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Alleviation of Cu and Pb Rhizotoxicities in Cowpea (*Vigna unguiculata*) as Related to Ion Activities at Root-Cell Plasma Membrane Surface

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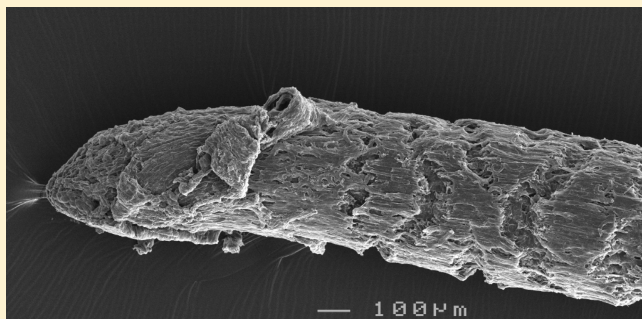
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S Supporting Information

ABSTRACT: Cations, such as Ca and Mg, are generally thought to alleviate toxicities of trace metals through site-specific competition (as incorporated in the biotic ligand model, BLM). Short-term experiments were conducted with cowpea (*Vigna unguiculata* L. Walp.) seedlings in simple nutrient solutions to examine the alleviation of Cu and Pb toxicities by Al, Ca, H, Mg, and Na. For Cu, the cations depolarized the plasma membrane (PM) and reduced the negativity of ψ_o° (electrical potential at the outer surface of the PM) and thereby decreased $\{Cu^{2+}\}_o^\circ$ (activity of Cu^{2+} at the outer surface of the PM). For Pb, root elongation was generally better correlated to the activity of Pb^{2+} in the bulk solution than to $\{Pb^{2+}\}_o^\circ$.

However, we propose that the addition of cations resulted in a decrease in $\{Pb^{2+}\}_o^\circ$ but a simultaneous increase in the rate of Pb uptake (due to an increase in the negativity of $E_{m,surf}$, the difference in potential between the inner and outer surfaces of the PM) thus offsetting the decrease in $\{Pb^{2+}\}_o^\circ$. In addition, Ca was found to alleviate Pb toxicity through a specific effect. Although our data do not preclude site-specific competition (as incorporated in the BLM), we suggest that electrostatic effects have an important role.



INTRODUCTION

Trace metals at elevated levels are toxic to both plants and animals in aquatic and terrestrial environments. However, various cations, and especially Ca^{2+} and Mg^{2+} , have been reported to alleviate the phytotoxicities of trace metals. Toxicity alleviation by ameliorants is widely attributed to site-specific competition, a concept incorporated into the biotic ligand model (BLM). The BLM assumes that the adverse effect of a toxicant is determined by the degree of binding to the site of action, specifically the plasma membrane (PM) (the 'biotic ligand').¹ Thus, the BLM assumes that the beneficial effects of ameliorating cations arise from competition between the ameliorant and the toxicant at the biotic ligand.^{1–3} Although initially developed for aquatic systems, the BLM has been extended to terrestrial environments (TBLM), including plants.^{4–6}

The PM expresses a negative electrical potential (ψ_o°) which influences the composition of the solution at the PM surface.⁷ Specifically, there is an increase in the activity of cations and a decrease in the activity of anions over that in the bulk solution

(hereafter, the activity of the intoxicating metal cation M^z in the bulk solution is designated as $\{M^z\}_b$ while its activity at the outer surface of the PM is $\{M^z\}_o^\circ$, where z is the charge of the ion). Further, the magnitude of ψ_o° is changed by the ionic composition of the solution bathing the root. That is, the addition of cations (such as Ca^{2+} and Mg^{2+}) reduces the negativity of ψ_o° with a concomitant decrease in $\{M^z\}_o^\circ$ when z is negative.⁷ Therefore, it is possible that the beneficial effects of ameliorants may be related to changes in ψ_o° rather than to site-specific competition *per se*. Indeed, there is increasing evidence to support this suggestion (for example, see Silva et al.⁸ and Kinraide⁷).

The aim of the current study was to examine the alleviating effects of Al, Ca, H, Mg, and Na on the toxicities of

