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REVIEW PAPER

Trace metal phytotoxicity in solution culture: a review

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Abstract

Solution culture has been used extensively to determine the phytotoxic effects of trace metals. A review of the literature from 1975 to 2009 was carried out to evaluate the effects of As(V), Cd(II), Co(II), Cu(II), Hg(II), Mn(II), Ni(II), Pb(II), and Zn(II) on plants grown in solution. A total of 119 studies was selected using criteria that allowed a valid comparison of the results; reported toxic concentrations varied by five orders of magnitude. Across a range of plant species and experimental conditions, the phytotoxicity of the trace metals followed the trend (from most to least toxic): Pb≈Hg > Cu >Cd≈As >Co≈Ni≈Zn >Mn, with median toxic concentrations of (μM): 0.30 Pb, 0.47 Hg, 2.0 Cu, 5.0 Cd, 9.0 As, 17 Co, 19 Ni, 25 Zn, and 46 Mn. For phytotoxicity studies in solution culture, we suggest (i) plants should be grown in a dilute solution which mimics the soil solution, or that, at a minimum, contains Ca and B, (ii) solution pH should be monitored and reported (as should the concentrations of the trace metal of interest), (iii) assessment should be made of the influence of pH on solution composition and ion speciation, and (iv) both the period of exposure to the trace metal and the plant variable measured should be appropriate. Observing these criteria will potentially lead to reliable data on the relationship between growth depression and the concentration of the toxic metal in solution.

Key words: Critical concentration, phytotoxicity, solution culture, trace metal.

Introduction

Trace metals are natural components of the environment, but elevated and potentially toxic levels sometimes occur. Some soils contain trace metals at naturally elevated concentrations, such as with Ni in soils formed from ultramafic (serpentine) minerals (Anderson *et al.*, 1973; Batianoff and Singh, 2001). In acid soils, elevated levels of soluble metals (particularly Al or Mn) may occur, whilst high concentrations of other metals (such as Cu or Pb) may be present in sites contaminated by agriculture, mining, industry, or transport. The presence of excess trace metals represents a serious environmental and financial problem, with *c*. 30% of the world's land affected by acidity (Sumner and Noble, 2003) and hundreds of thousands of metal-contaminated sites worldwide which require remediation at an estimated cost of up to US$35 billion (CEI, 2005). (Although strictly a metalloid, As will be grouped with the other trace metals for the purposes of this study.)

Many trace metals (such as Cu, Mn, and Zn) are essential for the growth of plants and animals, but are toxic at elevated concentrations. There are numerous reviews in the literature which examine the influence of trace metals on plant growth and function (for example, see Clemens, 2006; Babula *et al.*, 2008). However, underlying all studies is the requirement to expose the plant to a toxic, but appropriate, concentration of the trace metal. For example, some solution culture studies have used up to 1000 μM of the trace metal, despite published data demonstrating a complete cessation of growth at concentrations as low as 1 μM (see later discussion). There is a need, therefore, to establish criteria for determining which data on trace metal phytotoxicity in solution culture are likely to be reliable and to summarize these high-quality data.

Whilst the phytotoxicity of trace metals has been studied for over a century (for example, see Jensen, 1907), there remains considerable variation within the literature as to the concentrations of trace metals necessary to induce toxic effects. An initial examination of the literature on trace metal toxicities in solution culture revealed that the concentrations used to induce toxic effects varied by at least eight orders of magnitude, from 1 nM (Godbold, 1991) to high millimolar concentrations. Among factors likely to contribute to this variation are differences in: (i) the inherent toxicity of the various trace metals, (ii) tolerances among plant species, and (iii) the experimental techniques used in the various studies. Whilst it is these first two points (i.e. the toxicity of trace metals and the sensitivity of plants to them) which form the basis of many phytotoxicity studies, it appears that differences in 'true' toxic effects are often confounded by the experimental conditions employed. For example, Taylor and Foy (1985) reported that *c.* 30 μM Cu is required to reduce growth of wheat (*Triticum aestivum* L.) by 50%, whereas Wheeler *et al.* (1993) found that only 0.5 μM Cu was required for a 50% growth reduction in the same species. It is unlikely that such a large discrepancy could be due solely to genotypic effects.

The aim of the current study was to provide a comprehensive review of the literature to determine the range in concentrations over which nine trace metals [As(V), Cd(II), Co(II), Cu(II), Hg(II), Mn(II), Ni(II), Pb(II), and Zn(II)] have been reported to exert phytotoxic effects in solution culture. Although an important trace metal, Al was not included in the current study because its toxic effects result from soil acidification; neither were rare trace metals (such as Ga, Gd, and Sc) included. Also, Fe toxicity is confined to waterlogged soils, and whilst it may be of particular interest under paddy conditions, it was excluded from the present study. For arsenic, only arsenate, As(V), was considered; this species dominates under aerobic conditions. Given the wide range of concentrations which have been reported to be toxic, criteria were first established to minimize the influence of experimental conditions on apparent 'toxicity' of the nine trace metals. This review of the literature includes the results of only those studies meeting the criteria.

