Cell Membrane Surface Potential ($\psi_0$) Plays a Dominant Role in the Phytotoxicity of Copper and Arsenate1[W]

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Negative charges at cell membrane surfaces (CMS) create a surface electrical potential ($\psi_0$) that affects ion concentrations at the CMS and consequently affects the phytotoxicity of metallic cations and metalloid anions in different ways. The $\zeta$ potentials of root protoplasts of wheat (Triticum aestivum), as affected by the ionic environment of the solution, were measured and compared with the values of $\psi_0$ calculated with a Gouy-Chapman-Stern model. The mechanisms for the effects of cations (H+, Ca2+, Mg2+, Na+, and K+) on the acute toxicity of Cu2+ and As(V) to wheat were studied in terms of $\psi_0$. The order of effectiveness of the ions in reducing the negativity of $\psi_0$ was H+ > Ca2+ ≈ Mg2+ > Na+ ≈ K+. The calculated values of $\psi_0$ were proportional to the measured $\zeta$ potentials ($r^2 = 0.93$). Increasing Ca2+ or Mg2+ activities in bulk-phase media resulted in decreased CMS activities of Cu2+ ([Cu2+]0) and increased CMS activities of As(V) ([As(V)]0). The 48-h EA50[Cu2+]0 ([Cu2+]0) in bulk-phase media accounting for 50% inhibition of root elongation over 48 h) increased initially and then declined, whereas the 48-h EA50[As(V)]0 decreased linearly. However, the intrinsic toxicity of Cu2+ (toxicity expressed in terms of [Cu2+]0) appeared to be enhanced as $\psi_0$ became less negative and the intrinsic toxicity of As(V) appeared to be reduced. The $\psi_0$ effects, rather than site-specific competitions among ions at the CMS (invoked by the biotic ligand model), may play the dominant role in the phytotoxicities of Cu2+ and As(V) to wheat.

Current environmental quality criteria and risk assessment procedures for metals and metalloids are predominantly based on total or dissolved metal concentrations (De Schamphelaere and Janssen, 2002). However, it is widely recognized that total or dissolved metal concentrations are sometimes poor predictors of bioavailability and toxicity. The physicochemical characteristics of soil and water, such as the contents of common cations and organic matter, have important effects on the bioavailability and toxicity of metals. Therefore, modifying factors for the bioavailability and toxicity should be taken into account in the regulatory frameworks (Peijnenburg et al., 1997).

The biotic ligand model (BLM; Di Toro et al., 2001; De Schamphelaere and Janssen, 2002), as a useful construct for assessing the effects of metals on organisms, has gained increasing attention from both academic scientists and regulators. Recently, the U.S. Environmental Protection Agency (EPA) has incorporated the BLM into its regulatory framework, and some other countries are considering the implications of following suit (Slaveykova and Wilkinson, 2005). The most important assumption of the BLM is that metal toxicity occurs as a result of the reaction of a free metal ion (or other reactive metal species) with binding sites (biotic ligand [BL]) at the cell membrane surface (CMS; Di Toro et al., 2001; Santore et al., 2001). The magnitude of the toxic effect is proportional to the concentration of metal-BL complex. However, the actual toxic lesion may not be the interaction of the toxicant and BL. For example, the BL may be a binding site in a transport channel such that influx of the toxicant is proportional to the number of sites occupied. The actual lesion may occur intracellularly. In fact, the mechanisms of metal intoxication are generally very poorly understood. Decades of investigation have not revealed the principal mechanisms by which Cu2+ and Al3+ inhibit root elongation, for example, despite several known effects ranging from the induced synthesis of reactive oxygen species to alterations of cell membrane structure (effects that often require greater concentrations than those required to inhibit root elongation; Murphy et al., 1999).

The BLM provides a possible mechanism of ionic alleviation of toxicity. The ameliorative effectiveness of major cations, such as H+, Ca2+, Mg2+, Na+, and K+, on
the toxicity of metals has been viewed as a site-specific competition between toxic and ameliorative cations for binding sites at the CMS (Di Toro et al., 2001; Santore et al., 2001; De Schamphelaere and Janssen, 2002). However, the BLM fails to interpret the toxicity of metalloid anions, such as As(V) and selenate, or the enhancement of anion toxicity by the treatments that reduce the toxicity of cations (Yermiyahu and Kinraide, 2005; results presented in this article). Just as the toxic lesion itself may not be directly associated with the BL envisioned in the BLM (see above), the alleviation of cationic toxicity by ameliorative cations may not be related directly to site-specific competitions between toxican and ameliorants (Kinraide, 2006)—a topic considered in this article.