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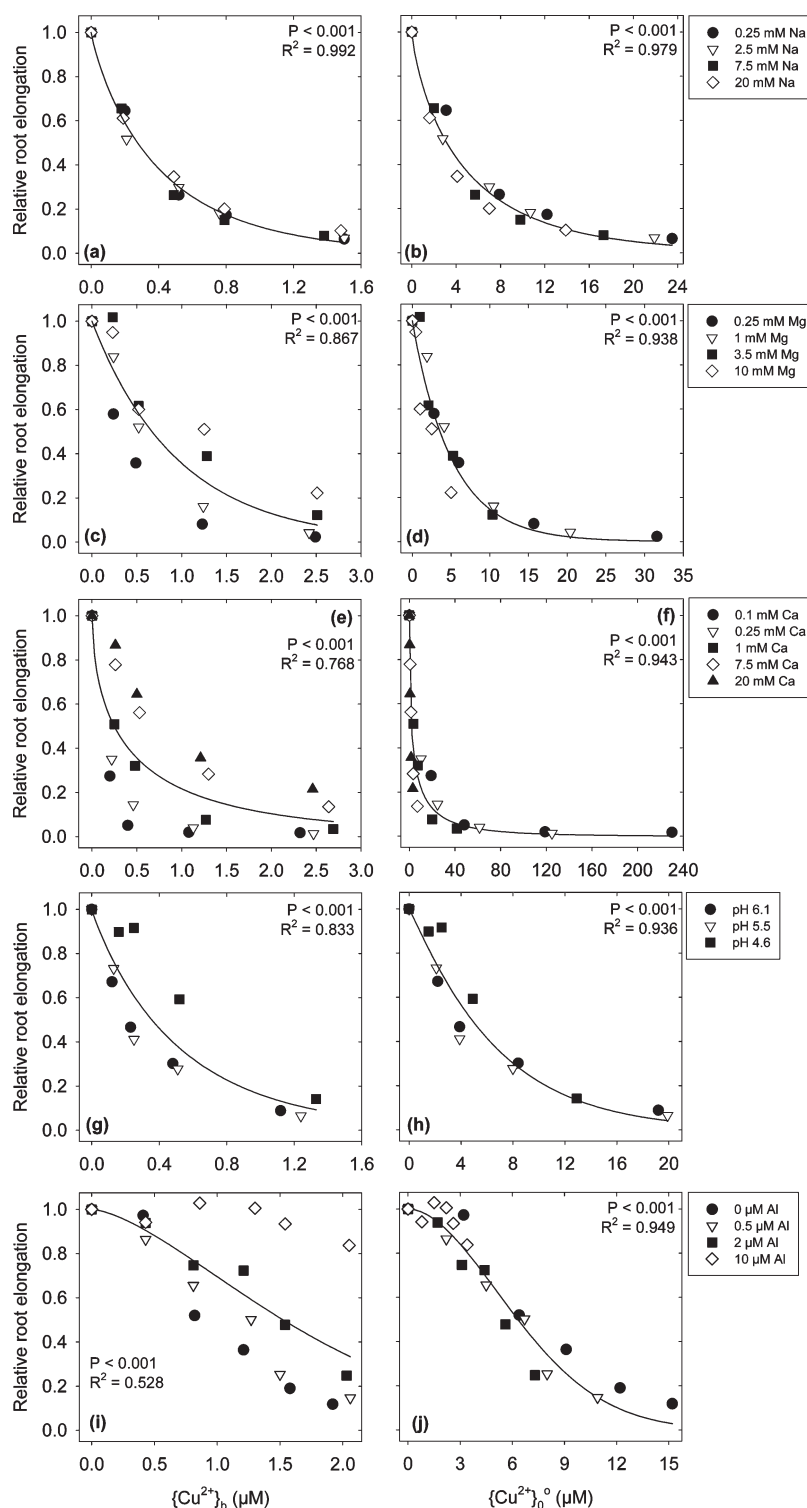


Figure 1. Influence of Na (a,b), Mg (c,d), Ca (e,f), H⁺ (g,h), and Al (i,j) concentrations on the elongation of cowpea roots exposed to Cu for 48 h. Root elongation is related to either the activity of the metal ion in the bulk solution, $\{Cu^{2+}\}_b$ (left), or its activity at the plasma membrane surface, $\{Cu^{2+}\}_o$ (right). The legends apply to the two panels on their left. All coefficients are given in Supporting Information Table S2.

Cu and Pb in short-term experiments using simple nutrient solutions. Reductions in root growth were considered relative to both $\{M^z\}_b$ and $\{M^z\}_o$. This was done to improve the understanding of the mechanism(s) by which cations alleviate the adverse effects of toxicants and to assist in the prediction of interactive effects.

MATERIALS AND METHODS

General Experimental Procedures. Solution culture experiments were conducted in a laboratory maintained at 25 °C at The University of Queensland, St. Lucia, Australia. The experiment was conducted as described by Kopittke et al.,⁹ with 3-d-old

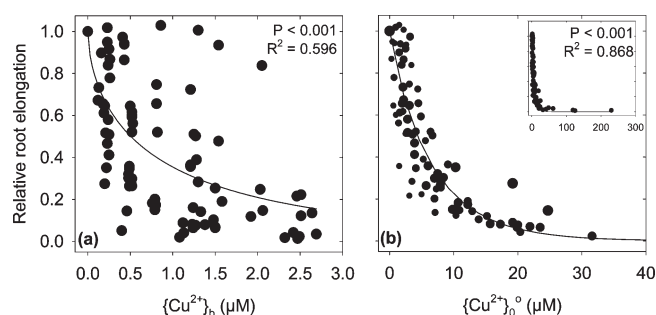


Figure 2. Elongation of cowpea roots exposed to Cu for 48 h, with all data from Figure 1 combined (i.e., from Na, Mg, Ca, H, and Al). Root elongation is related to either the activity of the metal ion in the bulk solution, $\{Cu^{2+}\}_e$ (left), or its activity at the PM surface, $\{Cu^{2+}\}_o$ (right). In (b), the size of the symbol is proportional to the ψ_o° ; symbols are larger at more negative values. All coefficients are given in Supporting Information Table S2.

cowpea (*Vigna unguiculata* L. Walp. cv. White Caloona) seedlings grown in Perspex strips (seven seedlings per strip) on top of 600 mL glass beakers with a basal solution of 1 mM $CaCl_2$ and 5 μM H_3BO_3 . The seedlings were grown for 18 h in this basal solution before transfer to the treatment solutions (in different beakers) for a further 48 h growth. Root length was assessed with digital photographs using ImageTool 3.0 (UTHSCSA, <http://ddstdx.uthscsa.edu/dig/itdesc.html>).