Materials and methods

An extensive data set was collected from the literature for solution culture studies examining the phytotoxicity of As(V), Cd, Co, Cu, Hg, Mn, Ni, Pb, and Zn. Two databases (ISI Web of Science and Google Scholar) were searched from 1975 onwards, with the final date of searching being July 2009. Next, using the ISI Web of Science, all articles citing the retrieved references and all articles cited in the retrieved references were searched for further relevant publications. Two sets of criteria were used in this study; acceptance criteria and evaluation criteria. Acceptance criteria aimed to exclude investigations which were incompatible with the purpose of the current study (i.e. to determine the range in concentrations over which trace metals exert phytotoxic effects in solution culture). Once a study had been accepted, evaluation criteria were then applied to ascertain the suitability of the test procedures.

Ten acceptance criteria were used to determine eligibility for inclusion into the dataset, thus limiting data to those that were appropriate for the aims of the current study. Specifically, it was decided that the study must: (i) be conducted using solution culture (thus, excluding studies using agar, filter paper, sand, or soil), (ii) provide a direct measurement of plant growth (e.g. biomass or elongation of the root or shoot), (iii) examine the growth of intact plants (i.e. not excised portions), (iv) include a control, which either contains no added metal or a basal (non-toxic) concentration in the case of essential trace metals, (v) utilize a minimum of four levels of the trace metal (inclusive of the control) with reported nominal or measured concentrations, (vi) report the duration of exposure and any non-exposure periods (e.g. during germination or early seedling growth), (vii) utilize metal concentrations sufficient to cause a significant decrease in growth, (viii) be the primary source of the data, (ix) utilize only a single stressor (or, if multiple stressors were examined, provide data for the stressors individually in addition to their combined effects), and (x) investigate the toxicity of the free, ionic metal. Regarding the last-named criterion, data from studies that examined the effects of chelation by organic complexes (such as EDTA) were excluded since chelation has marked effects on trace metal speciation (Parker and Norvell, 1999).

Those studies meeting the above criteria were subjected to further quality assurance using the evaluation criteria. First, it was considered necessary that the plants had been grown in a complete nutrient solution, or at least in solution containing Ca (i.e. studies were excluded in which control plants were grown in deionized water). Second, the pH of the nutrient solution needed to be reported, given the importance of pH on solubility and ionic speciation. Third, to be included in the database, in those instances where modelling with PhreeqcI 2.15.0 (Parkhurst, 2009) indicated the solution to be supersaturated with respect to the metal of interest, it was necessary for the solution to have been sampled, filtered, and the soluble metal concentration measured. Fourth, in many studies, total growth of plants was reported for a period which included both an establishment phase and a phase involving exposure to the trace metal. Results of these studies were incorporated into the database only if: (i) a minimum of 50% of the time was spent in the metal-containing solution, or (ii) growth was assessed only for the period in the metal-containing solution (e.g. root elongation rate during metal-exposure versus the total root biomass).

The following parameters were recorded for each study entered into the database: (i) publication details, (ii) trace metal stressor, (iii) total number of treatments per stressor, (iv) concentrations (or activities) of a stressor determined as being toxic, (v) pH of nutrient solution, (vi) P concentration in solution, (vii) duration of exposure, (viii) plant species, (ix) plant growth variable measured, and (x) approximate growth reduction caused by the stressor at the toxic concentration.

In most studies, the concentration of the trace metal considered to be toxic was reported in the text of the article; alternatively, the values were determined from the figures or tables. Where an analysis of variance had been used, the lowest metal concentration causing a significant reduction in growth was selected. Values in the range of EC_{25}–EC_{50} (i.e. 25–50% growth reduction) were selected from studies where the growth response had been modelled (e.g. by regression analysis). Where authors reported the toxicity of the stressor as the activity of the free ion (e.g. the activity of Cu^{2+}), this was noted, but no discrimination was made between values reported as concentrations or activities. It was surprising, and rather disappointing, that the concentration of the trace metal of interest was measured in very few studies; rather, studies often simply reported the nominal (added) concentrations. Where losses of the metal have occurred, for example, by precipitation, relating growth reductions to the nominal concentration will lead to an underestimation of the actual toxicity of the trace metal (Lee *et al.*, 2005).

A total of 119 studies were entered into the database, including 28 for Cu, 22 for Cd, 17 for Mn, 13 for Ni, 13 for Zn, 11 for As, eight for Hg, four for Co, and three for Pb (see Supplementary Table S1 at *JXB* online). There was an overall total of 180 limiting metal concentrations; some studies including data on a number of plant species. The most commonly investigated species was wheat which was included in 19 studies. The median number of trace metal treatments was six (ranging from 4 to 58).