Almost all cell surfaces are intrinsically negatively charged (Kinraide et al., 1998; Shomer et al., 2003; Hassler et al., 2004). These negative charges create negative potentials at the CMS (ψ0), which play important roles in plant-ion interactions (Gimmler et al., 2001; Kinraide, 2001, 2006; Kinraide et al., 2004; Yermiyahu and Kinraide, 2005). The ψ0 controls ion distribution between the CMS and the bulk-phase medium (BM): Negative values for ψ0 concentrate cations and deplete anions at the CMS. For instance, when ψ0 = −45.0 mV, mono-, di-, and trivalent cations will be concentrated, and anions of corresponding negative ions will be depleted 6-, 33-, and 191-fold, respectively. Although the cell wall is negatively charged, it has small effects upon ion activities at the CMS (Kinraide, 2004).

The surface potentials are also controlled by the ionic composition in the BM. Common cations, especially H+, Ca2+, and Mg2+, reduce the negativity of ψ0 by ionic screening and binding. These cations are known to alleviate the uptake and biotic effects of toxic cations (commonly metals; Kinraide, 2006) and to have the opposite effects upon toxic anions (Yermiyahu and Kinraide, 2005). It is difficult to measure ψ0 directly, but the electrical potential (ζ potential) at the hydrodynamic plane of shear at a small distance from the CMS can be determined by electrophoretic mobility (Delgado et al., 2007). In addition, a Gouy-Chapman-Stern (G-C-S) model is now available to calculate the ψ0 of plant cell membranes in response to the solution ionic environment (Kinraide et al., 1998; Kinraide and Yermiyahu, 2007).

The BLM considers the competition of coexisting cations to alleviate the toxicity of toxic cations, but the BLM neglects ψ0, which enriches toxic cations at the CMS. The BLM assumes that toxicity by cations requires them to bind to a hypothetical BL and the alleviation of toxicity by ameliorative cations requires them to bind to the same BL. The assumption might be unrealistic and, if true, difficult to verify. In fact, ψ0 effects can give a false appearance of competition in cases where competition is weak or does not occur at all (Kinraide, 2006). Thus, ψ0 effects should not be negligible and may be more important than the effects of site-specific competition.

Therefore, this study aims to (1) verify that a G-C-S model calculates values for ψ0 of wheat (Triticum aestivum) root that are at least proportional to measured ζ potentials; (2) investigate the role of ψ0 in the effects of common cations on the toxicity (inhibition of root elongation) of Cu2+ and As(V) and the probable mechanisms for the effects; and (3) establish the relationship between the toxicity threshold (EA50, activities producing 50% inhibition) for Cu2+ and As(V) and the ψ0, a relationship that can be used for risk assessment of Cu2+ and arsenate.

RESULTS AND DISCUSSION

Effects of Common Cations on ζ Potentials of Root Protoplasts

Cations in BM reduce the negative potential of the CMS by ionic binding and charge screening (Tatulian, 1999). The ζ potential reflects the electrical potential at the hydrodynamic plane of shear at a small distance from the CMS and, consequently, it is of somewhat lower magnitude than the ψ0. Measured ζ potentials of wheat root protoplasts as exposed with different concentrations of Ca2+, Mg2+, Na+, K+, and H+ are summarized in Supplemental Table S1. As shown in Figure 1, the negativity of ζ potentials declined significantly with increases of monovalent (Na+, K+, H+) and divalent (Ca2+, Mg2+) cations in the BM. The order of effectiveness for reducing the negativity of the ζ potential was H+ > Ca2+ ≈ Mg2+ > Na+ ≈ K+ > H+. This order is a function of ion charge and binding strength to the CMS (Kinraide and Yermiyahu, 2007). These trends can be well quantified with logarithmic equations and r2 values are 1.00, 0.99, 0.76, 0.85, and 0.91 for Ca2+, Mg2+, Na+, K+, and H+, respectively. The ζ potentials were not significantly decreased by Cu2+ at concentrations ≤4 μM.

The G-C-S model, combining classical electrostatic theory (Gouy-Chapman) with ion binding, was developed to calculate the ψ0 (Kinraide et al., 1998). Model parameters were derived or estimated from many studies (see further discussion below). The values of calculated ψ0 were compared with the measured ζ potentials, and significant linear correlations (r2 = 0.93) between the ζ potentials and ψ0 were obtained in all datasets (Fig. 2). This indicates that the adopted model parameters of the G-C-S model are, to a great extent, capable of computing ψ0 values for wheat root that are at least proportional to ζ potentials.