Unless otherwise stated, all solution concentrations given for Cu, Pb, and Al are the average values measured (0 and 48 h) by inductively coupled plasma mass spectroscopy (ICP-MS) (see Kopittke et al.⁹). Concentrations of Cu and Pb tended to decrease during the 48 h experimental period, presumably due to uptake/adsorption by the roots, and possibly through adsorption by the glass beakers. Across all treatments, Cu decreased by an average of 16% and Pb by 21% (Al concentrations did not decrease notably from 0 to 48 h). Solution concentrations of Ca, Mg, and Na were not measured; preliminary experiments had shown that measured values were close to the nominal values and that there was no detectable change in concentration during the 48-h experimental period.

Effects of Al, Ca, H, Mg, and Na on Toxicities of Cu and Pb.

A series of experiments were conducted to examine the effect of increasing concentrations of Al, Ca, H, Mg, and Na on the toxic effects of Cu and Pb to cowpea roots. The experiment consisted of 208 treatments replicated once, with seven roots per replicate (Supporting Information Table S1). The ameliorants (Al, Ca, H, Mg, and Na) were added at rates that reduced root elongation by <15% when compared to roots in a 1 mM $CaCl_2$ solution (data from preliminary experiment). Therefore, Al was supplied at four concentrations (ca. 0, 0.5, 2, and 10 μM), Ca at five concentrations (0.1, 0.25, 1, 7.5, and 20 mM), H^+ at three activities (pH 6.0, 5.4, and 4.6, corresponding to 1.0, 4.0, and 25 μM H^+), Mg at four concentrations (0.25, 1, 3.5, and 10 mM), and Na at four concentrations (0.25, 2.5, 7.5, and 20 mM). For each of these treatments, the toxicants (i.e., Cu or Pb) were supplied at five rates; the rates used for each treatment were varied so that the bulk solution activities were similar. For the Al-treatments, Cu or Pb were supplied at six rates.

For solutions containing Al, the standard experimental procedure was varied slightly as it was found that growth was reduced temporarily (<6 h) upon initial exposure to Al. Following the initial 18 h growth in 1 mM $CaCl_2$, the Perspex strips were

transferred into the treatment beakers without Cu or Pb (i.e., the beakers contained Ca, B, and Al at pH 4.5). After a further 24 h, and while the strip was removed from the beaker for photography, the Cu or Pb was added to the beaker (thereafter designated as '0 h'). The seedlings were grown in the Cu- or Pb-containing solutions for a further 48 h.

Solution pH (ca. 5.4) was not adjusted in any treatment except for solutions containing Al (pH 4.5) or the H-treatments. Solution pH was measured at the time of transfer (0 h) and after 48 h, and where necessary, adjusted using 0.1 M HCl (or 0.1 M HNO_3) or saturated $Ca(OH)_2$ (ca. 0.02 M). For treatments where the pH was modified, pH was also measured and readjusted as necessary after 24 h. Chloride-salts were used for Cu and Al, and nitrate-salts for Pb (AR-grade, Sigma Aldrich). Speciation with Phreeqc¹⁰ indicated that ca. 80% of Pb was present as Pb^{2+} in the treatment with the highest ionic strength when nitrate-salts were used (the remaining species largely $PbNO_3^+$), compared to 60% had chloride-salts been used (the remaining species largely $PbCl^+$).

Calculations of ψ_o° and $\{M^z\}_o$ were performed using the GCS computer program (available from T.B. Kinraide). Details of the computer program are provided in the Supporting Information. The GCS program was modified to allow calculation of activity coefficients using the extended Debye–Hückel equation rather than the Davies equation. The binding constant for Cu ($K_{R,Cu} = 400$) was taken from Kinraide,⁷ while the value for Pb ($K_{R,Pb} = 447$) was calculated from Kinraide.¹¹

Relative root elongation (RRE, i.e. the rate of root elongation relative to growth in the corresponding toxicant-free treatment) was analyzed using regression analysis, fitting curves of the form

$$RRE = 1/\exp[(c\{M^z\})^d] \quad (1)$$

where $\{M^z\}$ represents the activity of the ion either in the bulk solution ($\{M^z\}_e$) or at the outer surface of the PM ($\{M^z\}_o$), and c and d are coefficients determined by the model (see Kinraide⁷ for a discussion). Regression analyses were conducted using SYSTAT 12, and curves were plotted where R^2 values were ≥ 0.5 . No coefficients are reported where the 95% confidence interval encompassed zero.