Results and discussion

Concentrations of trace metals found to be phytotoxic

The review of scientific literature over the past 34 years showed that trace metal phytotoxicity followed the general trend (from most toxic to least toxic): Pb≈Hg >Cu > Cd≈As >Co≈Ni≈Zn >Mn (Fig. 1). The median toxic concentration varied by about two orders of magnitude among the nine metals, being (µM): 0.30 Pb, 0.47 Hg, 2.0 Cu, 5.0 Cd, 9.0 As, 17 Co, 19 Ni, 25 Zn, and 46 Mn (Fig. 1). This toxicity ranking is similar to that found in individual studies on the toxicity of a range of metals to a single species. For example, Wheeler *et al.* (1993) reported that wheat root mass was reduced by 50% in solutions containing (µM) 0.5 Cu, 19 Zn, or 600 Mn (toxic values were also reported for Sc, La, Ga, Al, Fe, and B). Similarly, Taylor *et al.* (1991) reported that root mass of wheat was reduced by 5% in solutions containing (µM) 0.02 Cd, 3.4 Cu, 11 Ni, 37 Mn, or 45 Zn (Al toxicity was also studied).

The median trace metal concentrations found to be toxic in the present review were all <100 µM (*c.* 1 µM for Hg and Pb to 47 µM for Mn). These concentrations are comparable with those in soil solutions from metal-toxic soils, for example, *c.* 1 µM Pb (Weng *et al.*, 2001; Degryse *et al.*, 2007), 5 µM Cu (Aguirre-Gomez *et al.*, 2006; Luo *et al.*, 2006), or 50 µM Ni (Anderson *et al.*, 1973; Proctor *et al.*, 1981). However, in the literature, numerous studies were found using concentrations of trace metals up to four orders of magnitude higher. For example, Zeid (2001) used up to 50 mM Co in a sand culture study on common bean (*Phaseolus vulgaris* L.); Chi Yu *et al.* (2005) used 10 mM Cu in solution culture to investigate the influence of nitric oxide on Cu toxicity and NH_4^+ accumulation in rice (*Oryza sativa* L.), and Sahi *et al.* (2007) used up to 4.7 mM Cu in solution culture when investigating rattlebush (*Sesbania drummondii* (Rydb.) Cory). Such studies are far removed from the metal-toxic field situation, and the results obtained are of little value in understanding metal phytotoxicity.

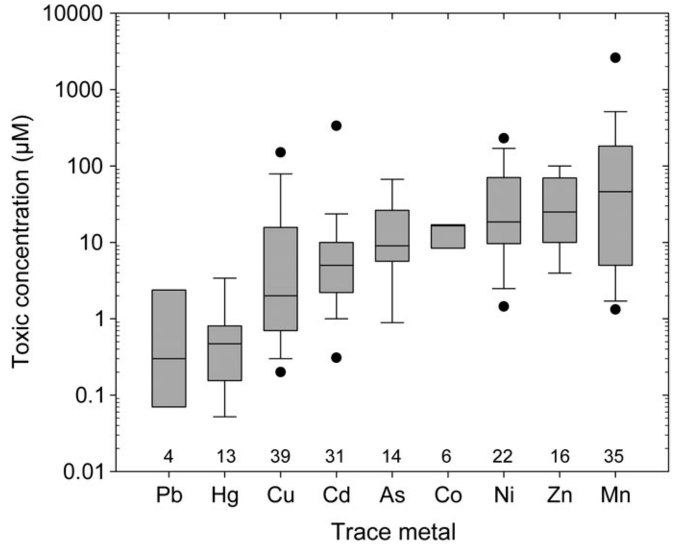

Fig. 1. Concentrations of nine trace metals that reduced the growth of plants in solution culture, obtained from a review of the literature from 1975 to 2009 (*n*=180). For each trace metal, the Tukey box plot represents the 25th and 75th percentiles (with the median contained therein). In cases where there are at least nine data points, the whiskers represent the largest and smallest values which are not outliers and, where shown, the dots represent the largest and smallest outliers. The numbers above each trace metal on the *x*-axis indicate the number of data points for that metal.

Whilst for each trace metal, the 25th and 75th percentile varied by about one order of magnitude (e.g. ranging from 2.2 µM to 10 µM for Cd), this variation in concentration required to induce toxic effects is not unexpected. Rather, there are several factors which may have contributed to this variability. These factors are related both to the plant species investigated and to the specific experimental conditions employed within each study.

Often, the aim of phytotoxicity studies is to identify genotypic differences in sensitivity (e.g. to aid in the identification of mechanisms, or to select resistant or tolerant plants for revegetation of contaminated lands). Indeed, there is a large variation in the sensitivity of plant species to trace metals under the same experimental conditions, or even among populations within a species. In a study involving four Australian tree species, Reichman *et al.* (2004) reported that shoot mass was reduced by 10% at 5.0 µM Mn for *Eucalyptus crebra* F. Muell. but only at 330 µM Mn for *Eucalyptus camaldulensis* Dehnh. Similarly, Edwards and Asher (1982) found 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 two monocots (maize, *Zea mays* L., and wheat) to 65 µM in a dicot (sunflower, *Helianthus annuus* L.). In a study with *Silene cucubalus* (Wib), de Vos *et al.* (1991) reported that the EC_{50} for root elongation was 4.0 µM Cu in a sensitive population but was 150 µM Cu in a tolerant population collected from a Cu-contaminated site.

