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Guidance document for the revegetation of land contaminated by metal(loid)s

P.M. Kopittke, F.P.C. Blamey and N.W. Menzies

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Executive summary

The revegetation of sites contaminated by metals (such as Cu, Zn, Ni, or Pb) and metalloids (such as As) is an important environmental challenge. On a global-scale, large investments are required in order to rehabilitate the soil to a productive and non-environmentally-damaging endpoint, and as a result, an ever increasing number of technologies have been developed. However, the successful implementation of a revegetation system requires a true multi-disciplinary effort, with collaboration between soil scientists, agronomists, hydrologists, ecotoxicologists, and economists.

The overall revegetation process can be separated into three broad steps:

(i) assessment of soil contamination

(ii) remediation, and

(iii) revegetation / plant selection.

Although all three steps are considered here, an emphasis is placed on the first and last of these. This document provides a brief review of current knowledge, with a particular emphasis on Australian plants and landscapes.

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1. Assessing soil contamination

An understanding of the toxic effects of trace metals on plants is essential for assessing the environmental risks associated with contaminated sites. However, risk assessments and revegetation efforts are hampered by a comparatively poor understanding of the chemical extractants which best estimate the phytoavailability (availability to plants) of trace metals and the relationship between the concentration of a particular measure of each individual trace metal in the soil and its uptake and concentration in the plant tissue. Commonly used extractants include measurements using 'total concentrations', DTPA, EDTA, and Mehlich.

A review was undertaken to examine the effectiveness of various chemical extractants to predict the phytoavailability of Cd, Zn, Ni, Cu, and Pb across a range of soil types and contamination levels. The data indicate that none of the chemical extractants examined were able to predict plant uptake (and hence the toxicity) of trace metals (Appendix 1). While low values unambiguously indicated low metal phyto-availability, elevated levels of extractable metal did not necessarily indicate that plants grown on these soils would suffer metal toxicity, or accumulate excessive levels of metal. Therefore, it is not currently possible to predict plant growth and metal uptake based upon the results of commonly used soil tests.

It is therefore suggested that, where soil tests indicate that a soil or overburden material has an elevated metal content, simple plant growth studies should be conducted using the material of interest to provide a direct indication of potential metal uptake and toxicity rather than using soil analyses alone to attempt predict plant performance and metal uptake. Some of the key issues to be considered when conducting plant-growth studies are outlined below.

Firstly, to ascertain whether simple plant-growth studies are required, the values published by the National Environment Protection Council (NEPC) in the National Environment Protection Measures (NEPMs) serve as an adequate starting point. These values are based upon the total metal concentration in the soil or other plant growth medium. Hence, they do not provide any clear indication as to the extent to which the plants will take up metals. They are conservative, however, and provide an indication as to whether further evaluation is warranted.

Careful consideration should be given to the material in which plant growth is to be investigated. Ideally, the soil or plant growth medium collected should be representative of the material of interest. This is often achieved by collecting subsamples from 5 to 10 locations before drying and mixing. Material which is not fully oxidised (for example, fresh tailings material) should not be utilised. Rather, only well-oxidised material should be examined (for example, the oxidised zone overlying an ore body or well-oxidised tailings). Where un-oxidised material are of interest, acid generation potential needs to be considered, and the materials may need to be permitted to oxidise fully before plant growth studies are undertaken. Further, saline materials should first be leached to remove excess soluble salts. Material obtained from core samples is generally adequate, provided it is well-oxidised.

Although a detailed assessment of the soil's fertiliser requirements would be beneficial for the final revegetation program, for simple plant-growth studies, a basal application of moderate rates of essential macro- and micro-elements will ensure that plant growth is not limited by nutritional constraints. The use of simple pot experiments also allows an assessment of the ability of soil amendments to reduce the uptake and toxicity of trace metals. For example, the addition of lime to acidic materials increases pH and generally reduces the solubility of metal-contaminants due to precipitation of insoluble metal compounds.

Whilst simple plant-growth experiments may be more time-consuming than using chemical extractants to measure metal concentrations in soil, plant-growth studies provide a direct measurement of the likelihood that the metal will either reduce plant growth or accumulate within the shoots to levels which are likely to be detrimental to animals which consume the plants. In contrast, commonly-used chemical extractants cannot be used with any certainty to predict the availability of trace metals to plants. Thus, the use of growth experiments will provide reliable data, support the planning of revegetation programs, and assist in ensuring environmental sustainability in the revegetation of contaminated sites.