RESULTS

Effect of Alleviating Cations on ψ_o° Negativity. The degree to which the addition of the alleviating cations was predicted to reduce ψ_o° negativity followed the order $Na < Ca = Mg < H < Al$ (Supporting Information Figure S1). Although both Ca and Mg had a similar influence on the negativity of ψ_o° , the range of Ca concentrations was greater than for Mg, and the Mg solutions contained a basal Ca concentration of 1 mM while the Ca-only solutions contained as little as 0.1 mM Ca.

Cu Toxicity. Root growth during the 48 h experimental period was good, with root elongation in a solution containing 1 mM Ca averaging 1.4 mm/h. In the 0 μM Cu and Pb controls, the addition of alleviating cations (i.e., Na, Mg, Ca, H, and Al) did not substantially alter root growth, with root elongation in these control solutions ranging between 1.2 and 1.5 mm/h.

The addition of Na had no apparent influence on the toxicity of Cu, with the addition of up to 20 mM Na having negligible influence on toxicity (Figure 1a). In contrast, the addition of 10 mM Mg, 20 mM Ca, 25 μM H^+ , or 10 μM Al resulted in decreases in the toxicity of Cu when related to the $\{Cu^{2+}\}_e$ (Figure 1c,e,g,i). While the effect on root growth was more

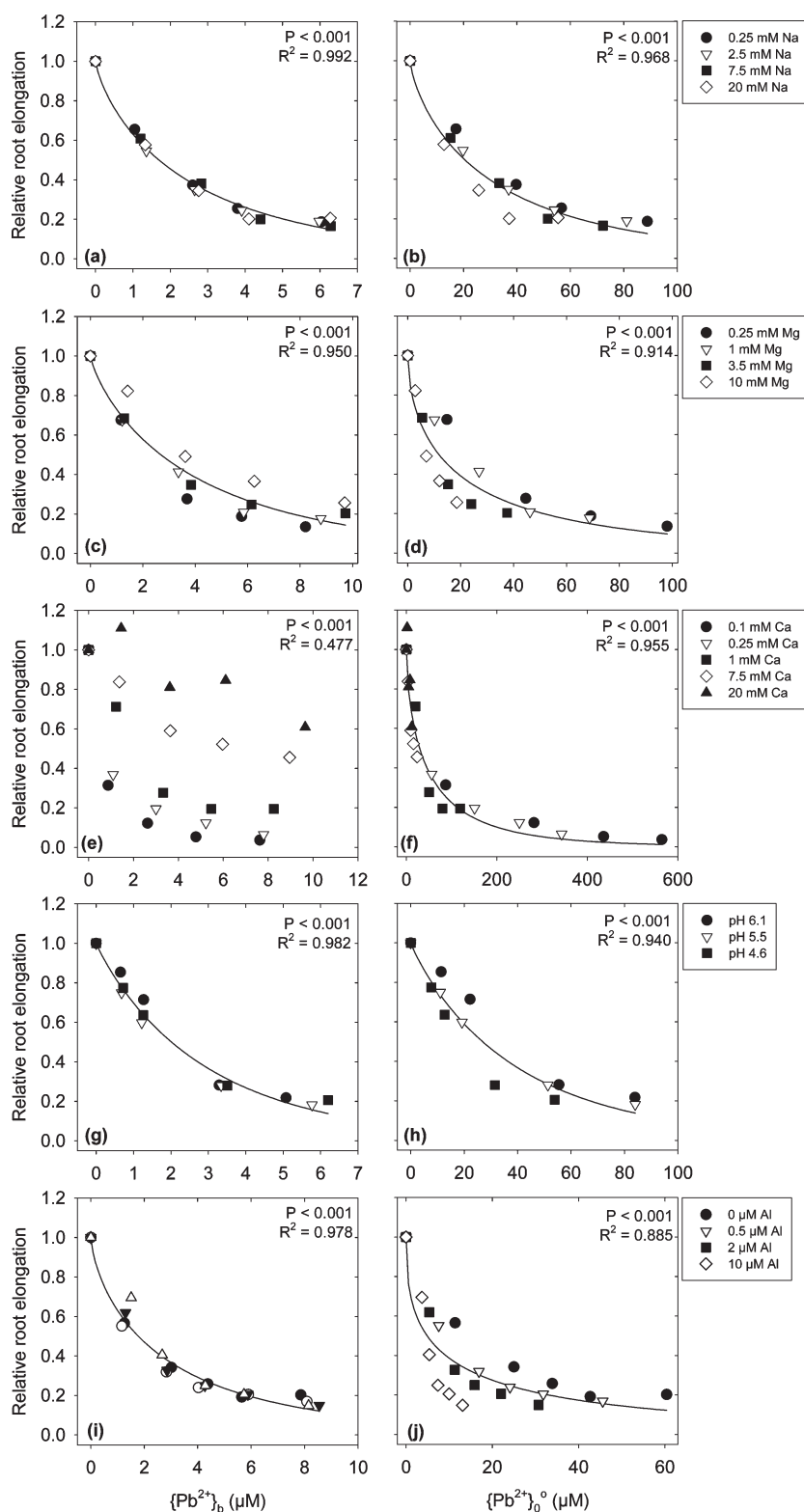


Figure 3. Influence of Na (a,b), Mg (c,d), Ca (e,f), H^+ (g,h), and Al (i,j) concentration on elongation of cowpea roots exposed to Pb for 48 h. Root elongation is related to either the activity of the metal ion in the bulk solution, $\{Pb^{2+}\}_b$ (left), or its activity at the PM surface, $\{Pb^{2+}\}_o$ (right). All coefficients are given in Supporting Information Table S2.