Ion Activities at the CMS

Negative ψ0 enriches cation and depletes anion activities at the CMS. In the studies, assessing Ca-Cu interactions, increasing the bulk activity of Ca2+ ([Ca2+]b) decreased ψ0 negativity from −50.9 to −5.9 mV. As a result, the enrichment factor of Cu2+ activity ([Cu2+]0)/([Cu2+]b) decreased from 51 to 1.6. With the increase of [Ca2+]b, [Ca2+]0 increased initially and then reached a...
plateau (Fig. 3A). In contrast, both \([\text{Mg}^{2+}]_0\) and \([\text{H}^+]_0\) declined markedly (Fig. 3, B and C) despite constant concentrations in the BM. In studies assessing K-Cu interactions, increasing \([\text{K}^+]_b\) from 0.08 to 8.9 mM decreased \(\psi_0\) negativity from -50.3 to -44.5 mV, and consequently the corresponding enrichment factor of Cu\(^{2+}\) activity decreased from 44.6 to 29.7. Meanwhile, \([\text{K}^+]_0\) increased considerably, but \([\text{Ca}^{2+}]_0\), \([\text{Mg}^{2+}]_0\) and \([\text{H}^+]_0\) declined modestly (Fig. 3, D–F). As for As(V), its predominant species at the CMS were \(\text{H}_2\text{AsO}_4^-\) and \(\text{HAsO}_4^{2-}\), which accounted for 98.2% and 1.76%, respectively, at \(\psi_0 = -45 \text{ mV}\). Increased negativity of \(\psi_0\) depleted the activity of \(\text{HAsO}_4^{2-}\) more than that of \(\text{H}_2\text{AsO}_4^-\) at the CMS.

Relative Root Elongation

Relative root elongations (RRLs) were fit with the equation \(\text{RRL} = 100/\exp\left[\frac{a \times T}{b}\right]\) with toxicant (T) expressed as bulk-phase or CMS activities. Figure 4 presents the plots of RRL as a function of \([\text{Mg}^{2+}]_b\) together with \([\text{Cu}^{2+}]_b\), \([\text{Cu}^{2+}]_0\), or \([\text{Cu}^{2+}]_0\). The correlation between root elongation and \([\text{Cu}^{2+}]_0\) (Fig. 4C) is clearly superior to the correlation between root elongation and \([\text{Cu}^{2+}]_b\) (Fig. 4B) or \([\text{Cu}^{2+}]_b\) (Fig. 4A). Substitution of \([\text{Cu}^{2+}]_b\), \([\text{Cu}^{2+}]_0\), or \([\text{Cu}^{2+}]_0\) into the equation yielded \(r^2\) values of 0.81, 0.87, and 0.91, respectively. From Figure 4, A and B, it is clear that the RRL increased initially, but then RRL decreased slightly with increasing \([\text{Mg}^{2+}]_b\) (i.e. black triangles were lower than white circles). Similar results were observed for the Ca dataset (data not shown). The initial alleviation of Cu\(^{2+}\) toxicity could be explained by decreases in \([\text{Cu}^{2+}]_0\) caused by a reduction in the negativity of \(\psi_0\) that accompanied an increase in \(\text{MgCl}_2\). The subsequent increase in toxicity may be due to the decrease of other competitive cations such as Ca\(^{2+}\) and \(\text{H}^+\) at the CMS (see discussion below), whereas \([\text{Mg}^{2+}]_0\) remained constant (i.e. plateaued as did \([\text{Ca}^{2+}]_0\) in Fig. 3A).

For the Cu studies, the 450 data points from all experiments were fit with RRL = 100/\exp\left[\frac{a \times [\text{Cu}^{2+}]_0}{b}\right]. The regression analysis yielded coefficients \(a = 0.0485\) and \(b = 0.96; r^2 = 0.90\). When RRL was assigned a value of 50\%, a corresponding \([\text{Cu}^{2+}]_0\) value of 14.1 \(\mu\text{M}\) was derived. This value is the computed activity of Cu\(^{2+}\)

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**Figure 1.** \(\zeta\) potentials of wheat root protoplasts as a function of the concentration of Ca\(^{2+}\) or Mg\(^{2+}\) (A), Na\(^+\) or K\(^+\) (B), or activity of H\(^+\) in the BM (C). Vertical bars represent the sos.