2. Remediating the soil

In contrast to organic contaminants, metal(loid)s do not undergo microbial or chemical degradation, and hence persist in the environment indefinitely. Therefore, remediation of sites contaminated by trace elements typically involves either the removal of the contaminant (for example, excavation, leaching, or phytoextraction) or stabilisation of the contaminant (for example, solidification or phytostabilisation). Although it is not within the scope of this document to review the methods used for remediating contaminated sites, techniques can usually be categorised as follows:

- Isolation – preventing transport of contaminants by containing them within a specified area (such as capping or subsurface barriers).
- Physical immobilisation – changing the mobility of contaminants by modifying the physical or leaching characteristics of the contaminated soil (such as solidification).
- Physical separation/extraction – separation of the contaminated fraction (or the contaminant itself) from the remaining soil (such as separation or washing).
- Toxicity/mobility reduction – reducing contaminant solubility and hence reducing the likelihood that it will be transferred through the food chain by being taken up by plants, leached into the groundwater, and/or available to soil organisms (such as chemical processes [pH adjustment, precipitation, ion exchange, organic amendments] or biological processes (phytoextraction, phytostabilisation)).

3. Revegetation and plant selection

When revegetating a contaminated site or conducting plant-growth studies, plants should be selected that are likely to be relevant to the location (for example, Rhodes grass (*Chloris gayana*) in subtropical Australia). Of critical importance to the success of any revegetation program is the selection of appropriate plant species. When selecting species, native plants have the advantage of being adapted to the site's climatic conditions. However, it is possible that introduced species may be more suited to the more hostile soil conditions often found in metal-contaminated sites. Several criteria should be considered to ensure that the most suitable species are chosen. In particular, the plant should:

- be tolerant of the contaminant
- minimise the movement of the contaminant into the shoots (i.e. the plant should not be a hyperaccumulator)
- be tolerant of other site-specific factors likely to influence plant growth (for example, drought, salinity, and acidity), and
- be fast growing (but not invasive) and self-propagate.

3.1 Tolerant of the contaminant

Ideally, plants used for revegetation should be tolerant of the contaminants onsite. For most sites, the objective is to achieve satisfactory groundcover rather than near-maximum plant growth, and hence 50% maximum growth is often considered suitable. For this purpose, the 'EC_{50}' (the half-maximal effective concentration) is useful as it identifies the concentration of the contaminant which causes a 50% reduction in plant growth. Therefore, for a particular contaminant, assessment of the EC_{50} allows comparison of the tolerance of a range of plants to that contaminant; higher EC_{50} values indicating a higher tolerance to the contaminant (Appendix 2 and 3).

3.2 Movement of contaminant into the shoots

For the revegetation of a contaminated site, the rate of contaminant transfer into the shoot should be minimized to reduce the risk of contaminant transfer through the ecosystem (the exception being phytoextraction-based systems, for which the aim is to maximise accumulation within the shoots). To provide an estimate of the potential movement of a contaminant through the ecosystem, two factors should be considered. Firstly, it is necessary to know the concentration of the contaminant which can be tolerated within the diet of animals. Typically, this is taken as the 'MTL' (maximum tolerable level), which is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and/or performance (National Research Council (U.S.) 2005). Secondly, it is necessary to estimate the concentration of the contaminant which could potentially accumulate within the shoots. This can be estimated as the 'PT_{50}' (the 50% phytotoxicity threshold), which is defined as the concentration of contaminant within the shoots corresponding to a 50% reduction in shoot growth.

If a plant has a low PT_{50} value, growth of the plant will be reduced before high concentrations of the contaminant accumulate within the shoot. In contrast, if a plant has a high PT_{50} value, plant growth will be reduced only after high levels of the contaminant have accumulated within the shoot. Although the PT_{50} does not represent the maximum concentration possible within the shoots, at concentrations higher than the PT_{50}, plant growth will be relatively poor and hence the mass of shoots available for consumption by animals will be low. In addition, the measurement of shoot concentrations allows the calculation of critical tissue concentrations for toxicity (which can aid in the assessment of the long-term potential for plant establishment).

Thus, for any given species, it is possible to compare the concentration of contaminant which can potentially accumulate within the shoots (PT_{50}) to the concentration of contaminant which can be tolerated in the diet of animals (MTL). Such a comparison estimates the likelihood (risk) that toxic levels of the contaminant will be transferred through the food chain.

