# Magnitude and kinetics of metal rhizotoxicity in cowpea

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## Abstract

Many difficulties exist in establishing the concentrations of metals in solution that are toxic to the growth of plant roots. To limit these difficulties, short-term solution culture experiments were conducted using the same technique on 3-d-old cowpea (*Vigna unguiculata* (L.) Walp.) cv. Caloona seedlings. These were grown for 48 h in solutions with ca. 1000 µM Ca and 5 µM B plus one of 24 metals, concentrations of which were determined after filtering (0.22 µm). The decrease in root elongation rate (RER) varied markedly among the metals tested, with a 50 % reduction in RER (i.e. EC50) evident at 0.02 µM Ag to 132 mM K. The rapidity with which RER was reduced varied also, as did the symptoms of rhizotoxicity. A range of metals caused rupturing of the rhizodermis and outer cortex in the roots elongation and transition zones within 2 – 24 h of exposure. These metals were all highly rhizotoxic, but not all highly rhizotoxic metals caused ruptures. We conclude that rhizotoxicity results from disruption of a range of underlying biochemical mechanisms. There were some common effects, but no metal could be considered an analogue for another in all respects.

## Key Words

Cell wall, plasmalemma, root, rymptoms, toxicity.

## Introduction

In the early 16th Century, Paracelsus stated: “All things are poison and nothing is without poison, only the dose permits something not to be poisonous”. This applies equally to metals of no known benefit to biota (e.g. Hg, Pb) and known for centuries to be toxic, as well as to those that are essential for life (e.g. Cu, Mn).

Kopittke *et al.* (Submitted) reviewed 34 y of published information on the toxicity of eight metals, and included only those data that met strict criteria which allowed comparison with other studies. There was decreasing trace metal toxicity in the order of Pb ≈ Hg > Cu ≈ Cd > As ≈ Co ≈ Ni ≈ Zn > Mn. Even with strict criteria for inclusion, there was an order of magnitude difference in the concentration of each metal reported to be toxic. For example, the 25 – 75 percentile phytotoxic range for Cd was 0.99 to 10 µM.

It is difficult to compare results on metal phytotoxicity across studies because of differences in (i) experimental conditions and (ii) sensitivity of plant genotypes to one or more metals. With regard to experimental conditions, increased concentrations of other ions reduce the toxicity of a metal in solution. This results from three mechanisms: (i) reduced electronegativity of the plasma membrane (PM), (ii) restoration of $Ca^{2+}$ activity at the PM, and (iii) other unknown mechanisms (Kinraide 2004). An example of this last-named mechanism is the alleviation of $Zn^{2+}$ toxicity by low concentrations (≤ 5 µM) of Mg (Pedler *et al.* 2004). The range in sensitivity among genotypes to elevated Mn in solution, for example, was demonstrated by Edwards and Asher (1982). This study showed that across 13 crop and pasture species, the external Mn concentration needed to reduce plant dry mass by 10 % varied from 1.4 µM in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) to 65 µM in sunflower (*Helianthus annuus* L.). To limit problems arising from differences in technique or genotypic sensitivity, this paper reports results using the same solution culture procedure on the rhizotoxic effects of 24 metals, both essential and non-essential, in cowpea seedlings.

## Methods

Solution culture experiments were conducted to determine root growth of 3-d-old cowpea cv. Caloona seedlings over a 48 h period when exposed to elevated concentrations of a range in metals (Kopittke *et al.* 2008). Initially, seedlings were grown for 16 h in continuously-aerated solutions nominally containing 1000 µM Ca (as $CaCl_2$ or $Ca(NO_3)_2$) and 5 µM B (as $H_3BO_3$) at ca. pH 5. After this acclimatisation period, the seedlings (seven per 650 mL of solution) were transferred to solutions with the same concentrations of Ca and B plus one of 10 nominal concentrations of 24 metals. Solutions were sampled at the beginning and end of the experimental period (i.e. 48 h); mean data are presented. In some cases there was considerable loss of metal from solution, making it difficult to determine the actual concentration of metal detrimental to root growth. In these instances, mean measured values at the beginning and end of the experiment was used. The 10 mL samples were filtered to 0.22 µm (Millipore, Millex-GS), acidified with 20 µL concentrated HCl or $HNO_3$. The concentrations of metals were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectroscopy (ICP-MS), or by flow injection mercury system atomic absorption spectroscopy (FIMS-AAS, for Hg). Solution pH was measured at the beginning and end of the experimental period, but was not adjusted in any instance (other than for Al, where pH was reduced to 4.5 in all treatments using 0.1 M HCl).