**Figure 2.** Relationship between the \(\zeta\) potentials and the CMS electrical potentials (\(\psi_0\)) calculated with the G-C-S model. Dashed lines indicate 95% confidence limits.
ion at the CMS that produces 50% inhibition. In the case of arsenate, root elongation was better correlated with $\{\text{As(V)}\}_0$ ($r^2 = 0.93$) than with $\{\text{As(V)}\}_b$ ($r^2 = 0.90$) or $[\text{As(V)}]$ ($r^2 = 0.88$; Fig. 5). Hormesis, a stimulation of response at low doses followed by inhibition at high doses, was observed in all sets in the As series. Therefore, a modified equation of $RRL = 100/\exp[a(\{\text{As(V)}\}_0)^2 + b]$ was used to fit RRL. The regression analysis yielded coefficients $a = 2.26$, $b = 1.10$, and $c = 0.12$; $r^2 = 0.93$ and $n = 360$. When $RRL = 50\%$, a value of $0.434$ mM $\{\text{As(V)}\}_0$ was determined. When the $c_0$ was considered, the activities of ionic toxicants at the CMS rather than their activities in the BM were better predictors of solution Cu$^{2+}$ and arsenate toxicity.

Effects of $\psi_0$ on the Toxicity of Copper and Arsenate

Treatments that reduce the negativity of $\psi_0$, such as increases in salt concentration or decreases in pH, reduce $[\text{Cu}^{2+}]_b$ and increase $[\text{As(V)}]_b$. This alleviates Cu$^{2+}$ toxicity (Fig. 3, A and D), but aggravates As toxicity (Fig. 6, A and D). With the increase of $[\text{Ca}^{2+}]_b$, the 48-h EA50$[\text{Cu}^{2+}]_0$ was significantly increased initially and then reduced (Fig. 3A). This indicates that increasing $[\text{Ca}^{2+}]_b$ initially alleviated and then aggravated the Cu$^{2+}$ toxicity. A similar phenomenon of initial alleviation and subsequent aggravation of toxicity was also observed with increasing $[\text{Mg}^{2+}]_b$ (Fig. 4, A and B, where black triangles were lower than white circles).

The 48-h EA50$[\text{Cu}^{2+}]_0$ instead of the usual EA50$[\text{Cu}^{2+}]_b$ was introduced to possibly distinguish the $\psi_0$ effects from competition effects. In addition, the values of 48-h EA50$[\text{Cu}^{2+}]_0$ were also regarded as the intrinsic toxicity of metals to plants. As seen in Figure 3A, $[\text{Cu}^{2+}]_0$ increased initially and then reached a plateau at about 1.0 mM $[\text{Ca}^{2+}]_b$ as the $[\text{Ca}^{2+}]_b$ increased. However, the 48-h EA50$[\text{Cu}^{2+}]_0$ declined at all times (Fig. 3, B and C), accompanied by decreases in the CMS activities of other cations (Mg$^{2+}$, H$^+$, Na$^+$, and K$^+$). This raises the possibility that the decreased EA50$[\text{Cu}^{2+}]_0$ (Fig. 3, B and C) was due to the decreased competition from Mg$^{2+}$ or some other cation at the CMS (discussed in the next section).

For K$^+$, because it is less depolarizing than Ca$^{2+}$ or Mg$^{2+}$, the $\psi_0$ declined only slightly, from −50.3 to −44.5 mV; and $[\text{Ca}^{2+}]_0$, $[\text{Mg}^{2+}]_0$, and $[\text{H}^+]_0$ declined slightly as $[\text{K}^+]_b$ increased from 0.08 to 8.9 mM (Fig. 3, E and F; $[\text{Mg}^{2+}]_0$ not shown). The slightly decreased EA50$[\text{Cu}^{2+}]_0$ (Fig. 3, E and F) may be related also to the slight decrease of Ca$^{2+}$, Mg$^{2+}$, and H$^+$ at the CMS. The 48-h EA50$[\text{Cu}^{2+}]_0$ was expected to increase about 2-fold (the amount that $[\text{Cu}^{2+}]_0$ was estimated to increase), but it did not. This expected increase in EA50$[\text{Cu}^{2+}]_0$ may have been offset by the weakened competition from Ca$^{2+}$ and Mg$^{2+}$ (discussed in the next section).

For arsenate bioassays, increases of Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, or a decrease of pH resulted in aggravated arsenate toxicity (only the effects of Ca$^{2+}$ and Na$^+$ are shown in Figure 3).
Fig. 6). As shown in that figure, the 48-h EA50\([\text{As(V)}]\) decreased linearly with increasing \([\text{Ca}^{2+}]\) \((r^2 = 0.96)\) and \([\text{K}^{+}]\) \((r^2 = 0.59)\). However, the 48-h EA50\([\text{As(V)}]\) was better corrected with \(\psi_0\) than with \([\text{Ca}^{2+}]\) or \([\text{K}^{+}]\).