Whilst the comparison of the PT_{50} and the MTL is useful for the selection of specific plants, comparatively few data are available for species suitable for revegetation in Australian landscapes (Appendix 3). Therefore, it is useful to compare the MTL to 'generalised' PT_{50} values which have been estimated from a range of plants. Whilst these general PT_{50} values enable broad assumptions to be made regarding which of the contaminants are likely to be more toxic to plants and which are likely to be more toxic to animals consuming the plant shoots, they do not take into account differences between plant species. Thus, using generalised values, it would appear that the risk of toxic concentrations being transferred through the ecosystem (due to consumption of plant material) is greatest for Cd-contaminated sites, and least for As- and Cu-contaminated sites.

Table 1. Approximate maximum tolerable level (MTL) and general 'PT' (phytotoxicity threshold) for a range of trace metals.

	PT – plant toxicity[1] (mg/kg)	MTL – fauna toxicity[2] (mg/kg)	Toxicity more likely to animals or plants	Likelihood that toxic levels of contaminant will be transferred into wider ecosystem
As	5-20	30	plants	low
Cd	10-100	5-10	animals	high
Cu	15-30	40	plants	low
Mn	200-2000	2,000	both	moderate
Ni	25-100	100	both	moderate
Pb	50-100	100	both	moderate
Zn	100-1000	500	both	moderate

[1] *Macnicol and Beckett (1985) and Kabata-Pendias and Pendias (2001).*

[2] *National Research Council (U.S.) (2005).*

3.3 Tolerant to other site-specific factors

For the revegetation of contaminated sites, it is important to identify all factors limiting plant growth. In addition to being tolerant to the contaminant, plants selected for the stabilisation of a site should be tolerant to other site-specific factors, such as low soil fertility, salinity, drought, and extreme pH (acidity or alkalinity).

In some instances, it may be possible to address some of the limitations by covering the contaminated material with a relatively thin layer of clean soil. Unlike a capping layer, which limits access of the plant roots to the waste, in this situation the clean soil provides a portion of the root zone where plants can obtain nutrients, while the waste acts as a subsoil. However, this is an expensive process, and it may not be possible to obtain sufficient soil to cover large sites. Alternatively, it may be possible to amend the existing (contaminated) soil sufficiently to allow plant growth. The application of appropriate amendments may improve the likelihood of establishing vegetation on the contaminated site, and may include fertilisers (to overcome nutrient deficiencies), lime (to overcome soil acidity), or organic materials (to add nutrients or improve the physical properties of the soil). Information regarding the salinity tolerance of a range of species can be found in Shaw (1999), whilst information regarding the tolerance of grasses to a variety of growth-limiting conditions (such as low fertility, soil acidity, etc) can be found in Cook et al. (2005).

3.4 Fast-growing, non-invasive, and self-propagate

Perennial grasses provide a quick groundcover to assist in limiting erosion (both water and wind). Furthermore, grasses are generally more tolerant of many contaminants than are broadleaf species (e.g. Kukier and Chaney 2004). Whilst trees are slower growing, over the longer-term they provide a canopy cover and a deep root network to help stabilise the soil.

Whilst native species offer the advantage of being well adapted to climatic conditions of the site, though introduced species may be faster growing and/or more tolerant of the hostile conditions commonly found in contaminated sites. However, if utilising introduced species, care must be taken to ensure the plant is not invasive and thereby decrease regional biodiversity. Furthermore, for the long-term effectiveness of a revegetation program, it is essential to ensure that the species selected will self-propagate.

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APPENDIX A.

Review of trace metal toxicity in soil

Summary

The accurate estimation of the phytoavailability (availability to plants) of trace metals in soils and solid wastes is important in assessing their risk to the environment. A large dataset consisting of 5700 individual data points was taken from the literature, and the effectiveness of various chemical extractants to predict the phytoavailability of Cd, Zn, Ni, Cu, and Pb was examined across a range of soil types and contamination levels. The data suggest that generally, the total soil trace metal content, and trace metal concentrations determined by complexing agents (such as the widely used DTPA and EDTA extractants) or acid extractants (such as 0.1 M HCl and the Mehlich extractants) are only poorly correlated to plant phytoavailability. Further, although it would appear that neutral salt extractants (such as 0.01 M $CaCl_2$, 0.1 M $NaNO_3$, and 1.0 M NH_4OAc) provide a better indication of metal phytoavailability across the range of metals of interest, trace metal concentrations determined by these neutral salt extractants were also only poorly correlated to plant phytoavailability in most instances. Thus, the data presented here indicate that none of the commonly used chemical extractants can be used to consistently predict availability of trace metals to plants.