Seedlings were grown for 48 h and digital images captured at the time of transfer (0 h) and 4, 8, 12, 24, 36, and 48 h thereafter. The length of each root in each treatment was measured using ImageTool 3.0. These data were used to calculate the root elongation rate (RER) of each root for each time period. All roots were harvested after 48 h growth, and stored in 10 % ethanol in deionised water prior to examination using light microscopy. In some instances, scanning electron microscopy (SEM) was used to produce high resolution images of the symptoms evident close to the root tip. There were two replicates of each treatment, with data being presented as either the mean ± standard error (SE) or as non-linear relationships estimated by GenStat 7.2 (GenStat 2003). Metal ions were classified according to The Chemogenesis Web Book available at http://www.meta-synthesis.com/webbook/43_hsab/HSAB.html.

## Results

The EC50 for cowpea RER ranged from 0.02 µM Ag to 130 mM K using the same technique to assess metal rhizotoxicity (Table 1). It is noteworthy also that some hard, soft, and borderline metals were highly rhizotoxic, and that this classification provided no clear distinction among metals in the magnitude of their rhizotoxic effects. However, the least rhizotoxic metals were all hard metals – but not necessarily those essential for plant growth.

**Table 1. Rhizotoxicity of 24 metal ions in cowpea in decreasing order of toxicity, with their classification as hard (H), soft (S) or borderline (B) cations and whether (✓) or not (✗) they cause ruptures in the root's elongation and transition zones.**

| Metal Ion | Hard, soft, or borderline | Ruptures | EC50 (µM) |
|---|---|---|---|
| Ag(I) | S | ✓ | 0.02 |
| Tl(I) | S | ✗ | 0.44 |
| Cu(II) | B | ✓ | 0.5 |
| Hg(II) | S | ✓ | 1.0 |
| In(III) | H | ✓ | 1.5 |
| Ni(II) | B | ✗ | 1.5 |
| Cd(II) | S | ✗ | 2.0 |
| La(III) | H | ✓ | 2.0 |
| Sc(III) | H | ✓ | 2.0 |
| Gd(III) | H | ✓ | 3.0 |
| Cs(I) | H | ✗ | 3.1 |
| Pb(II) | B | ✗ | 3.5 |
| Co(II) | H | ✗ | 4.0 |
| Ru(III) | H | ✓ | 10 |
| Zn(II) | B | ✗ | 28 |
| Al(III) | H | ✓ | 30 |
| Ga(III) | H | ✓ | 30 |
| Ba(II) | H | ✗ | 3200 |
| Sr(II) | H | ✗ | 3400 |
| Li(I) | H | ✗ | 12000 |
| Mg(II) | H | ✗ | 25000 |
| Ca(II) | H | ✗ | 48000 |
| Na(I) | H | ✗ | 69000 |
| K(I) | H | ✗ | 130000 |

