



Defining appropriate methods for studying toxicities of trace metals in nutrient solutions



Zhigen Li^a, Peng Wang^{a,b,*}, Neal W. Menzies^a, Peter M. Kopittke^a

^a The University of Queensland, School of Agriculture and Food Sciences, St. Lucia, Queensland, 4072, Australia

^b Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, Jiangsu 210095, China

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ABSTRACT

The use of inappropriate experimental conditions for examining trace metal phytotoxicity results in data of questionable value. The present study aimed to identify suitable parameters for study of phytotoxic metals in nutrient solutions. First, the literature was reviewed to determine the concentration of six metals (Cd, Cu, Hg, Ni, Pb, and Zn) from solution of contaminated soils. Next, the effects of pH, P, Cl, NO₃, and four Fe-chelators were investigated by using thermodynamic modelling and by examining changes in root elongation rate of soybean (*Glycine max* cv. Bunya). The literature review identified that the solution concentrations of metals in soils were low, ranging from (μM) 0.069–11Cd, 0.19–15.8 Cu, 0.000027–0.000079 Hg, 1.0–8.7 Ni, 0.004–0.55 Pb, and 0.4–36.3 Zn. For studies in nutrient solution, pH should generally be low given its effects on solubility and speciation, as should the P concentration due to the formation of insoluble phosphate salts. The concentrations of Cl, NO₃, and various chelators also influence metal toxicity through alteration of metal speciation. The nutrient solutions used to study metal toxicity should consider environmentally-relevant conditions especially for metal concentrations, with concentrations of other components added at levels that do not substantially alter metal toxicity.

1. Introduction

Trace metals, such as Cd, Cu, and Hg are natural components of the environment but are toxic at elevated concentrations. Indeed, trace metals are amongst the most frequently encountered environmental contaminants (EEA, 2007) and many are of high priority (ATSDR, 2011). Plant-based approaches (including phytostabilization) for the remediation of contaminated land are attracting attention in view of their potential benefits in terms of cost, ability to improve soil conditions, and public acceptance as a ‘natural’ and ‘green’ method of remediation. However, the toxicities of metals and their potential movement through the food chain can only be managed if their interactions with plants are understood.

Due to the complex nature of the soil solid phase, many studies utilize nutrient solution systems to investigate the phytotoxicity of trace metals. In contrast to soil, nutrient solution systems are comparatively well-defined and homogenous (Parker and Norvell, 1999) thereby providing an easier approach for examining phytotoxicity and interaction mechanisms. However, care must be taken when utilizing nutrient solutions that the composition of the solution is appropriate and

does not result in experimental artefacts. For example, it is not uncommon for studies published in the literature to use nutrient solutions that contain concentrations of trace metals that are orders of magnitude higher than those found in soil solutions. Whilst the potential reasons for this are discussed in detail later, one possibility is that the use of inappropriate pH values or P concentrations can result in marked changes in solution composition due to precipitation. Studies such as these are often of questionable value.

The importance of the composition of the nutrient solution has been discussed previously, both in general terms (Kopittke et al., 2010; Twiss et al., 2001) but also more specifically in regard to experiments investigating the phytotoxicity of Al (Kopittke and Blamey, 2016; Shaff et al., 2010) and Pb (Kopittke et al., 2008). These previous studies are extended here, with the current focus being on Cd, Cu, Hg, Ni, Pb, and Zn - these six metals being among the most commonly encountered environmental contaminants (ATSDR, 2011). Aluminum and Mn are not considered here, with toxicities of these two trace metals being important in acid agricultural soils rather than soils contaminated directly by anthropogenic activity per se. Consideration also needs to be given to the speciation of trace metals in soil solutions (Cancès et al.,

Abbreviation: RER, root elongation rate

* Corresponding author at: Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, Jiangsu 210095, China.

E-mail address: p.wang3@njau.edu.cn (P. Wang).