Introduction

Trace metals (also known as heavy metals) include metals such as zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), nickel (Ni), and cadmium (Cd). Some of these trace metals (in particular, Mn, Cu, Zn and Ni) are essential elements for the growth of plants, while these four elements together with chromium (Cr) and selenium (Se) are essential for animals (Underwood 1975). Though trace metals are natural components of rocks and soils, they are generally in forms which are of low availability to plants and animals. In some instances, however, soils may 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). Elevated concentrations of trace metals may also be present in sites contaminated by anthropogenic activities. The presence of excess trace metals represents a serious environmental and financial problem, with hundreds of thousands of contaminated sites globally requiring remediation at an estimated cost of up to US$35 billion (CEI 2005). The release of trace metals is also a serious environmental problem in Australia, although the contaminant load associated with various types of land use and industry activity in Australia is not known (Australian SoE Committee 2001). In contrast to organic contaminants, trace metals do not undergo microbial or chemical degradation. As a result, metals (and their toxic effects) persist in the environment indefinitely.

The accurate estimation of the phytoavailability of trace metals in soils and solid wastes is important in assessing their risk to the environment. In particular, it is useful to be able to estimate the extent to which metals will accumulate within the shoots of plants growing on contaminated soil.

Indeed, to provide an indication of the impact of trace metal contamination on the overall ecosystem, two factors need to be considered:

- the approximate concentration in plant tissue at which a decrease in growth occurs, and
- the concentration of metal that can be tolerated in an animal's diet.

Where there is the potential for plant tissues to accumulate more than the threshold level for animal toxicosis (for example, Cd), this threshold level should be used in addition to, or rather than, the threshold for a plant growth response. Approximate plant-toxic tissue concentrations were determined from Macnicol and Beckett (1985) and Kabata-Pendias and Pendias (2001), whereas the indicative animal toxicosis value was determined from guidelines published by the US National Research Council (2005) (Table 2).

Table 2. Approximate indicative threshold concentrations (all values on a dry weight basis) in the dietary intake for the onset of metal toxicoses in animals, and an indication of the plant tissue concentrations at which yield decrease occurs.

	Indicative threshold – fauna toxicity[1] (mg/kg)	Indicative threshold – plant toxicity[2] (mg/kg)	Toxicity more likely to animals or plants
As	30	5 – 20	plants
Cd	5	10 – 100	animals
Cu	40	15 – 30	plants
Mn	2,000	200 – 2000	both
Ni	100	50 – 100	both
Pb	100	50 – 100	both
Zn	500	200 – 1000	both

[1] *National Research Council (U.S.) (2005).*

[2] *Macnicol and Beckett (1985) and Kabata-Pendias and Pendias (2001).*

Note: When examining the relationship between the soil-extractant concentration and the plant tissue concentration (Figure 2 and Figure 6), the lower of the plant toxicity values were used.

The assessment of the phytoavailability of trace metals remains complicated; within the soil, trace metals are located in different 'pools' which differ markedly in the extent to which they are available to plants. Therefore, the availability of trace metals within soils varies not only with the total concentration of metals within the soil, but also with other soil properties such as pH, organic matter content, and clay content. Thus, measurement of the total concentration alone is not usually a good indicator of the amount available for the plant. Nevertheless, 'investigation levels' adopted by governments often rely on the measurement of the total concentration of metal within the soil. As a result of this poor ability to predict phytoavailability based on the total metal concentration and the need to be conservative, investigation levels based on the total metal concentration may be lower than the background concentration. For example, the Eco-SSL (US EPA) investigation value for Mn in plants (220 mg/kg) is lower than the median background concentration of Mn in western USA soils (ca. 600 mg/kg). Thus, there is a need to more accurately assess the phytoavailability of trace metals in soil to improve the ability to assess the risk of trace metal contaminants to the environment.

