity and alleviation. Irrespective of whether the mechanisms invoked by the BLM play a role in any given situation, electrostatic effects surely do and should be incorporated into any model, including the BLM, of ion toxicity and the ionic alleviation or enhancement of toxicity.

Materials and Methods

Uptake and Toxicity Bioassay Experiments

Data for ion uptake and root elongation in response to the ionic environment were complied for analysis from previous studies employing solution culture (references in Table I) and soil culture. In addition, data for uptake and toxicity of Cu and As(V) in wheat roots were collected from experiments conducted as part of the current study (Expts. 3 and 11 for Cu, and Expts. 10 and 15 for As(V)). The plant species used and a summary of culture conditions are presented in Table I. For detailed experimental materials and methods (except for the current study) refer to the corresponding references in Table I.

Experiments 3 and 11 – Effects of Ca$^{2+}$, Mg$^{2+}$, and pH on Cu$^{2+}$ uptake and toxicity (current study)

Three sets of uptake and toxicity bioassay experiments were performed, one each for three Ca$^{2+}$ concentrations (0.25, 0.98, and 3.78 mM), three Mg$^{2+}$ concentrations (0.26, 1.0, and 4.0 mM), and three pH values (5.1, 5.5, and 6.0). In each medium, six Cu$^{2+}$ concentrations (0.25 to 2.0 $\mu$M) and a control were tested. Further details of the growth experiments and precautions for the preparation of the test solutions have been presented previously (Wang et al., 2008). For acute toxicity tests, 2-day-old wheat (cv. Yangmai 14) seedlings with uniform root length (1 to 2 cm) were cultured in darkness for 48 h at 25°C in acid-washed polyethylene beakers containing 500 mL of test medium. Each treatment was performed.
with three or four replicates and eight seedlings per replicate. At termination, the two longest roots of
each seedling were measured and the mean value of 16 measured values per replicate was recorded. The
plants were transferred to a 10 mM Ca(NO₃)₂ solution for 10 min to remove CW-bound Cu. Roots were
dried at 40°C, weighed, and digested with 5 mL ultra pure concentrated HNO₃. Copper was determined
by flame atomic absorption spectrometry (F-AAS; Varian 220Z).

Experiments 10 and 15 – Effects of Ca²⁺, Mg²⁺, Na⁺, and pH on As(V) uptake and toxicity (current
study)

For As(V) uptake, three sets of experiments were performed, one each for three Ca²⁺ concentrations
(0.24, 1.0, and 4.1 mM), three Mg²⁺ concentrations (0.26, 0.83, and 3.7 mM), and three pH values (4.4,
5.3, and 6.0). In each medium, five H₂AsO₄⁻ concentrations (0.75 to 3.0 μM) and a control were tested.
For As(V) toxicity experiments, four sets of experiments were performed, one each for Ca²⁺ (0.27 to 4.3
mM), Mg²⁺ (0.26 to 4.0 mM), Na⁺ (1.4 to 20.1 mM), and pH (4.5 to 6.6). In each medium, five arsenate
concentrations (0.67 to 26.7 μM) and a control were tested. These uptake and growth experiments were
similar to Experiments 3 and 11. At termination, the plants were transferred to an ice-cold phosphate
buffer solution for 10 min to remove CW-bound As (Zhang et al., 2009). Roots were dried at 40°C,
weighed, and digested with 5 mL ultra pure concentrated HNO₃. Arsenic was determined by atomic
fluorescence spectrometry (AF-160A, Beijing Haichuang Analytical Instrument Co., China).

Data Treatment and Statistics

Ion species and activities were calculated using the visual MINTEQ (version 2.51) chemical
equilibrium program (US EPA). The speciation calculations included atmospheric CO₂ (pCO₂ = 10⁻³.₅
atm). Relative root elongation was calculated by the formula $RRE = 100 \left( RL_T - RL_S \right) /
\left( RL_C - RL_S \right)$, in which $RL_T$ is the mean root length ($RL$) in the presence of toxicants, $RL_C$
is the $RL$ in the corresponding toxicant-free control, and $RL_S$ is $RL$ at the time of seedling transfer to the test media. Uptake and toxicity were plotted, and curved surfaces were fitted by regression analysis using Origin Professional 6.0 and
MATLAB 7.0. Significance levels are $p < 0.05$ for all reported regressions and coefficients.