The linear regression for EA50\([\text{As(V)}]\) versus \(\psi_0\) yielded \(r^2 = 1.00\) for the Ca set and 0.77 for the K set (Fig. 6, B and E). The EA50\([\text{As(V)}]\) increased slightly with increasing \([\text{Ca}^{2+}]\), \([\text{Mg}^{2+}]\), or \([\text{H}^{+}]\), whereas the values of EA50\([\text{As(V)}]\) did not change significantly as \([\text{Na}^{+}]\) or \([\text{K}^{+}]\) increased (Figs. 6, C and F, and 8A).

Figure 4. RRL in response to \(\text{Cu}^{2+}\) and \(\text{Mg}^{2+}\). \(\text{Mg}^{2+}\) is expressed as concentration in the rooting medium and \(\text{Cu}^{2+}\) is expressed as concentration in the medium \(([\text{Cu}^{2+}])_b\); A), activity in the medium \((\{\text{Cu}^{2+}\})_b\); B), or activity at the CMS \((\{\text{Cu}^{2+}\})_c\); C).

Figure 5. RRL in response to arsenate \([\text{As(V)}]\) concentration in the medium \(([\text{As(V)}])_b\); A), activity in the medium \((\{\text{As(V)}\})_b\); B), or activity at the CMS \((\{\text{As(V)}\})_c\); C).
These effects may be related to the different physiological mechanisms of these ions; the root length in control treatments of each set increased with the increasing Ca\textsuperscript{2+} or Mg\textsuperscript{2+}, but did not change significantly with the increasing Na\textsuperscript{+} or K\textsuperscript{+} (data not shown).

Mechanisms for the Cation Effects on the Toxicity of Copper and Arsenate

The results of this study and previous studies indicate several mechanisms for ion-toxicant interactions. These are listed below. These mechanisms are intended to account for ionic alleviation or aggravation of intoxication by ionic toxicants. Some commonly studied cationic toxicants include H\textsuperscript{+}, Cu\textsuperscript{2+}, Pb\textsuperscript{2+}, Hg\textsuperscript{2+} (and other heavy metals), trivalent metals (La\textsuperscript{3+}, but especially Al\textsuperscript{3+}), Na\textsuperscript{+} (at higher concentrations), and some anions (such as selenate and arsenate). The first mechanism listed below is the principal mechanism considered in our study, and we consider it to be very well substantiated now.

1) Electrostatic interactions: The cationic components of salts commonly cause reductions in the negativity of CMS electrical potentials (\(\psi_0\)). The anionic components (commonly Cl\textsuperscript{−} or SO\textsubscript{4}\textsuperscript{2−}) generally have small effects because of weak binding to the CMS and small concentrations at the CMS because of electrostatic repulsion. The depolarizing effectiveness of some common cations follows the order Al\textsuperscript{3+} > H\textsuperscript{+} > Ca\textsuperscript{2+} > Mg\textsuperscript{2+} > Na\textsuperscript{+} ≈ K\textsuperscript{+}, depending upon charge and strength of binding to the CMS. The reduced negativity of \(\psi_0\) causes reductions at the CMS of cationic toxicants and increases of anionic toxicants. In some cases, these effects appear to account almost entirely for ion-toxicant interactions (Yermiyahu and Kinraide, 2005; Kinraide, 2006).

2) Site-specific competition: This mechanism accounts for ion-toxicant interactions according to the BLM, at least for cation-cation and anion-anion interactions. The operation of mechanism 1 above does not negate the operation of site-specific competition. Addition of Ca\textsuperscript{2+} to an intoxicating Cu\textsuperscript{2+} solution could both reduce [Cu\textsuperscript{2+}]\(_{0}\) and displace Cu\textsuperscript{2+} from surface ligands through direct competition. SO\textsubscript{4}\textsuperscript{2−}, although it would do little to enhance \(\psi_0\) negativity, could compete with SeO\textsubscript{4}\textsuperscript{2−} for binding sites, including transport sites. The electrostatic interactions and the site-specific competition models may be combined by incorporating into the equations of the BLM (\(I^f\)) instead of \(I^f\), where \(I^f\) is any ion I of charge Z (Kinraide, 2006).
(3) Other mechanisms: One may envision several additional ion-toxicant interactions. Consider a case where one ion, say Ca\(^{2+}\), alleviates Cu\(^{2+}\) toxicity according to the two mechanisms above, but a third ion, say Mg\(^{2+}\), alleviates toxicity by an additional mechanism (perhaps through an especially great affinity for a site involved in Cu\(^{2+}\) uptake, but not a site to which Cu\(^{2+}\) binds). Additions of Ca\(^{2+}\) to solutions of constant \([\text{Cu}^{2+}]_b\) and \([\text{Mg}^{2+}]_b\) would alleviate toxicity by reducing \([\text{Cu}^{2+}]_0\) but would enhance toxicity by reducing \([\text{Mg}^{2+}]_0\).