Whilst comparisons in a specific experiment are possible, comparing metal toxicity between studies is often difficult because of differing experimental conditions which may markedly affect the concentration of metal found to be toxic. As part of the quality assessment in the current study, several evaluation criteria were developed to identify those studies where it is possible to compare results. It is proposed that these criteria should underpin all experiments on the phytotoxicity of trace metals.

Nutrient solution composition

The composition of the base nutrient solution has marked effects on the perceived toxicity of trace metals. Unfortunately, this does not seem to have been considered in many studies, resulting in toxicity data which are of limited value. Thus, besides ensuring appropriate trace metal concentrations, there is a need to pay particular attention to the overall composition of the nutrient solution.

Since plants can draw on their nutrient reserves for short periods of time, it is possible to conduct meaningful metal-toxicity experiments in simplified nutrient solutions which do not contain all the essential elements. However, because Ca does not move towards the root tip, it must be present in the test solution to maintain structural and functional integrity. Indeed, it has been noted that root growth is reduced rapidly when placed in solutions lacking Ca (Burstrom, 1953; Kinraide, 1998; del Amor and Marcelis, 2003). Root tips of six tropical legumes were thick and blackened with <12 μM Ca in solution; indeed, symptoms were evident within 2 d at 2 μM Ca (Bell *et al.*, 1989) and there was poor lateral root development at 2 μM Ca also. Spehar and Galwey (1997) found that, in the absence of Ca, the primary root length of eight soybean [*Glycine max* (L.) Merr.] lines was only 34±4 mm after 7 d but ranged from 99 mm to 147 mm with 500 μM Ca; at least 100 μM Ca was needed to discriminate among lines varying in root growth. The absence of B in nutrient solutions also 'leads to morphological changes … within hours or days' (Goldbach *et al.*, 2001). Therefore, at a minimum, the nutrient solution must contain Ca and B. However, examination of the literature revealed numerous studies where roots were grown in deionized water with no nutrients added. For example, Yildiz *et al.* (2009) conducted a study in which roots of onion (*Allium cepa* L.) were grown in deionized water for 4 d.

The composition of a nutrient solution should ideally mimic that of a soil solution (Table 1) (Parker and Norvell, 1999). This is especially important if the aim of the solution culture experiment is to study the effects of a toxic metal on plant growth in the field. However, for reasons of convenience, many well-known and commonly-used nutrient solutions, such as that of Hoagland and Arnon (1950), employ high initial concentrations of nutrient salts. This allows a large total supply of nutrients in a conveniently small volume of solution, but the concentrations are typically 1–3 orders of magnitude higher than those commonly found in soil solutions (Table 1). This is particularly so for P, which is typically present in soil solution at a low concentration relative to those used in many nutrient solution culture studies. Soil solution P concentration is often <2 μM in unfertilized forest soils and in highly weathered soils (Gillman and Bell, 1978; Menzies and Bell, 1988) (Table 1). In agricultural soils, soil solution P is increased by fertilizer use, but the soil solution P concentration is still generally <10 μM. For example, 80% of 149 samples in the data compilation of Reisenauer (1966), and 80% of those in a study of 33 soils by Kovar and Barber (1988), fell below 10 μM P (Table 1). It is only in soils which have recently received P fertilizer that soil solution P concentration of *c.* 100 μM is evident (Wiklander and Andersson, 1974; Adams *et al.*, 1980; Wheeler and Edmeades, 1995). However, toxicities of trace metals such as Pb would not occur in these highly fertile (high-P) soils due to the precipitation of metal-phosphates. Indeed, P-fertilization is one method of reducing soluble Pb concentrations when remediating contaminated sites (Zhu *et al.*, 2004). Yet, in the solution culture studies reviewed, the median P concentration was 100 μM (ranging from 0 μM to 4000 μM) (Table 1).

In the studies reviewed, the median ionic strength was found to be 4.7 mM (ranging from 0.29 mM to 46 mM), with soil solutions typically having an ionic strength of *c.*

Table 1. Comparison of the composition of Hoagland's No. 2 solution, a dilute nutrient solution, and soil solutions extracted from a Krasnozem (Oxisol) from Queensland, Australia and eight soils from New Zealand

Ionic strength was calculated using Phreeqcl where sufficient data were available.