pronounced for Ca than Mg, the range of Ca concentrations (0.1 to 20 mM) was greater than for Mg (0.25 to 10 mM with basal 1 mM Ca). Thus, for Cu toxicity, alleviatory effects of other cations could not be entirely explained by changes in $\{Cu^{2+}\}_b$

($R^2 = 0.596$, Figure 2a). Indeed, the EA_{50-b} (i.e., the effective bulk activity causing a 50% reduction in growth) values varied by ca. 1 order of magnitude depending upon the addition of alleviating cations, ranging from 0.14 to $>2.8 \mu M \{Cu^{2+}\}_b$.

For any given alleviatory cation (except Na), correlation between root growth and the activity of Cu^{2+} improved when related to $\{\text{Cu}^{2+}\}_0^\circ$ rather than $\{\text{Cu}^{2+}\}_b$ (Figure 1 and Figure 2). For example, for Ca addition to Cu solutions the R^2 value improved from 0.768 for $\{\text{Cu}^{2+}\}_b$ to 0.943 for $\{\text{Cu}^{2+}\}_0^\circ$ (Figure 1e,f). Further, root elongation was strongly correlated with the $\{\text{Cu}^{2+}\}_0^\circ$ regardless of the alleviating cation and its concentration ($R^2 = 0.868$, Figure 2b). These results suggest that the alleviation of Cu toxicity caused by the addition of these cations results from changes in $\{\text{Cu}^{2+}\}_0^\circ$, is nonspecific, and varies according to the extent to which each cation reduces the negativity of ψ_0° . Using the fitted curves, an $\text{EA}_{50 \text{ p.m.}}$ value (i.e., the effective PM activity causing a 50% reduction in growth) can be calculated as $4.4 \mu\text{M} \{\text{Cu}^{2+}\}_0^\circ$ (Figure 2b). This value is similar to the value of $5 \mu\text{M} \{\text{Cu}^{2+}\}_0^\circ$ by Kinraide et al.¹² in wheat (*Triticum aestivum* L.).

Pb Toxicity. In contrast to Cu toxicity, it appeared that only Ca influenced the toxicity of Pb (Figure 3). Indeed, additions of Na (Figure 3a), Mg (Figure 3c), H (Figure 3g), or Al (Figure 3i) had no apparent influence on root growth in Pb-solutions when related to the $\{\text{Pb}^{2+}\}_b$. Further, other than for Ca, correlations between root growth and Pb^{2+} activity were generally worse for $\{\text{Pb}^{2+}\}_0^\circ$ than for $\{\text{Pb}^{2+}\}_b$ (compare Figure 3i,j). Interestingly the converse was true for Ca-alleviation; root elongation was more highly correlated to $\{\text{Pb}^{2+}\}_0^\circ$ than to $\{\text{Pb}^{2+}\}_b$, with R^2 improving from 0.477 to 0.955 (Figure 3e,f). These observations regarding Pb toxicity raise two important questions: (1) why is $\{\text{Cu}^{2+}\}_0^\circ$ a better predictor than $\{\text{Cu}^{2+}\}_b$ while $\{\text{Pb}^{2+}\}_b$ is generally better than $\{\text{Pb}^{2+}\}_0^\circ$ (a reduction in $\{\text{M}^{2+}\}_0^\circ$ from a decrease in negativity of ψ_0° is nonspecific), and (2) why does Ca apparently alleviate Pb toxicity but Na, Mg, H, and Al do not.

Wang et al.¹³ suggest that a change in ψ_0° has a dual effect on the uptake of metals and metalloids by plant roots: (i) it influences the activity of the metal at the PM surface (eq 1), and (ii) it influences the surface-to-surface transmembrane potential difference, $E_{\text{m,surf}}$ (i.e., the difference in potential between the inner and outer surfaces of the PM). It is noteworthy that $E_{\text{m,surf}}$ provides the driving force for cation transport across the PM, and hence a change in $E_{\text{m,surf}}$ influences the uptake of cations. Thus, the depolarization of the PM causes a decrease in ψ_0° which results in a decrease in the activity of cations at the outer surface of the PM but a simultaneous increase in the $E_{\text{m,surf}}$ (i.e., increase in the driving force for cation uptake) – these two factors at least partially offsetting each other. Thus, to investigate the possible role of $E_{\text{m,surf}}$ in Pb toxicity, data were fitted to

$$\text{RRE} = 1/\exp([c_0(1 + c_1\psi_0^\circ)\{\text{Pb}^{2+}\}_0^\circ]^d) \quad (2)$$