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Figure Legends

Figure 1. Profile of the electrical potentials and ion distributions at the PM (top part). Conceptualized ion \((M^{n+})\) transport across the PM, including dissociation/association with the active binding site \((R_{cell};\text{e.g., transporter, ion channel})\) on the surface of the PM (bottom part). Lines 1 and 2 illustrate the potential profile through the PM and electric double layer before (line 1) and after (line 2) the addition of depolarizing solutes to the bulk-phase medium. \(\psi_0^o\) is the potential difference between the bulk-phase medium and the external PM surface. The \(E_m\) is the transmembrane potential difference from the bulk-phase medium to the cell interior. \(E_{m,\text{surf}}\) is the potential difference through the PM from surface to surface for line 1 (\(E_{m,\text{surf}}\) not shown for line 2). The cell wall has been omitted for simplicity. Lines 3 (cations) and 4 (anions) represent the profile of activities from the bulk solutions to the PM exterior surface. The \(k_a\), \(k_d\), and \(k_{int}\) are the association, dissociation, and internalization rate constants.

Figure 2. Uptake and toxicity in response to Cu\(^{2+}\) and Ca\(^{2+}\) in wheat seedlings, showing (A) uptake of Cu\(^{2+}\) as a function of Cu\(^{2+}\) activity in the bulk-phase medium (\([\text{Cu}^{2+}]_b\)) at different \([\text{CaCl}_2]\), (B) effect of Ca\(^{2+}\) on EA50\([\text{Cu}^{2+}]_b\) (\([\text{Cu}^{2+}]_b\) causing 50% reduction in root elongation), and (C) effect of Ca\(^{2+}\) on EA50\([\text{Cu}^{2+}]_0\) (PM-surface \{Cu\(^{2+}\)\} causing 50% reduction in root elongation). Error bars indicate 95% confidence intervals. Different letters indicate significant differences among means (\(p < 0.01\)). Data from Expts. 3 and 11.

Figure 3. Predicted electrical potentials and PM surface activity of Cu\(^{2+}\) in responses to Ca\(^{2+}\) activity in the bulk-phase medium. (A) The PM surface potentials (\(\psi_0^o\)) and surface-to-surface transmembrane potentials (\(E_{m,\text{surf}}\)); (B) the calculated PM surface activity of Cu\(^{2+}\) at 1.0 \(\mu\)M CuCl\(_2\). Data from Expts. 3 and 11.

Figure 4. Uptake (left column) and relative root elongation (\(RRE\), right column) of Cu\(^{2+}\) by wheat roots as functions of Cu\(^{2+}\) activities in the bulk-phase medium (\([\text{Cu}^{2+}]_b\)), first row), Cu\(^{2+}\) ion activities at the PM exterior surface (\([\text{Cu}^{2+}]_0^o\), second row), and Cu\(^{2+}\) uptake or \(RRE\) calculated from electrostatic models (third row). Uptake and \(RRE\) as functions of \(\psi_0^o\) and Cu\(^{2+}\) surface activity (bottom row). In D and H, the curved surfaces denote uptake or \(RRE\) calculated from the electrostatic models whilst the open circles represent the measured values. Data from Expts. 3 and 11.

Figure 5. Measured Ca\(^{2+}\) uptake plotted against (A) bulk-phase activities of Ca\(^{2+}\) (\([\text{Ca}^{2+}]_b\)), (B) PM surface activities of Ca\(^{2+}\) (\([\text{Ca}^{2+}]_0^o\)), (C) Ca\(^{2+}\) uptake calculated from the EUM (Eq. 4), or (D) as a function of \(\psi_0^o\) and \([\text{Ca}^{2+}]_0^o\). In D, the curved surface denotes uptake calculated with the EUM whilst
the open circles represent the measured uptake. Data were from Huang et al. (1994, 1996) (Expts. 1 and 2), who measured Ca\(^{2+}\) influx into PM vesicles from wheat roots in a basal medium of 0.1 mM NaCl and 1.0 mM K\(_2\)SO\(_4\) at pH 7.0 (for La\(^{3+}\), Mg\(^{2+}\) and Sr\(^{2+}\)) or pH 4.5 (for Al\(^{3+}\)). The \(E_m\) in all solutions was clamped at a constant \(-100\) mV.