	Hoagland's No. 2 solution[a] (μM)	**Dilute nutrient solution[b] (μM)**	**Soil solution[c] (unfertilized) (μM)**	**Soil solution[d] (0 kg P ha^{-1} year^{-1}) (μM)**	**Soil solution[d] (80 kg P ha^{-1} year^{-1}) (μM)**
Ionic strength	26 000	2 700	4 900	–	–
NO_3^- – N	14 000	450	1 740	–	–
NH_4^+ – N	1 000	150	320	–	–
K	6 000	300	850	250	240
Ca	4 000	500	520	370	450
S	2 000	600	310	89	91
Mg	2 000	100	700	150	150
P	1 000	2.5	0.13	5	45
B	46	3	–	–	–
Fe	25	2.5	24	6.2	5.2
Cl	18	0	860	–	–
Mn	9	0.5	3.2	1.6	0.9
Zn	0.8	0.5	–	–	–
Cu	0.3	0.1	–	–	–
Na	0	0	250	490	510

[a] See Hoagland and Arnon (1950) or Parker and Norvell (1999).
[b] Taken from Wheeler *et al.* (1993).
[c] Surface soil of a highly weathered Krasnozem (Oxisol) from Queensland, Australia (Menzies and Bell, 1988).
[d] Average values of soil solutions collected from eight surface soils (0–50 mm) from New Zealand receiving P fertilizer at either 0 or 80 kg P ha^{-1} year^{-1} for 4 years (Wheeler and Edmeades, 1995).

0.5–10 mM (Edmeades *et al.*, 1985; Menzies and Bell, 1988; Bruce *et al.*, 1989; Agbenin, 2003). High ionic strength solutions often affect trace metal toxicity as the concentration of other nutrients has an influence on the toxicity of the metal. For example, Lock *et al.* (2007*b*) reported that the activity of Ni^{2+} required to reduce root length of barley (*Hordeum vulgare* L.) by 50% increased 20-fold (from 5.05 μM to 105 μM) as the solution Mg concentration increased from 0.05 mM to 3.9 mM. Similarly, a study using the technique of Kopittke *et al.* (2008*b*) on short-term root growth in cowpea [*Vigna unguiculata* (L.) Walp. cv. Caloona] showed that an increase in the activity of Ca^{2+} increased the EC_{50} of Cu^{2+} activity from *c.* 0.24 μM to 0.59 μM (Fig. 2). This cation amelioration of cation toxicity probably does not result from changes in metal-speciation, but is attributable to changes in cation activity both in the bulk solution (Taylor *et al.*, 1998) and, perhaps more importantly, at the root-cell plasma membrane surface (Kinraide, 2006). For example, the data in Fig. 2 show the influence of cation composition, in this case Ca concentration, on the toxicity of Cu^{2+} [as determined from the activity of Cu^{2+} either in the bulk solution (Fig. 2A) or at the root-cell plasma membrane surface (Fig. 2B)]. However, in this review, no relationship was found between the concentration of metal which is toxic and solution ionic strength (data not presented). It is likely that the toxic values decrease in high ionic strength solutions, but we consider that the data from the reviewed studies is confounded by other variables (e.g. differences in sensitivity among plant genotypes). The effects of specific ions should also be considered. For example, phosphate inhibits arsenate uptake due to a competitive interaction (Asher and Reay, 1979; Tamaki and Frankenberger, 1992); hence, the phytotoxicity of As is likely to be underestimated where high P concentrations are used. It is interesting that Zn toxicity was alleviated in wheat and radish (*Raphanus sativus* L.) by as little as 1–5 μM Mg, concentrations too low to affect Zn activity in the bulk solution or at the plasma membrane (Pedler *et al.*, 2004). While the ameliorative mechanism in this instance remains unknown, it appears distinct from that of Ca (illustrated in Fig. 2).

Solution pH and trace metal speciation

The pH of the nutrient solution is an extremely important property in regulating the solubility, speciation, and toxicity of trace metals; hence, the results of a study are of limited value without knowledge of solution pH. Perhaps rather surprisingly, about one-third of the studies did not list the pH used, including recently published studies (Israr *et al.*, 2006; Sahi *et al.*, 2007; Krantev *et al.*, 2008). Given that metal toxicity is most commonly encountered on acidic soils, studies should typically be conducted at low pH. Indeed, the median pH of studies included in the database was 5.5 (ranging from pH 4.0 to pH 7.5).