In this equation, ψ_0° is linearly related to $E_{\text{m,surf}}$ given that the transmembrane electrical potential from bulk medium to cell interior (E_{m}) likely remained essentially constant under the conditions of our experiments^{14,15} (see Wang et al.¹³ for more details). Fitting the Pb-data (other than for Ca) to eq 2 yielded an R^2 value of 0.965 (cf. R^2 of 0.830 when considering only $\{\text{Pb}^{2+}\}_0^\circ$ and not $E_{\text{m,surf}}$, Figure 4b), with $c_0 = 0.127$, $c_1 = 0.024$, and $d = 0.771$ (Figure 5a,b and Supporting Information Table S2). The positive value of c_1 (0.024) indicates that a decrease in the negativity of ψ_0° (i.e., an increase in the negativity of $E_{\text{m,surf}}$ and hence an increase in the driving force for cation uptake across the PM) enhanced toxicity and decreased root elongation. However, this R^2 value decreased from 0.965 to 0.890 upon inclusion of the Ca-alleviation data (Figure 5a,b,c). Indeed, it was

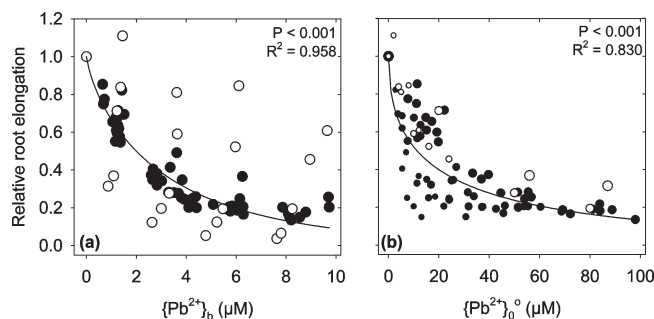


Figure 4. Elongation of cowpea roots exposed to Pb for 48 h, with all data from Figure 3 combined other than for the Ca treatments (i.e., from Na, Mg, H, and Al). Root elongation is related to either the activity of the metal ion in the bulk solution, $\{\text{Pb}^{2+}\}_b$ (left), or its activity at the PM surface, $\{\text{Pb}^{2+}\}_0^\circ$ (right). In (b), the size of the symbol is proportional to the ψ_0° ; symbols are larger at more negative values. Data for the Ca treatments (white symbols) are presented but are not included in the regression analyses. All coefficients are given in Supporting Information Table S2.

noted that at any given values for $\{\text{Pb}^{2+}\}_0^\circ$ and ψ_0° (as a surrogate for $E_{\text{m,surf}}$), measured root elongation tended to be higher than predicted from eq 2 (Figure 5c). To investigate whether Ca had an additional (specific) alleviatory effect, eq 2 was modified

$$\text{RRE} = 1/\exp([c_0(1 + c_1\psi_0^\circ)\{\text{Pb}^{2+}\}_0^\circ/(1 + e\{\text{Ca}^{2+}\}_0^\circ)]^d) \quad (3)$$

Equation 3 incorporates a term specific for Ca where an increase in $\{\text{Ca}^{2+}\}_0^\circ$ influences Pb toxicity. Fitting all Pb-data (including Ca) to eq 3 yielded an R^2 value of 0.963, with $c_0 = 0.179$, $c_1 = 0.012$, $e = 0.324$, and $d = 0.809$ (Figure 5d and Supporting Information Table S2). As noted previously, the positive value of c_1 (0.012) indicates that a decrease in the negativity of ψ_0° enhanced the toxicity of Pb, but the positive value of e (0.324) indicates that an increase in $\{\text{Ca}^{2+}\}_0^\circ$ reduced toxicity and increased root elongation.

To further investigate the role of Ca in the alleviation of Pb toxicity, data were fitted to

$$\text{RRE} = 1/\exp([c_0\{\text{Pb}^{2+}\}_b/(1 + e\{\text{Ca}^{2+}\}_b)]^d) \quad (4)$$

Equation 4 is based upon eq 1 but incorporates a term specific for Ca (as does eq 3 when using PM activities). Fitting all Pb-data (including Ca) to eq 4 yielded an R^2 value of 0.949, with $c_1 = 1.183$, $e = 3.693$, and $d = 0.751$ (as for eq 3, the positive value of e (3.693) indicates that an increase in $\{\text{Ca}^{2+}\}_b$ reduced toxicity). Thus, the R^2 value for this equation with bulk activities (0.949) was marginally lower than when considering PM activities and the dual effects of ψ_0° (eq 3, R^2 of 0.963).

It is noteworthy that the Cu-data were also fitted to eq 2 to determine if Cu toxicity was influenced solely by $\{\text{Cu}^{2+}\}_0^\circ$ (eq 1, Figure 2b), or if $E_{\text{m,surf}}$ was also important. Interestingly, the addition of a term to account for changes in $E_{\text{m,surf}}$ (eq 2) improved the correlation marginally, from an R^2 value of 0.868 (Figure 2b) to 0.871 with the coefficient c_1 significantly different to zero (Supporting Information Table S2). Again, the positive value for c_1 (0.008) indicates that a decrease in the negativity of ψ_0° (i.e., an increase in the negativity of $E_{\text{m,surf}}$ and hence an increase in the driving force) enhanced toxicity. However, this