**Figure 6.** Measured relative root elongation (RRE) of wheat seedlings exposed to Al\(^{3+}\) affected by Ca\(^{2+}\) and Mg\(^{2+}\). Data were taken from Kinraide et al. (1992) (Expt. 14). The RRE is plotted as functions of (A) Al\(^{3+}\) activities in the bulk phase medium (\([\text{Al}^{3+}]_b\)), (B) Al\(^{3+}\) activities at the exterior surface of the cell membrane (\([\text{Al}^{3+}]_0\)), or (C) calculated Al\(^{3+}\) toxicity based on the ETM, or (D) \(\psi_0\) and \([\text{Al}^{3+}]_0\).
Table I. Overview of studies used to examine ion uptake and toxicity. For each study, the pH, total concentration of ions ([I]₀), and the calculated electrical potential (ψ₀) at the exterior surfaces of the plasma membrane are reported. The ψ₀ was calculated for each medium with the standard GCS model.

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<tr>
<td>Ca²⁺ influx into plasma membrane vesicles of wheat roots (Huang et al., 1994)</td>
<td>1</td>
<td>7.0</td>
<td>[AlCl₃] 0 - 20</td>
<td>0.01 - 5.0</td>
<td>[MgCl₂] 0 - 0.5</td>
<td>–87.6 to –18.9</td>
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<tr>
<td>Ca²⁺ influx into plasma membrane vesicles of wheat roots (Huang et al., 1996)</td>
<td>2</td>
<td>4.5</td>
<td>[LaCl₃] 0 - 20</td>
<td>0.01 - 3.0</td>
<td>[MgCl₂] 0.26 - 4.0</td>
<td>–34.7 to –5.49</td>
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<tr>
<td>Cu²⁺ uptake by wheat seedlings (current study)</td>
<td>3</td>
<td>5.1 - 6.0</td>
<td>0.25 - 3.8</td>
<td>[MgCl₂] 1.0</td>
<td>–44.4 to –19.3</td>
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<td>Zn²⁺ uptake by pea seedlings (Wu, 2007)</td>
<td>4</td>
<td>4.3 - 6.0</td>
<td>0.19 - 1.8</td>
<td>[MgCl₂] 0.26 - 4.0</td>
<td>–57.1 to –14.9</td>
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<td>Ca²⁺ uptake by wheat roots (Davenport et al., 1997)</td>
<td>5</td>
<td>6.5</td>
<td>0 - 3.1</td>
<td>5.0 - 150</td>
<td>–86.4 to –6.6</td>
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<td>Na⁺ uptake by wheat roots (Davenport et al., 1997)</td>
<td>6</td>
<td>6.5</td>
<td>0.08 - 11</td>
<td>5.0 - 150</td>
<td>–61.4 to –6.6</td>
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<td>Cd²⁺ uptake by pea seedlings (Wu, 2007)</td>
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<td>4.0 - 6.0</td>
<td>0.04 - 1.6</td>
<td>[MgCl₂] 0.26 - 3.7</td>
<td>–76.0 to –8.7</td>
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<td>Ni²⁺ uptake by pea seedlings (Wu, 2007)</td>
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<td>4.0 - 6.0</td>
<td>0.04 - 1.8</td>
<td>[MgCl₂] 0.26 - 3.7</td>
<td>–76.0 to –8.2</td>
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<tr>
<td>Cu²⁺ uptake by pea seedlings (Wu, 2007)</td>
<td>9</td>
<td>4.0 - 6.0</td>
<td>0.04 - 1.9</td>
<td>[MgCl₂] 0.26 - 3.7</td>
<td>–75.9 to –8.3</td>
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<td>H₂AsO₄⁻ uptake by wheat seedlings (current study)</td>
<td>10</td>
<td>4.4 - 6.0</td>
<td>0.24 - 4.1</td>
<td>[MgCl₂] 0.26 - 3.7</td>
<td>–43.8 to –19.1</td>
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<td>Substance</td>
<td>Study Year</td>
<td>pH Range</td>
<td>[Salt] Range</td>
<td>[MgCl₂] Range</td>
<td>Concentration Range</td>
<td>Toxicity Range</td>
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<tr>
<td>Cu²⁺ rhizotoxicity to wheat seedlings (current study)</td>
<td>11</td>
<td>5.1 - 6.0</td>
<td>0.25 - 3.8</td>
<td>[MgCl₂] 0.26 - 4.0</td>
<td>1.0</td>
<td>−44.4 to −19.3</td>
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<td>Cu²⁺ rhizotoxicity to wheat seedlings (Parker et al., 1998)</td>
<td>12</td>
<td>4.5 - 6.0</td>
<td>0.20 - 4.0</td>
<td>[MgCl₂] 0 - 4.7</td>
<td>−56.6 to −12.7</td>
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<tr>
<td>Cu²⁺ rhizotoxicity to wheat seedlings (Kinraide, 2006)</td>
<td>13</td>
<td>4.5 - 5.7</td>
<td>0.50 - 8.0</td>
<td>0 - 10</td>
<td>−33.4 to −8.7</td>
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<td>Al³⁺ rhizotoxicity to wheat seedlings (Tyler wheat, archived data)</td>
<td>14</td>
<td>4.3</td>
<td>[AlCl₃] 0.40 - 4.0</td>
<td>[MgCl₂] 0 - 3.6</td>
<td>−25.3 to 0.8</td>
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<tr>
<td>H₂AsO₄⁻ rhizotoxicity by wheat seedlings (current study)</td>
<td>15</td>
<td>4.5 - 6.0</td>
<td>0.27 - 4.3</td>
<td>[MgCl₂] 0.26 - 4.0</td>
<td>0.53 - 20</td>
<td>−44.1 to −19.1</td>
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Table II. Comparison of three models for ion uptake. For the free ion activity model (FIAM), uptake is expressed in terms of the activity in the bulk-phase rooting medium according to the equation $J_{int} = a\{M\}_b/(K_M + \{M\}_b)$. For the surface ion activity model (SIAM), uptake is expressed in terms of the activity at the PM exterior surface according to the equation $J_{int} = a\{M\}_0/(K_M + \{M\}_0)$. For the EUM, uptake is expressed in terms of the PM surface activity and electrical potentials ($\psi_0$) according to the equation $J_{int} = a(1 + b\psi_0)/(K_M + \{M\}_0)$. \{M\} refers to one of the ions below; $a$, $K_M$, and $b$ are coefficients ($a$ resembles in some ways the $J_{max}$ in the Michaelis-Menten equation); and $n$ is the number of datum points. All of the presented values for coefficients are statistically significant (95% confidence intervals do not encompass zero).