Firstly, solution pH has a major influence on the solubility of many trace metals, this being well known for Al. This is particularly important for Pb (Fig. 3) among the trace metals examined in the current review. Lead phosphates are highly insoluble (Kopittke *et al.*, 2008*a*), and large amounts of Pb would have precipitated in the study of Malone *et al.* (1974) who added up to 4.8 mM Pb to Hoagland's solution (1000 μM P) even at pH 3.5–4.0 when investigating Pb toxicity in maize. Similarly, investigating the toxicity of Pb to *Beta vulgaris* L., Larbi *et al.* (2002) noted the 'immediate formation of a white precipitate cloud' following the addition of up to 2 mM Pb to a nutrient solution at pH 5.5. The importance of pH can also be seen in the study of Wong and Bradshaw (1982) (which, as of July 2009, has been cited 100 times). The concentrations of Al, Fe, Mn, or Pb reported to reduce root growth of ryegrass (*Lolium perenne* L.) by 50% in 3 mM $Ca(NO_3)_2$

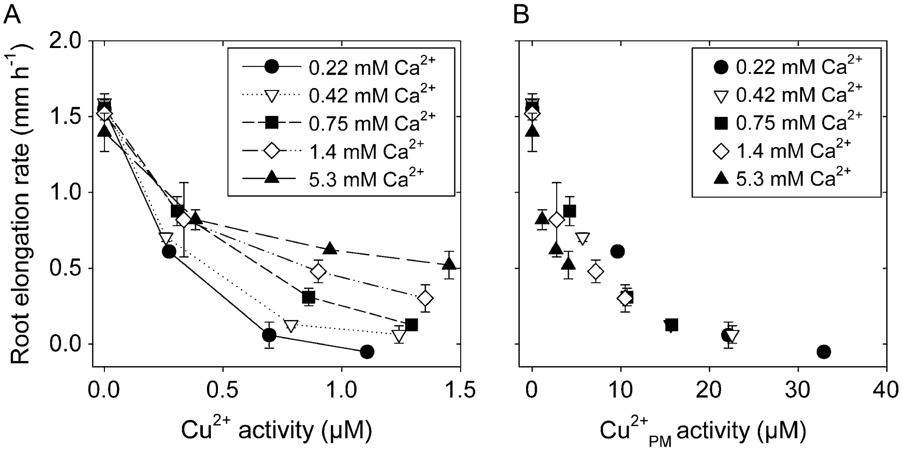

Fig. 2. Effects of the activities of Cu^{2+} [in either the bulk solution (A) or at the plasma membrane surface (B)] and Ca^{2+} and on the average root elongation rate (0–24 h) of 3-d-old cowpea seedlings grown in a solution containing 5 μM H_3BO_3 at pH 5.3. All bulk solution Ca^{2+} and Cu^{2+} activities were calculated using PhreeqcI from measured concentrations (see Kopittke *et al.*, 2008*b*, for more details). The Cu^{2+} activity at the plasma membrane surface was calculated as described by Kinraide (2006). Vertical bars represent the standard deviations of the arithmetic mean of two replications (where not visible, the vertical bars are smaller than the symbol). The Ca was supplied as $CaCl_2.2H_2O$ and the Cu as $CuCl_2.2H_2O$.