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2003). For instance, the toxicity of trace elements toxicity is often closely related to the free metal ion (M^{2+}) activity in soils, and in the biotic ligand model, it is assumed that M^{2+} binds to the site of action (i.e. the 'biotic ligand') within the plants root (Thakali et al., 2006). Both in soil solutions and at the biotic ligand, the activity of M^{2+} is influenced by the presence of other ions, including Ca^{2+} and K^+ . However, a detailed description is beyond the scope of the present study to examine this in detail (for example, see Morel, 1983; Paquin et al., 2002).

The aim of the present study was to identify appropriate nutrient solution compositions for the examination of trace metal phytotoxicity. Specifically, we aimed to provide information on appropriate methods for conducting solution culture studies that are predictive of trace metal toxicity in soils. Consideration was first given to the concentrations of trace metals found in solutions of field-contaminated soils, with this providing an initial starting-point to guide the selection of concentrations for use in nutrient solutions. Next, thermodynamic modelling was used to investigate the theoretical effects of solution pH on trace metal solubility and speciation, P concentration on solubility, Cl concentration speciation, and chelator on speciation. Finally, a simple solution culture system was used to measure changes in the elongation rate of soybean (*Glycine max* cv. Bunya) roots when grown in solutions that varied in pH, P, Cl, and chelator concentration. This information will assist others in the development of rigorous studies for the investigation of trace metal phytotoxicity.

2. Materials and methods

2.1. Soil solution composition

The literature was reviewed in order to identify studies in which the concentrations of Cd, Cu, Hg, Ni, Pb, or Zn were measured in field-contaminated soils. Studies that examined laboratory-spiked soils were excluded. The review was not intended to be exhaustive, but rather, it aimed to identify indicative concentrations for each of the six trace metals from solutions of contaminated soils.

2.2. Modelling

Thermodynamic modelling was conducted using Phreeqc v 3.1.7 (Parkhurst, 2014) with the default phreeqc database. The equilibrium constants ($\log K$) for metals was updated as listed in Supplemental Table S1. Briefly, where available, constants were taken from the International Union of Pure and Applied Chemistry (IUPAC), as provided by Powell et al. (2011) for Cd, Powell et al. (2007) for Cu, Powell et al. (2005) for Hg, Powell et al. (2009) for Pb, and Powell et al. (2013) for Zn. For Ni, constants were taken from Gamsjäger et al. (2005). All other constants were left as the default values as listed in the phreeqc database.

Unless otherwise stated, the six trace metals were used at the median concentration reported to be phytotoxic in the meta-analysis of Kopittke et al. (2010), with all solutions in equilibrium with atmospheric O_2 and CO_2 . First, the effect of pH on solution composition was modelled in a simple solution containing 1 mM $CaCl_2$. Second, the effect of P concentration was examined in a simple solution containing 1 mM $CaCl_2$ at pH 5.5. Third, the effect of up to 10 mM Cl and NO_3^- -N on speciation was examined in solutions at pH 5.5. The maximum concentration of 10 mM was selected for Cl and NO_3^- -N as concentrations in the soil solution typically do not exceed this except in highly saline soils (Wolt, 1994). Finally, for a complete, dilute nutrient solution (Blamey et al., 2015), the effect of the chelate used for the supply of Fe (6 μ M) on the speciation of the trace metals was examined using Geochem-EZ (Shaff et al., 2010). Four different chelates were examined, being ethylenediaminetetraacetic acid (EDTA), 1,2-diaminocyclohexanetetraacetic acid (CDTA), N-(2-hydroxyethyl) ethylenediamine-N,N',N'-triacetic acid (HEDTA), and nitrilotriacetic acid (NTA).

2.3. Measurements of root elongation rate

Seeds of soybean (cv. Bunya) were rolled in paper towel, moistened with tap water, and suspended vertically for 3 d. The seedlings were removed and placed in a Perspex strip on a glass beaker filled with 650 mL of basal solution containing 1 mM $CaCl_2$ and 5 μ M H_3BO_3 . The seedlings were grown in this basal solution for 18 h before being transferred to new beakers containing the treatment solution (see below). Root elongation rates (RER) were calculated from root lengths determined from digital images captured at the time of transfer (0 h) and after 48 h. Root lengths were measured using ImageJ v1.45 s (available without charge at: <http://imagej.nih.gov/ij/>). Each Perspex strip held seven seedlings and formed one experimental unit. All solutions were continuously aerated, and unless otherwise stated, the pH of the initial basal solution (ca. pH 5.6) was not adjusted but was measured to be pH 5.6. Preliminary experiments indicated that pH changed by < 0.2 units during the 48-h experimental period.