Typically, the phytoavailability of trace metals is estimated using chemicals which extract only a portion of the metal from the soil. Although numerous extractants have been proposed, it remains unclear as to which is/are the most useful in predicting the phytoavailability of trace metals. Ideally, the chemical should extract only the metals which are available to the plants (i.e. the chemical should extract the trace metals under conditions similar to those exerted by the plant in the soil surrounding the root) (Alloway and Jackson 1991). Indeed, the usefulness of any soil extractant to predict the phytoavailability of trace metals depends upon the ability to predict (from that extractant) the extent to which plants will accumulate that given trace metal. Although comparisons between various treatments (and studies) can be problematic due to variations in soil types and treatment durations (Krishnamurti et al. 2000), an effective extractant should be able to assess the phytoavailability of the trace metal under a variety of conditions.

Older testing methods for trace elements are frequently aggressive by design because earlier analytical methods were generally too insensitive to detect low levels of elements in the extracts (McBride et al. 2003). Comparatively recently, metal phyto-availability has often been estimated using mild-extraction methods, such as neutral salts, mild acids, organic extractants, and resin-based techniques, all with only limited/ varying success. Although good correlations have recently been reported for a number of novel extractants such as diffusive gradients in thin films (DGT) (Nolan et al. 2005; Song et al. 2004; Zhang et al. 2001), and lux-marked bacteria (Palmer et al. 1998)), too little data is currently available to adequately assess these new approaches. In addition to the single chemical extractants, several solid (e.g. sequential, spectroscopic) and solution phase speciation methods have been used to estimate metal phytoavailability (McLaughlin et al. 2000). Although the sequential extraction approach is unlikely to provide precise information on the mineral phases to which trace metals are bound, it does provide information on potential mobility of metal contaminants. Attempts to quantitatively predict phytoavailability and toxicity from sequential extraction data alone have not typically been successful (see McLaughlin et al. (2000) and references therein). This is not only due to limitations of analytical speciation techniques, but also to the complexity of the interactions between metals and biota, and needs to be taken into account when estimating metal phytoavailability.

This report examines the effectiveness of a variety of extracts used for predicting the phytoavailability of trace metals. A dataset consisting of approximately 5700 individual data points was collected from the literature for Cd, Zn, Ni, Cu, and Pb, and the relationship between the extractable concentration and plant tissue concentration (or plant yield) examined for various extractants. The data presented here may be used (for appropriate extractants) to predict the likelihood that the metal will accumulate within the shoots to levels which are likely to either reduce plant growth or cause harm to animals which consume the plant tissue. The approach described here offers the advantages of providing a very diverse set of test samples. However, datasets in publications are more commonly provided in order to establish that an extractant does provide an effective prediction of phytoavailability, than to establish that it does not. Thus, collection of data from published papers has a tendency to bias the result toward successful prediction, as the data from studies where the extractant was not successful are less available.

Methods

Dataset

An extensive data set encompassing a wide range of levels of contamination and many different substrates was collected from the literature. A variety of databases were searched (including ISI Web of Science, Google Scholar, Geobase, GeoRef, and Biological Abstracts); July 2009 was the final date of searching. In addition, the bibliographies of retrieved references were scanned for further relevant publications. Data were extracted from both the figures (by calculation) and the tables of the collected literature, and placed into structured summary tables for future reference. Several selection criteria were used to determine eligibility for inclusion into the dataset (see 'Assessment criteria' section, p. 21). The minimum dataset required for inclusion was species, plant tissue or yield data, and extractable concentration. Unpublished data (from the authors) was also collected and included in the analysis. Although database searches initially retrieved approximately 600 studies, the majority of these studies varied from the standard extraction procedures (predominantly due to a change in the extractant concentration; see Table 3) or the dataset presented was inadequate for this study. A total of 125 studies (approximately 5700 individual data points) met the selection criteria and were included in the dataset.

Table 3. Extractants previously used (and reported in the current study) to predict the phytoavailability of trace metals.

	Concentration (M)	Soil: extractant	Time (min)	Reference
Total				Hossner (1996)
DTPA	0.005	1:2	120	Lindsay and Norvell (1978)
EDTA	0.05	1:10	60	Quevauviller et al. (1997)
HCl	0.1			Baker and Amacher (1982)
$CaCl_2$	0.01	1:10	120	Novozamsky et al. (1993) and Houba et al. (2000)
NH_4OAc	1	1:1.5	20	Sanka and Dolezal (1992)
NH_4NO_3	1	1:10	60	Symeonides and McRae (1977)
NH_4Cl	1			Krishnamurti et al. (1995)
$NaNO_3$	0.1	1:2.5	120	Sanka and Dolezal (1992)
Mehlich 1				Korcak and Fanning (1978)

Individual studies often examined several extractants and plant species, although there was often overlap between studies in regards to the plant species examined. The most commonly studied plant species included lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* L.), alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* L. Merr.) for the dicots, and maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) for the monocots.