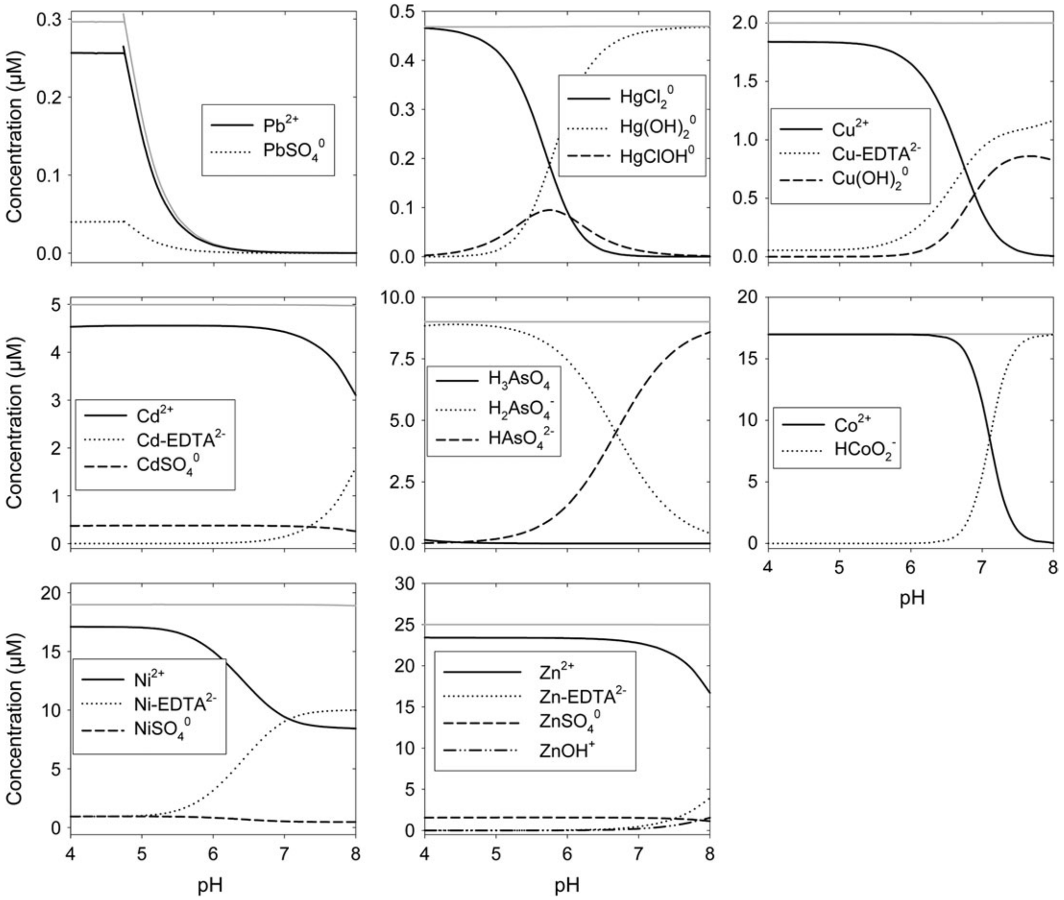

Fig. 3. Speciation of nine trace metals in a dilute nutrient solution containing a total of (μM): 0.30 Pb, 0.47 Hg, 2.0 Cu, 5.0 Cd, 9.0 As, 17 Co, 19 Ni, 25 Zn, and 46 Mn (the median toxic concentrations listed in Fig. 1). The solid grey line represents the total soluble concentration (for Pb, the soluble concentration decreased markedly with increasing pH due to precipitation as $Pb_5(PO_4)_3Cl$). The solutions were modelled using PhreeqcI 2.15.0 (Parkhurst, 2009), with the Minteq database (other than for Co), using a dilute nutrient solution containing (μM): 680 $\mathrm{NO_3^-} - \mathrm{N}$, 120 $\mathrm{NH_4^+} - \mathrm{N}$, 650 Ca, 502 S, 302 K, 140 Cl, 50 Mg, 10 Fe (as EDTA), 3 B, 2 P, 2 Mn, 1 Zn, 0.2 Cu, and 0.02 Mo (Kopittke *et al.*, 2008*a*) and in equilibrium with atmospheric O_2. The Minteq database contained no constants for Co, so the 'llnl' database (prepared by Jim Johnson, Lawrence Livermore National Laboratory) supplied with PhreeqcI 2.15.0 was used. Only the soluble species with the highest concentrations are presented. Solutions were not modelled for Mn as the relationship between measured and predicted concentrations is often poor (Norvell, 1988).

adjusted to pH 7.0 were considerably higher than those predicted to have remained in solution. Indeed, of the 30.8 μM Al added to reduce growth by 50%, it is predicted using PhreeqcI that <1 μM remained in solution. Similarly, <1 μM of the 256 μM Fe (assuming Fe^{2+} was oxidized to Fe^{3+} and no chelators were used) and 2.5 μM of the 8.2 μM Pb is predicted to have remained in solution. Although much of the Mn was also likely to have precipitated, Mn solutions were not modelled as the relationship between measured and predicted concentrations is often poor (Norvell, 1988). It is possible, therefore, that the solutions in the studies of Malone *et al.* (1974), Wong and Bradshaw (1982), and Larbi *et al.* (2002) may not have reached equilibrium. This further emphasizes the need to measure the soluble trace metal concentrations in filtered solutions, thereby establishing with greater certainty the concentrations or activities that are toxic to plants.

Comparatively few studies have considered trace metal speciation when examining their phytotoxicity. For the nine trace metals included in this study (and within the pH range commonly employed), consideration of speciation is particularly important for Hg, since it is unlikely that the free Hg^{2+} will be the dominant ion. Rather, solutions will tend to be dominated by $\mathrm{HgCl_2^0}$ or $\mathrm{Hg(OH)_2^0}$ (Fig. 3). The influence of Fe-chelators (such as EDTA) on solution speciation should also be considered, particularly in solutions at ⩾ *c.* pH 5.5 (Fig. 3).

Finally, a decrease in solution pH decreases the adsorption of metals onto and absorption into plant roots (Rengel, 2002). This was reflected, for example, in the study of Lock *et al.* (2007*a*) in which the root growth EC_{50} in barley for Cu^{2+} activity was 0.083 μM at pH 7.7, but this increased to 0.44 μM at pH 4.5. Weng *et al.* (2003) also reported that the EC_{50} for Ni^{2+} activity increased from 1.7 μM to 23 μM with a decrease from pH 7.0 to pH 4.0. Similarly, a short-term study, similar to that of Kopittke *et al.* (2008*b*), showed that poor root elongation rate (0.2 mm h^{-1}) was evident at pH 4.0 irrespective of Cu concentration (data not shown). At higher pH, however, the EC_{50} for Cu^{2+} toxicity in cowpea increased from 0.52 μM to 0.87 μM Cu^{2+} as the pH decreased from 5.3 to 4.6 (Fig. 4A). As with the effect of Ca (Fig. 2B), this effect of pH can potentially be explained due to a change in the activity of the toxicant (in this case, Cu^{2+}) at the plasma membrane surface (Fig. 4B). Interestingly, the data from the effects of Ca (Fig. 2B) and pH (Fig. 4B) on Cu toxicity can be combined to produce a single relationship between root elongation rate and changes in Cu^{2+} activity at the plasma membrane surface.