<table>
<thead>
<tr>
<th>Ion</th>
<th>Expt. No.</th>
<th>$a$</th>
<th>$K_M$</th>
<th>$b$</th>
<th>$n$</th>
<th>$R^2$</th>
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<th>J_int</th>
<th>M</th>
<th>Jint</th>
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All units of \(\{M\}\) and \(J_{\text{int}}\) were those used in the references. For Experiments 1 and 2, \(J_{\text{int}}\) was expressed in nmol mg\(^{-1}\) min\(^{-1}\); for Experiments 3, 4, and 7 to 9 \(J_{\text{int}}\) was expressed in mg g\(^{-1}\) root dry weight; for Experiments 5 and 6, \(J_{\text{int}}\) was expressed in \(\mu\)mol g\(^{-1}\) root fresh weight h\(^{-1}\). All activities were expressed in \(\mu\)M except for Experiment 6 in which Na\(^+\) activities were expressed in mM.
Table III. Comparison of three models for ion toxicity. For the free ion activity model (FIAM), toxicity is expressed in terms of the activity in the bulk-phase rooting medium according to the equation $RRE = 100/\exp[(\alpha M)_{b}]^{\beta}$). For the surface ion activity model (SIAM), toxicity is expressed in terms of the activity at the PM exterior surface according to the equation $RRE = 100/\exp[(\alpha M)_{0}^{\beta}]$. For the ETM, toxicity is expressed in terms of the PM surface activity and electrical potentials ($\psi_{0}^{\beta}$) according to the equation $RRE = 100/\exp[(\alpha(1 + b \psi_{0}^{\beta}) M)_{0}^{\beta}]$. \(M\) refers to one of the ions below; $\alpha$, $\beta$, and $b$ are coefficients; $\alpha$ is a strength coefficient and $\beta$ a shape coefficient. All of the presented values for coefficients are statistically significant (95% confidence intervals do not encompass zero). All activities were expressed in µM.