For Cd, Zn, and Ni, a linear regression (GenStat 2003) was used to examine the relationship between the extractable trace metal concentration and the plant shoot tissue trace metal concentration. Examination of the dataset revealed that often the soil contamination levels resulted in plant tissue levels that were several orders of

magnitude greater than that which is acceptable in the diets of animals. In many respects, the ability of a soil test to discriminate at these high levels of contamination is of little value; it is typically sufficient to identify the soil substrate as being contaminated to an extent that the indicative threshold will be greatly exceeded. A more critical assessment is to consider how well soil tests are able to discriminate between materials with contamination levels that result in plant tissue concentrations close to (or lower than) the indicative threshold. Therefore, all statistical analyses were limited to data with values less than double that of the lower of the two indicative toxicity threshold levels. Limiting the statistical analysis to the lower data points also serves to

- exclude data greatly in excess of the thresholds which may have high leverage and thus skew the statistical analysis, and
- exclude data from heavily contaminated soils where the ligand may have become saturated (for example, see Clayton and Tiller (1979)).

For Cu and Pb, a regression analysis using the Mitscherlich (exponential) model was used to examine the relationship between the extractable trace metal concentration and the relative plant yield (GenStat 2003). For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset was noted.

Although initially collected for a total of nine trace metals and a wide range of extractants, there were insufficient datasets published in the literature to permit analysis for several of the trace metals/extractants. As a result, data is presented for Cd, Cu, Ni, Pb, and Zn with a variety of extractants as shown in Table 2. Similarly, although data was collected for both monocots and dicots, only the data for monocots are presented herein (except for Figure 1). Therefore, of the 5700 initially collected, approximately 2000 data points are presented in this study.

Assessment criteria

Screening methods have been used in the development of most national guidelines, including the Australian and New Zealand water quality guidelines (ANZECC) and the USEPA ECOTOX database. These screening methods were reviewed and updated by Hobbs et al. (2005) who provided criteria for the assessment of aquatic toxicity data. As part of a review of the NEPM and the ecological investigation levels (EILs), Heemsbergen et al. (2008) (see also Heemsbergen et al. (2009)) adapted the screening method of Hobbs et al. (2005) to suit terrestrial ecotoxicology data (both organic and inorganic). Whilst the schemes of Heemsbergen et al. (2008) and Hobbs et al. (2005) provide a basic framework for the generalised assessment of eco-toxicology data, there is a need for a set of criteria developed specifically studies investigating the phytotoxicity of trace metals.

Acceptance criteria

Acceptance criteria were developed to exclude studies where the test results were incompatible with the purpose to examine the effectiveness of a variety of extracts used for predicting the phytoavailability of trace metals (Table 4).

Table 4. Acceptance criteria used to assess studies for examining the relationship between the soil-extractant concentration and the plant tissue concentration.

No.	Acceptance criteria
1	The study must be the primary source of the data
2	The test medium is soil
3	The concentration of the trace metal in the soil has been measured using at least one chemical extractant and the extraction procedure used in the study conformed to the standard procedure for that extractant (for example, extractant concentration, extraction time, and extractant:soil ratio)
4	The plant species grown in the contaminated soil is reported (including scientific name)
5	The study relates the soil-extractant concentration to either the concentration of trace metal in the plant shoot tissue or the relative plant yield

No evaluation criteria were applied in the current study for investigating the relationship between the soil-extractant metal concentration and the shoot metal concentration (or plant growth). This is in contrast to other criteria applied for assessment of terrestrial toxicology data. For example, the ECOTOX (USEPA Eco-SSL, which is based upon the total trace metal content of the soil) excluded studies where the pH of the soil is < 4 or > 8.5 or studies where the organic matter content is > 10%. Rather, the approach was taken that, ideally, a chemical should extract only the metals which are available to the plants and that an effective extractant should be able to assess the phytoavailability of the trace metal under a variety of conditions. Therefore, all studies which met the acceptance criteria were included, giving a wide range in soil properties and experimental conditions.