The apparent enhancement of intrinsic Cu\(^{2+}\) toxicity illustrated in Figure 7A and the apparent alleviation of intrinsic arsenate toxicity illustrated in Figure 8A are problematic. A simple explanation is a systematic computational error caused by an overestimate of \(R^*\), which is the CMS density of negative charges in units \(\mu\text{mol/m}^2\). The use of smaller values for \(R^*\) for the computation of \(\phi_0\) reduce the slopes in both Figures 7A and 8A without reducing the slopes in Figures 7B and 8B. Alternative explanations for the apparent change in the intrinsic toxicities may be related to the changes in the activities of other influential ions, both positive and negative, as suggested in mechanism 3 above.

A slight enhancement of intrinsic Cu\(^{2+}\) toxicity by Ca\(^{2+}\) has been noted previously (Kinraide, 2006). Even more significant, and not subject to modeling error, is the Ca\(^{2+}\) enhancement of extrinsic toxicity (expressed in terms of \([\text{Cu}^{2+}]_b\)) noted by Lock et al. (2007a), as well as possible enhancements by Ca\(^{2+}\) of extrinsic Co\(^{2+}\) toxicity (Lock et al., 2007b) and extrinsic Ni\(^{2+}\) toxicity (Lock et al., 2007c). Despite these occasional oddities, which are now under investigation, a very large number of studies (for review, see Kinraide, 2006; Yermiyahu and Kinraide, 2005) demonstrate the superiority of expressing both the toxicity and uptake of cations and anions in terms of CMS activities rather than in terms of BM activities.

### Prediction of Copper and Arsenate Toxicity

As shown in Figures 7B and 8B, the 48-h EA50s (expressed as cupric and arsenate ion activities in BM) can be predicted on the basis of \(\phi_0\) calculated with the G-C-S model. For Cu\(^{2+}\) toxicity, the 48-h EA50 can be expressed as \(\text{EA50}[\text{Cu}^{2+}]_b = \exp(0.27\phi_0 + 0.36)\) (n = 25). The dashed line in A presents a predicted EA50\([\text{Cu}^{2+}]_b\) value of 14.1 \(\mu\text{M}\); the full line in B presents the function \(\ln(\text{EA50}[\text{Cu}^{2+}]_b) = 0.27\phi_0 + 0.36\) (n = 25); and the dashed lines in B present 95% confidence limits.

The G-C-S Model

The G-C-S model combines classical electrostatic theory (Gouy-Chapman theory) with ion binding so that the electrical potential at the CMS (\(\psi_0\)) can be computed (Kinraide et al., 1998; Tatulian, 1999). The \(\psi_0\) appears to be little influenced by the cell wall (Kinraide, 2004). Up-to-date parameters for the model are presented in Kinraide and Yermiyahu (2007). On the basis of several lines of evidence, including \(\zeta\) potential measurements and adsorption measurements, we are quite confident of model parameters relating to ion binding strength at the CMS (Kinraide and Yermiyahu, 2007). Another critical parameter, the surface charge density of negative charges (\(R^\ast = 0.3074\ \mu\text{mol/m}^2\) is currently assumed in our model) appears to be more variable and may be influenced by species, tissue, and preparation in the case of vesicles and protoplasts. Consequently, the values for \(\psi_0\) and the CMS ion activities computed from \(\psi_0\) with the Nernst equation, may be only proportional to the actual values. (Figure 2 in Kinraide et al. [1998] illustrates this proportionality.) This limitation appears not to reduce seriously the great superiority of expressing plant-ion interactions (including uptake, toxicity, and the alleviation of toxicity) in terms of CMS ion activities rather than bulk-phase ion activities (Kinraide, 2001, 2006; Yermiyahu and Kinraide, 2005).

CONCLUSION

The parameters adopted in the G-C-S model for computing \(\psi_0\) can be substantially verified by measured \(\zeta\) potentials. The \(\text{Ca}^{2+}\) depolarizing effectiveness on reducing the negativity of \(\psi_0\) was the same as that by \(\text{Mg}^{2+}\). The \(\psi_0\) provides a new avenue for exploring the mechanisms of ion interactions and the biotic effects of toxic ions. In fact, ignoring the electrostatic effects (tantamount to assuming \(\psi_0 = 0\)) must surely lead to greater errors of interpretation than the small errors (relative to assuming \(\psi_0 = 0\)) in estimates of \(\psi_0\).