<table>
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<th>Ion</th>
<th>Expt. No.</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$b$</th>
<th>n</th>
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<td><strong>Surface Ion Activity Model (SIAM)</strong></td>
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Figure 1. Profile of the electrical potentials and ion distributions at the PM (top part). Conceptualized ion ($M^{n+}$) transport across the PM, including dissociation/association with the active binding site ($R_{cell}$; e.g., transporter, ion channel) on the surface of the PM (bottom part). Lines 1 and 2 illustrate the potential profile through the PM and electric double layer before (line 1) and after (line 2) the addition of depolarizing solutes to the bulk-phase medium. $\psi_0^o$ is the potential difference between the bulk-phase medium and the external PM surface. The $E_m$ is the transmembrane potential difference from the bulk-phase medium to the cell interior. $E_{m, surf}$ is the potential difference through the PM from surface to surface for line 1 ($E_{m, surf}$ not shown for line 2). The cell wall has been omitted for simplicity. Lines 3 (cations) and 4 (anions) represent the profile of activities from the bulk solutions to the PM exterior surface. The $k_a$, $k_d$, and $k_{int}$ are the association, dissociation, and internalization rate constants.
Figure 2. Uptake and toxicity in response to Cu\(^{2+}\) and Ca\(^{2+}\) in wheat seedlings, showing (A) uptake of Cu\(^{2+}\) as a function of Cu\(^{2+}\) activity in the bulk-phase medium (\([\text{Cu}^{2+}]_b\)) at different [CaCl\(_2\)], (B) effect of Ca\(^{2+}\) on EA50\([\text{Cu}^{2+}]_b\) (\([\text{Cu}^{2+}]_b\) causing 50% reduction in root elongation), and (C) effect of Ca\(^{2+}\) on EA50\([\text{Cu}^{2+}]_o\) (PM-surface \([\text{Cu}^{2+}]\) causing 50% reduction in root elongation). Error bars indicate 95% confidence intervals. Different letters indicate significant differences among means (\(p < 0.01\)). Data from Expts. 3 and 11.
Figure 3. Predicted electrical potentials and PM surface activity of Cu$^{2+}$ in responses to Ca$^{2+}$ activity in the bulk-phase medium. (A) The PM surface potentials ($\psi_0^o$) and surface-to-surface transmembrane potentials ($E_{m,surf}$); (B) the calculated PM surface activity of Cu$^{2+}$ at 1.0 µM CuCl$_2$. Data from Expts. 3 and 11.
Figure 4. Uptake (left column) and relative root elongation (RRE, right column) of Cu^{2+} by wheat roots as functions of Cu^{2+} activities in the bulk-phase medium ([Cu^{2+}]_b, first row), Cu^{2+} ion activities at the PM exterior surface ([Cu^{2+}]_o, second row), and Cu^{2+} uptake or RRE calculated from electrostatic models (third row). Uptake and RRE as functions of \( \phi_0 \) and Cu^{2+} surface activity (bottom row). In D and H, the curved surfaces denote uptake or RRE calculated from the electrostatic models whilst the open circles represent the measured values. Data from Expts. 3 and 11.
Figure 5. Measured Ca$^{2+}$ uptake plotted against (A) bulk-phase activities of Ca$^{2+}$ ($[Ca^{2+}]_b$), (B) PM surface activities of Ca$^{2+}$ ($[Ca^{2+}]_0$), (C) Ca$^{2+}$ uptake calculated from the EUM (Eq. 4), or (D) as a function of $\psi_0$ and $[Ca^{2+}]_0$. In D, the curved surface denotes uptake calculated with the EUM whilst the open circles represent the measured uptake. Data were from Huang et al. (1994, 1996) (Expts. 1 and 2), who measured Ca$^{2+}$ influx into PM vesicles from wheat roots in a basal medium of 0.1 mM NaCl and 1.0 mM K$_2$SO$_4$ at pH 7.0 (for La$^{3+}$, Mg$^{2+}$ and Sr$^{2+}$) or pH 4.5 (for Al$^{3+}$). The $E_m$ in all solutions was clamped at a constant –100 mV.
Figure 6. Measured relative root elongation (RRE) of wheat seedlings exposed to Al\(^{3+}\) affected by Ca\(^{2+}\) and Mg\(^{2+}\). Data were taken from Kinraide et al. (1992) (Expt. 14). The RRE is plotted as functions of (A) Al\(^{3+}\) activities in the bulk phase medium (\([Al^{3+}]_b\)), (B) Al\(^{3+}\) activities at the exterior surface of the cell membrane (\([Al^{3+}]_0\)), or (C) calculated Al\(^{3+}\) toxicity based on the ETM, or (D) \(\psi_0\) and \([Al^{3+}]_0\).