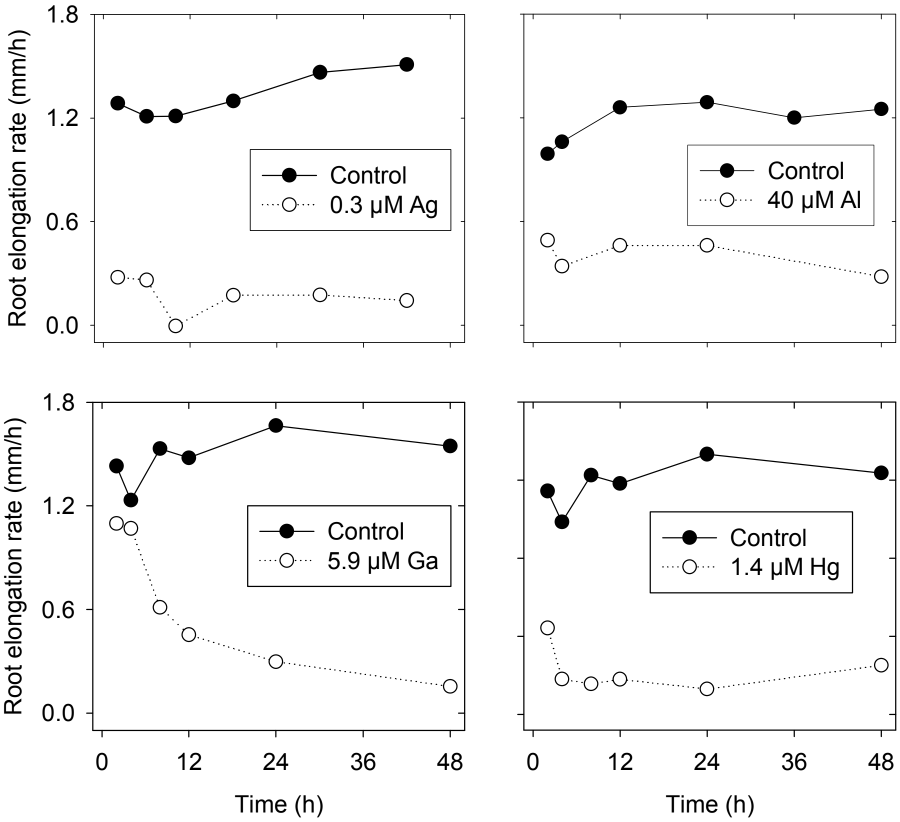

**Figure 1. Kinetics of reduced root elongation rate (RER) in cowpea seedlings exposed to Ag, Al, Ga, and Hg sufficient to reduce root elongation by ca. 85 %.**

Besides a reduction in RER, 10 metals caused ruptures to the rhizodermis and outer cortex (Table 1). This occurred with many, but not all, of the highly rhizotoxic metals. None of the least rhizotoxic metals did so, however, and these were all hard metals. Classification of metals as hard, soft, and borderline did not discriminate among the metals that rupture cowpea roots.

There were marked differences in the rapidity with which the metals reduced root growth. It was evident that Ag, Al, and Hg reduced root growth within 4 h of exposure (Figure 1). In contrast, there was a continuingly detrimental effect of Ga on root growth for up to 48 h.

Some metals caused ruptures to the rhizodermis and outer cortex of the root's transition and elongation zones (Figure 2). These ruptures were evident over an extended range of 30 – 600 µM Al, but this was not the case with most metals (e.g. 0.85 to 1.8 µM Cu or 2.0 to 5.5 µM La) (Kopittke *et al.* 2008). This study also showed that rupturing occurred within 4 h of exposure to the EC50 Al concentration, but only after 24 h on exposure to Cu and La also at EC50. It appeared that no ruptures developed where metal concentrations caused complete cessation of root elongation. This suggests that ruptures result from a decrease in wall loosening of the outer cells in the root's elongation zone while cells of the stele and inner cortex continue to grow.