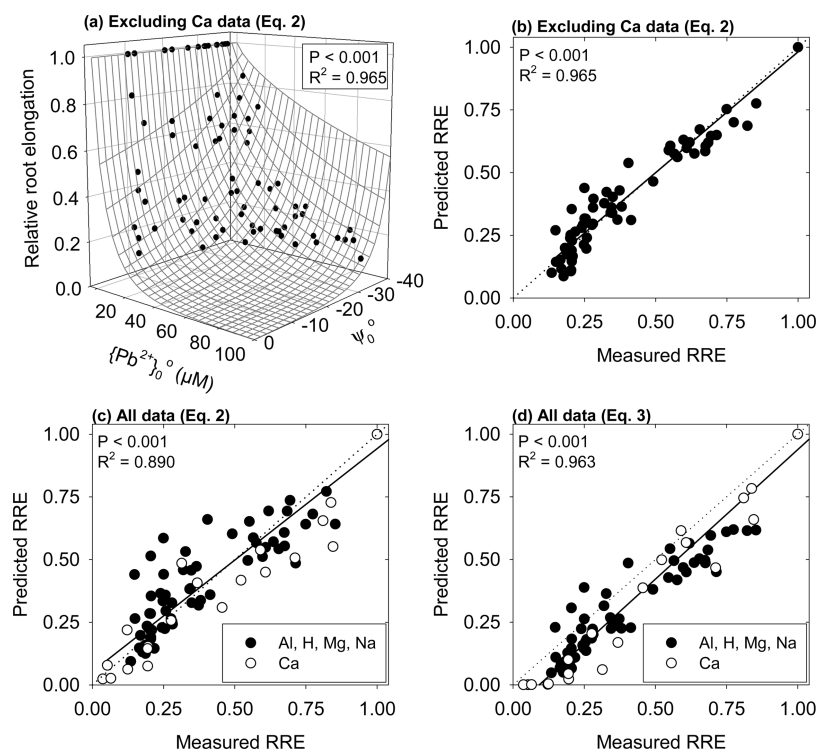


Figure 5. Comparison of measured and predicted values for relative root elongation (RRE) for roots exposed to Pb (expressed as the activity of Pb^{2+} at the outer surface of the PM, $\{\text{Pb}^{2+}\}_o^\circ$). In (a) and (b) data were analyzed using eq 2 (i.e., incorporating the dual effects of ψ_o°) although the Ca-alleviation data were excluded from the analysis, while in (c) the Ca-alleviation data were included in the analysis. For (d) data were analyzed using eq 3 (i.e., incorporating the dual effects of ψ_o° plus a term for specific alleviation by Ca). In (b), (c), and (d), the solid line is the linear regression, and the dotted line is the 1:1 line. (b) $y = 0.96x + 0.017$; (c) $y = 0.89x + 0.053$; (d) $y = 1.0x - 0.095$. All coefficients are given in Supporting Information Table S2.

effect was not as pronounced as it was for Pb where R^2 values improved from 0.830 to 0.965.

DISCUSSION

The addition of cations to the growth solution tended to alleviate the toxicity of Cu in cowpea, although the degree to which the toxicity was alleviated by different cations varied. Generally, this alleviatory affect could not be explained by changes in $\{\text{Cu}^{2+}\}_b$ (see left-hand panels in Figure 1 and Figure 2). Thus, the addition of cations did not alleviate Cu toxicity by an ionic strength effect (i.e., an increase in ionic strength decreasing the activity of the free metal ion in the bulk phase). Rather, their addition reduced the negativity of ψ_o° and thereby decreased $\{\text{Cu}^{2+}\}_o^\circ$ (see right-hand panels of Figure 1 and Figure 2). Indeed, when the data were combined for all alleviating cations, and in contrast to $\{\text{Cu}^{2+}\}_b$, a good correlation was observed between root growth and $\{\text{Cu}^{2+}\}_o^\circ$ irrespective of the alleviating cation (R^2 of 0.868 versus 0.596, Figure 2a,b).

In contrast to Cu, the addition of Na, Mg, H, and Al did not improve root growth of cowpea in Pb-toxic solutions when expressed as $\{\text{Pb}^{2+}\}_b$ (Figure 3 and Figure 4). Two hypotheses are offered regarding these observations for Pb. First, it is possible that the mechanism of Pb toxicity differs and that it is indeed determined by $\{\text{Pb}^{2+}\}_b$ rather than $\{\text{Pb}^{2+}\}_o^\circ$, and that the alleviation of Pb toxicity by Ca (Figure 3e) is a specific effect (the apparent relationship for $\{\text{Pb}^{2+}\}_o^\circ$ with Ca-alleviation may be somewhat coincidental). However, a potential difficulty with this hypothesis is that it is not apparent why Pb toxicity would not be influenced by nonspecific changes in ψ_o° (and $\{\text{Pb}^{2+}\}_o^\circ$) as