Experiment 1 provided dose-response curves for six trace metals. Following the initial 18-h growth in basal solution, seedlings were transferred to solutions containing six to eleven concentrations of each of the six metals. These solutions contained (μ M): 1, 2, 3, 5, 7.5, 10, or 15 for Cd; 0.25, 0.5, 1, 1.5, 2, or 3 for Cu; 0.5, 1, 2, 3, 4, or 5 for Hg; 0.5, 0.75, 1, 1.5, 2, 3, 5, 7.5, 10, 15, or 20 for Ni; 1, 2, 3, 5, 7.5, 10, 15, or 20 for Pb; 10, 20, 30, 40, 50, 75, 100, 200, or 250 for Zn, with all supplied as Cl-salts. These concentrations were selected from preliminary experiments. Thus, the experiment consisted of 48 treatments including a control (to which no trace metals were added), each with two replicates (with seven seedlings per replicate), yielding a total of 96 experimental units. The pH, initially 5.6, was not adjusted for any treatment. Solutions were sampled after 0 and 48 h, filtered (0.22 μ m, Millipore Millex GV), and acidified with concentrated HCl (6 M). Samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) for Cd, Cu, Ni, Pb, or Zn and flow injection mercury system atomic absorption spectrometry (FIMS-AAS) for Hg.

Experiment 2 examined the effect of pH on RER, with pH potentially changing both solubility and speciation of the trace metals. After growth in basal solutions, treatment solutions also containing 1 mM $CaCl_2$ and 5 μ M H_3BO_3 were prepared with trace metals added at concentrations that reduced RER (over 48 h) in basal solutions by 75%, being 5 μ M Cd, 0.9 μ M Cu, 1.3 μ M Hg, 13 μ M Ni, 9 μ M Pb, and 250 μ M Zn. With all six trace metals and four pH values, this yielded a total of 24 treatments. Solution pH was adjusted to 5.0, 6.0, 7.0, or 8.0 using 0.1 M HCl or 0.1 M NaOH, with solutions buffered using MES (2-[N-morpholino] ethane sulfonic acid, 2.0 mM) for pH ≤ 6 and MOPS (3-[N-morpholino] propane sulfonic acid, 3.6 mM) for pH > 6 . Solutions were collected and analyzed as described for Experiment 1.

Experiment 3 aimed to examine changes in soybean RER as influenced by P concentrations. Given that modelling had indicated that P would only be likely to influence Pb concentrations, Experiment 3 only examined Pb. Seedlings were grown in a basal solution before being transferred to the treatment solutions containing 1 mM $CaCl_2$ and 5 μ M H_3BO_3 . The experiment consisted of one Pb concentration (9 μ M, which was sufficient to reduce RER by 75% over 48 h at 0 μ M P), six P concentrations (0, 0.1, 1, 20, 50, 100 μ M, added as KH_2PO_4), with two replications. Solution pH was adjusted to 5.5 using 0.1 M HCl. As described previously, solution samples were collected after 0 and 48 h.

Experiment 4 examined the influence of Cl and NO_3^- on plant growth. For this experiment, only three trace metals were investigated, being Hg, Cd, and Pb. The three trace metals were added at concentrations of 1.3 μ M Hg, 5 μ M Cd, and 9 μ M Pb (these concentrations being sufficient to reduce RER by 75% over 48 h). Across all treatments, ionic strength was kept constant such that differences between treatments were not due to changes in activity coefficients. Therefore, ionic strength in all treatments was 15 mM, with Cl^- added (as $CaCl_2$) at rates of 0, 0.01, 0.1, 1, 2, 5, and 10 mM with corresponding NO_3^- addition [as $Ca(NO_3)_2$] of 10, 9.99, 9.9, 9, 8, 5, and 0 mM. Across all treatments,

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