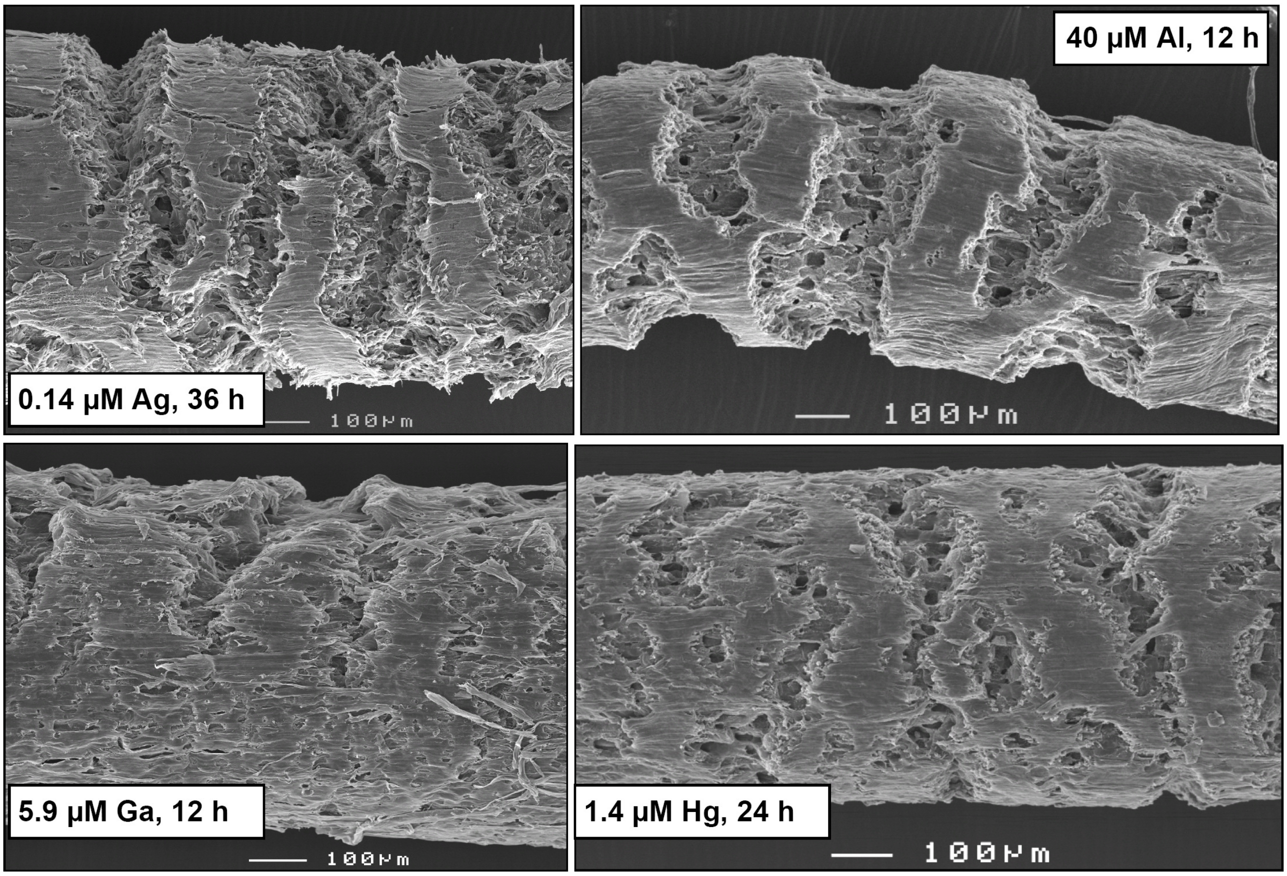

**Figure 2. Ruptures developed in the rhizodermis and outer cortex of the elongation and transition zones when exposed to rhizotoxic concentrations of some metals (Table 1) as evident in scanning electron micrographs of roots exposed to Ag, Al, Ga, and Hg.**

## Conclusions

A range of over six orders of magnitude in EC50 rhizotoxicity was found, from 0.02 µM Ag to 132 mM K, among the 24 metals tested using the same technique. It was evident that elevated concentrations of the metals caused a range in effects, with many (but not all) highly rhizotoxic metals causing ruptures to the epidermis and outer cortex. We conclude that the metals' rhizotoxic effects result from the disruption of many underlying biochemical mechanisms, with some commonality among some metals. However, no metal could be considered an analogue for another in all respects.

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