An increase in cations or a decrease in pH in BM reduces the negativity of \(\psi_0\), reduces the surface activity of \(\text{Cu}^{2+}\), and increases the surface activity of arsenate by electrostatic mechanisms. As a result, the extrinsic toxicity of \(\text{Cu}^{2+}\) was alleviated, whereas the extrinsic toxicity of arsenate was aggravated. Effects upon the intrinsic toxicities of \(\text{Cu}^{2+}\) and arsenate may have been opposite to the effects upon extrinsic toxicity, but those effects are not certain and require further study. We also analyzed published studies (Lock et al., 2007a, 2007b, 2007c) and obtained compatible results. Exploring the mechanisms for an apparent enhanced intrinsic toxicity was under way. In addition, only cereals are discussed in this article, the results applying to measurements in dicots require further study.

MATERIALS AND METHODS

Experimental Design

In order to verify the reliability of the G-C-S model for calculating the \(\phi_0\) and to investigate the independent effect of different cations on \(\text{Cu}^{2+}\) and arsenate toxicity, only one cation concentration varied at a time while all other cation concentrations were kept as nearly constant as possible. Seven sets of \(\zeta\) potential measurements were determined, one each for \(\text{Ca}^{2+}\), \(\text{Mg}^{2+}\), \(\text{Na}^+\), \(\text{K}^+\), \(\text{pH}\), and \(\text{Cu}^{2+}\) (Supplemental Table S1).

For \(\text{Cu}^{2+}\) toxicity tests (Cu series), five sets of \(\text{Cu}^{2+}\) bioassays were performed, one each for \(\text{Ca}^{2+}\), \(\text{Mg}^{2+}\), \(\text{Na}^+\), \(\text{K}^+\), and \(\text{pH}\) (Supplemental Table S2). In each medium, five \(\text{Cu}\) concentrations (0.25, 0.50, 1.0, 2.0, 4.0 \(\mu\text{M})\) and a control were tested. For the arsenate toxicity tests, five sets of bioassays were performed, one each for \(\text{Ca}^{2+}\), \(\text{Mg}^{2+}\), \(\text{Na}^+\), \(\text{K}^+\), and \(\text{pH}\) (Supplemental Table S3). In each medium, five arsenate concentrations (0.67, 1.3, 3.3, 6.7, 26.7 \(\mu\text{M})\) and a control were tested. The selected cation concentrations reflected the variability occurring in natural soil solutions (Swab, 2009).

Preparation of the Test Solutions

In all experiments, the salts used were analytical grade and deionized water was used throughout. Except for pH tests, medium was buffered with 2.0 mM MES adjusted to pH 6 with NaOH (Lock et al., 2007a, 2007b). For each toxicity assay, CuCl\(_2\) and NaH\(_2\)AsO\(_4\) were added to the prepared test medium. All media were prepared 1 d before the start of tests to obtain near-equilibrium solutions. The chemical characteristics of different test media are summarized in Supplemental Tables S2 and S3. Solution pH was measured before and after each test. The concentrations of Cu were determined by graphite furnace atomic absorption spectrophotometry (Varian 220). The concentrations of arsenate and other cations (Ca, Mg, Na, and K) were determined by inductively coupled plasma-atomic emission spectroscopy (POEMS-II; TJ). Preliminary tests showed that the measured concentrations did not differ significantly from their nominal value and variability of concentrations before and after 24 h of test was found to be 10%.

Determination of \(\zeta\) Potentials of Root Protoplasts

Two-day-old seedlings with uniform root length were transferred to the culture solution containing 0.25 mM CaCl\(_2\), 0.25 mM MgCl\(_2\), 0.5 mM NaCl, and 0.5 mM KCl. The medium was buffered with 2.0 mM MES adjusted to pH 6 with NaOH. Seedlings were grown at 25 \(^\circ\text{C}\) for 2 d. Wheat (Triticum aestivum) root tips were collected, washed with distilled water, and cut into 1- to 2-mm sections. Three grams of root material were incubated for 12 h in 30 mL of enzyme solution containing 2% cellulase Onozuka R-10 (w/v), 1% pectinase (w/v), 1% bovine serum albumin (w/v), 1% polyvinylpyrrolidone (w/v), 0.5 mM mannitol, 2.0 mM MES, 0.25 mM CaCl\(_2\) at pH 6.0. The mixture of protoplasts and enzyme solution was filtered through two-layer nylon net (0.1-mm pore diameter) and the filtrate was centrifuged at 800 rpm for 5 min. The supernatant was discarded and the protoplasts in the residue were suspended and washed by three successive additions of 15 mL of a protoplast washing solution containing 0.4 mM mannitol, 2.0 mM MES, and 25 \(\mu\text{M}\) CaCl\(_2\) at pH 6.0. After washing, the protoplasts in the residue were suspended again in 10 mL of protoplast washing solution. Twenty milliliters of 20% Suc were injected into the bottom of the centrifuge tube with a long-needled syringe for density gradient centrifugation. The mixture was centrifuged at 800 rpm for 5 min and the pure protoplasts were collected from the two-phase solution interface. The collected protoplasts were suspended again and washed several times with protoplast washing solution. The final preparation contained at least 5,000 pure protoplasts/mL.