are toxicities of the other metals, including for Al, As(V), Cu, Zn.^{7,12,16} Thus, a second hypothesis is offered regarding Pb toxicity; the toxicity of Pb was determined by three factors: (i) $\{\text{Pb}^{2+}\}_o^\circ$, (ii) $E_{m,\text{surf}}$ and (iii) $\{\text{Ca}^{2+}\}_o^\circ$ (Figure 5a,b,c,d). The first two of these factors are influenced by ψ_o° (and are hence termed the ‘dual effects of ψ_o° ’); a decrease in the negativity of ψ_o° decreases $\{\text{Pb}^{2+}\}_o^\circ$ but increases the driving force for the transport across the PM ($E_{m,\text{surf}}$).¹³ As before, $\{\text{Ca}^{2+}\}_o^\circ$ is important because it appears to alleviate Pb toxicity through a specific mechanism (Figure 5a,b,c,d). For this second hypothesis, it is somewhat coincidental that $\{\text{Pb}^{2+}\}_b$ is a better predictor than $\{\text{Pb}^{2+}\}_o^\circ$ (although the effects of $\{\text{Pb}^{2+}\}_o^\circ$ and $E_{m,\text{surf}}$ partially offset each other and potentially give the appearance of no alleviation). This hypothesis is consistent with the study of Wang et al.¹³ who reported that these dual effects of ψ_o° could account for the uptake and/or toxicity of a range of nutrients and toxicants, including Al, As(V), Ca, Cd, Cu, Na, Ni, and Zn. Regardless, it was observed that Ca alleviated Pb toxicity more than could be accounted for simply from changes in $\{\text{Pb}^{2+}\}_b$ (Figure 3e) or from the dual effects of ψ_o° (Figure 5c). This Ca-alleviation of Pb toxicity is thus a specific effect as is the case of H^+ and Na^+ toxicity¹² and possibly results from competition between Ca^{2+} and Pb^{2+} due to similarities in their hydrated ionic radii (412 pm for Ca^{2+} and 401 pm for Pb^{2+} ¹⁷) (further work specifically investigating Ca–Pb interactions are required).

The results discussed above suggest that the alleviation of Cu toxicity by Al, Ca, H, Mg, and Na is due to a nonspecific reduction in the negativity of ψ_o° resulting in a decrease in $\{\text{Cu}^{2+}\}_o^\circ$, and this is consistent with previous reports.^{7,16} For Pb, we propose that the addition of cations also caused a reduction in the

negativity of ψ_0° (as for Cu), although the corresponding decrease in $\{Pb^{2+}\}_0^\circ$ was largely offset by an increase in $E_{m,surf}$ (the driving force for transport across the PM) which increased Pb uptake and increased toxicity. This additional effect for Pb (i.e., increased toxicity from an increase in negativity of $E_{m,surf}$) was not observed to the same extent for Cu. Although the coefficient c_1 was positive in eq 2 for Cu, R^2 improved only marginally from 0.868 to 0.871. This difference between Cu and Pb regarding the potential role of $E_{m,surf}$ results perhaps from a difference in the mechanism through which these two metals are toxic in cowpea. For example, it is possible that the primary site of Cu toxicity is in the cell wall or at the PM, while the primary site of Pb toxicity is in the cytoplasm (and hence transport across the PM is required for toxicity). However, further investigations are required in this regard. Interestingly, recent studies using fresh, hydrated cowpea roots have identified that Cu accumulates almost entirely extracellularly and that this is where it appears to exert its toxic effect (at least in part).^{9,18} However, these results regarding the importance of $E_{m,surf}$ for Cu in the present study are in contrast to that reported for Cu rhizotoxicity in wheat and pea (*Pisum sativum* L.),¹³ thereby suggesting the possibility of differences among species.

Although it is well-established that Ca, Mg, and H alleviate metal toxicities, few authors have reported that the addition of low rates of Al alleviates metal toxicities in intact plants; Fortunati et al.¹⁹ reported that Al alleviated Cu toxicity in wheat, and Liebigh et al.²⁰ reported Al alleviated Cu toxicity in citrus (scientific name not stated). This scarcity of information likely results from the fact that while Al is effective at alleviating toxicities (due to its pronounced effect on the negativity of ψ_0° , Supporting Information Figure S1), it is also highly rhizotoxic and hence even small additions of Al may result in toxicity. To our knowledge, the results presented here are the first demonstration that the addition of Al at rates not sufficient to cause a reduction in short-term root growth can markedly alleviate toxicities of other metals in plants; the addition of ca. 10 μ M Al did not reduce root growth in Cu-free solutions (roots grew at an average of 1.3 mm/h in both the 10 μ M Al-treatment and the corresponding control) but improved relative root growth from 12% to 83% at 2.0 μ M $\{Cu^{2+}\}_b$ (Figure 1j).

Finally, it is important to note some of the limitations of the present study. The plants used in the current study were only seedlings, and were 5-d-old at the conclusion of the study. Hence, these plants were not physiologically mature, and most of the nutrients were supplied from the seed reserve rather than from solution. In addition, the nutrient solutions were free of organic ligands, the presence of which would likely alter the properties of the root surface.^{21,22} Therefore, care must be taken when extrapolating the present results to other conditions.

■ ASSOCIATED CONTENT

Supporting Information. Composition of the 208 solutions investigated, coefficients for all regressions, and the effect of cations on the calculated ψ_0° . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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