Time of exposure to metals

The length of time that roots are exposed to trace metals is important in determining their toxicity; the median duration of metal-exposure for studies incorporated into the database was 14 d (ranging from 2 d to 90 d). Many trace metals exert toxic effects within minutes or hours (Rengel, 1996; Blamey *et al.*, 2004; Kopittke *et al.*, 2008*b*, 2009), and the relative magnitude of their influence on plant growth increases with time of exposure. For example, Charpentier *et al.* (1987) reported that the EC_{50} for duckweed (*Lemna polyrrhiza* L.) exposed to Cd decreased from 1.5 μM after 4 d exposure to 0.8 μM after 14 d exposure. The length of exposure is particularly important in studies where plants are initially grown in a toxicant-free environment before transfer to metal-containing solutions and growth is measured as a 'bulk' variable. For example, root elongation rate during the metal-exposure period would be a more sensitive indicator of toxicity than the total mass of roots (i.e. including those produced during the non-exposure period). This does not seem to have been considered in the study of Mourato *et al.* (2009) who grew yellow lupin (*Lupinus luteus* L.) for 49 d in a toxicant-free environment before exposing them to excess Cu for 15 d. These authors reported that a Cu concentration of ≤50 μM did not affect the total biomass of the plant. This most likely occurred not because 50 μM Cu is not toxic (see Fig. 1), but because most of the biomass had been produced in the toxicant-free environment with insufficient time allowed for differences to develop between treatments.

Conclusions

A review of the literature over the past 34 years showed that the concentration required to reduce plant growth followed the general trend (from most to least toxic): Pb≈Hg >Cu >Cd≈As >Co≈Ni≈Zn >Mn. The median toxic concentration was (μM): 0.30 Pb, 0.47 Hg, 2.0 Cu, 5.0 Cd, 9.0 As, 17 Co, 19 Ni, 25 Zn, and 46 Mn, but the 25th and 75th percentile causing toxicity varied by about one order of magnitude for each trace metal. We conclude that this was due to differences among plant species and in experimental conditions, the latter sometimes making it difficult to apply the results to field situations. To improve the utility of the data, it is suggested that studies investigating trace metal phytotoxicity in solution culture: (i) use a solution containing, as a minimum, Ca and B, but ideally one which mimics the composition of the soil solution, particularly for P, (ii) carefully monitor and report solution pH, (iii) filter the solution and measure the actual concentration of metal in solution and assess the influence of pH on solution composition and speciation, and (iv) use an appropriate period of exposure and ensure that the plant variable measured is a sensitive indicator of toxicity.

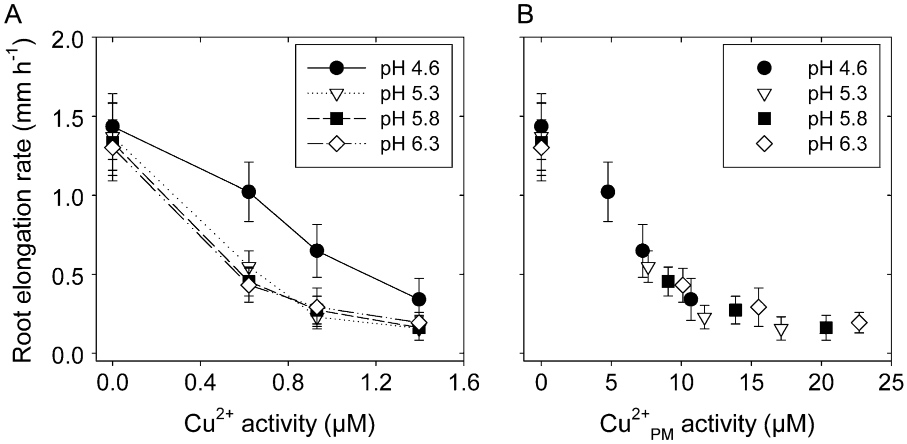

Fig. 4. Effects of solution pH and the activity of Cu^{2+} (in either the bulk solution (A) or at the plasma membrane surface (B)) on root elongation rate (0–26 h) of 3-d-old cowpea seedlings grown in solution containing 1000 μM Ca and 5 μM H_3BO_3. All bulk solution Cu^{2+} activities were calculated using PhreeqcI from measured concentrations (see Kopittke *et al.*, 2008*b*, for more details). The Cu^{2+} activity at the plasma membrane surface was calculated as described by Kinraide (2006). Vertical bars represent the standard deviations of the arithmetic mean of two replications. The Ca was supplied as $CaCl_2.2H_2O$ and the Cu as $CuCl_2.2H_2O$.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Table S1. Data set collected from the literature (1975–2009) for solution culture studies examining the phytotoxicity of As, Cd, Co, Cu, Hg, Mn, Ni, Pb, and Zn.

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