The protoplasts were suspended (at least \(10^7\) protoplasts/mL) in the test medium (Supplemental Table S1) and incubated for 1 h at 25 \(^\circ\text{C}\) ± 1 \(^\circ\text{C}\). The \(\zeta\) potentials of the root protoplasts were measured using a J94H microelectrophoresis meter (Powereach Instruments; Wang et al., 2008). The equilibrated suspension was agitated and transferred to the electrophoresis vessel after the electrode was wetted to avoid disturbance caused by air bubbles. The migration of the protoplast particles under a potential gradient of about 10
As for arsenate, $H_2AsO_4$ is toxic to saturate growth-inhibitory processes. The RL at which $S$ is nearly equal to RL at (Kinraide et al., 2004). If growth is limited by $T$, then $RRL = 100/\exp\left(\frac{-c}{RT}\right)$]. The resulting curves are often negatively sigmoidal and have been expressed by a Weibull equation, such as the activity of free cupric or arsenate ion at the time of seedling transfer to the test medium. Growth can be plotted against $T$ and $B$ to give $RRL = 100/\exp\left(\frac{-c}{RT}\right)\left[\frac{\exp\left(\frac{-c}{RT}\right)}{R} T^B\right]$. The chemical equilibrium program (EPA) was used to compute the activity of free cupric or arsenate ion in BM, denoted $EA50({T}_{b})$, or the activity at the CMS ($EA50({T}_0)$). The resulting curves are often negatively sigmoidal and have been expressed by a Weibull equation (Kinraide et al., 2004). If growth is limited by $T$, then $RRL = 100/\exp\left(\frac{-c}{RT}\right)$, where $a$ and $b$ are curve-fitting parameters.

### Computation of Ion Activities at the CMS

The activity of ion $I^Z$ in the CMS ($I^Z_0$) was computed from the activity of $I^Z$ in the BM ($I^Z_b$) according to the Nernst equation ($I^Z_0 = I^Z_b \exp\left[\frac{Z\varphi_s}{RT}\right]$). The $\varphi_s$ was calculated with a G-C-S model (Kinraide et al., 1998; Yermiyahu and Kinraide, 2005, 2007). The resulting curves are often negatively sigmoidal and have been expressed by a Weibull equation (Kinraide et al., 2004). If growth is limited by $T$, then $RRL = 100/\exp\left(\frac{-c}{RT}\right)$, where $a$ and $b$ are curve-fitting parameters.

### Data Treatment and Statistics

Ion species and activities were calculated using the visual MINTEQ (version 2.51) chemical equilibrium program (EPA). The equilibrium phases in the precipitation calculation included atmospheric CO$_2$ ($P_{CO_2} = 10^{-3.3}$ atm). As for arsenate, $H_2AsO_4$ (90.6%) and $HAAsO_4$ (9.36%) are the predominant and thermodynamically stable species at pH 6.0. The 48-h EA50 values expressed as the activity of free cupric or arsenate ion in BM, denoted $EA50(Cu^{2+})$, or $EA50(As(V))$, are then calculated from the observed root growth at each calculated free cupric or arsenate ion activity in the BM ($Cu^{2+}_b$), or $As(V)_b$). The 48-h EA50 values expressed as the activity of free cupric or arsenate ion in the CMS, denoted $EA50(Cu^{2+})$, or $EA50(As(V))$, were then calculated from $EA50(Cu^{2+})$, or $EA50(As(V))$, incorporated into the Nernst equation (see above). EA50s were calculated by fitting a sigmoid curve to the dose-effect relationships according to the model of Haanstra et al. (1985). $RRL$ was related to toxicant intensity in different phases (e.g. $T_L$, $T_H$, $T_{0}$) by regression analysis ($RRL = 100/\exp\left(\frac{c}{RT}\right)$) using Origin Professional 6.0. All the values for the regression coefficients in the Results and Discussion section are at a significance level of $P < 0.05$.

### Supplemental Data

The following materials are available in the online version of this article.

#### Supplemental Table S1.
Measured $\zeta$ potentials of wheat root protoplasts as exposed with different ion environments.

#### Supplemental Table S2.
Copper toxicity assays.

#### Supplemental Table S3.
Arsenate toxicity assays.

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**LITERATURE CITED**


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