Results and discussion

Differences between plant species

An immediate difficulty in comparing data is that different plant species growing under the same conditions will accumulate different concentrations of trace metals in their tissues. For example, leafy vegetables (such as lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), and radish tops (*Raphanus sativa* L.)) tend to accumulate more Cd and Zn than monocots and dicots (Figure 1). Identification of this source of variability is important when considering the revegetation of contaminated lands and wastes. In order to reduce the variability caused by having multiple species types (Figure 1), all data presented in this section (other than for Figure 1) is for monocots only. This is based on the assumption that monocots, particularly grasses, are the most frequently used species for revegetation of contaminated lands. Thus, if considering the growth of a leafy vegetable on a contaminated soil, the relationship between extractable trace metal and plant tissue trace metal would differ from that which has presented in this study.

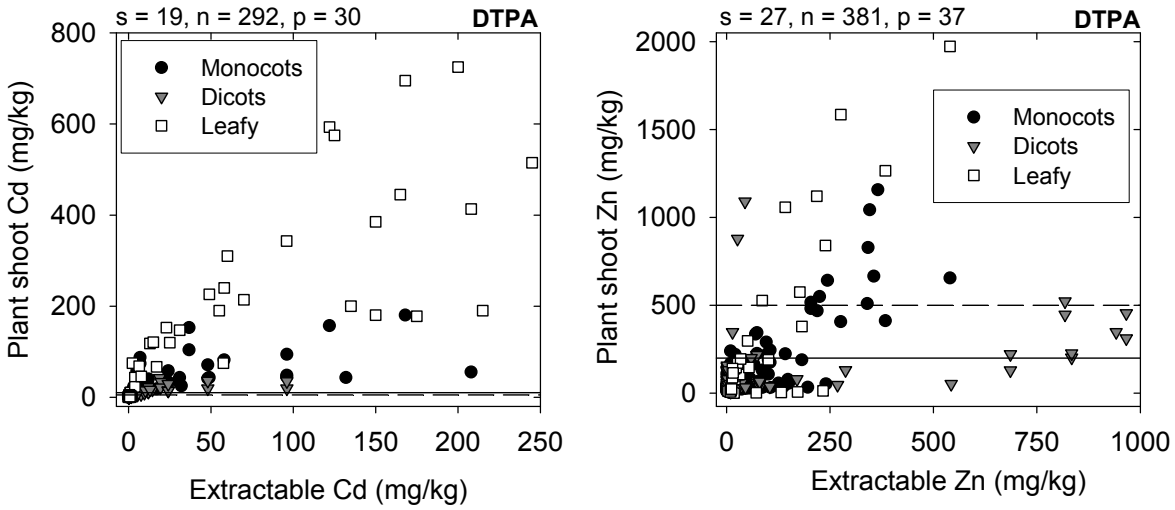

Figure 1. Relationship between the soil DTPA extractable concentration and the plant shoot concentration for both Cd and Zn for a range of monocots, leafy vegetables, and dicots.

Note: The horizontal dashed line represents the indicative toxicity threshold level in animal diets, while the solid horizontal line represents the indicative level for plant toxicity. The number of studies (s), data points (n), and plant species (p) comprising that dataset is given.

Differences between trace metals

In this report, the metals of interest are separated into two basic groupings: those that accumulate in the plant tops (and for which a reasonable relationship can be developed between the shoot tissue metal concentration and plant yield (Cd, Zn, and Ni)), and those that accumulate primarily in the roots in most plants (and for which the relationship between shoot tissue metal concentration and yield is often poor (Cu and Pb)).

For trace metals which accumulate in the shoots, trace metal toxicity is of interest due to a reduction in plant growth, and/or a toxicity to grazing animals. Thus, in order to examine the effectiveness of the various extractants for these trace metals, the extractant concentration is best related to the concentration in the plant shoot (Figure 1 to Figure 4). However, for trace metals which accumulate in the roots, plant shoot concentrations are typically low (< 25 to 50 mg/kg) (Godbold and Kettner 1991; Kopittke and Menzies 2006) and generally do not present a toxicity risk to grazing animals. Furthermore, shoot concentrations of these root-accumulating trace metals may not necessarily reflect the supply (and phytotoxicity) of that trace metal. Thus, in order to assess the effectiveness of the various extractants at predicting the phytotoxicity of these root-accumulating trace metals, it is first necessary to determine what plant parameter (shoot concentration, root concentration, or relative shoot yield) should be related to the trace metal concentration in the extractant.

For trace metals which accumulate in the root, shoot concentrations are typically relatively unresponsive to supply, and hence do not necessarily reflect the degree of toxicity and growth limitation resulting from excess supply (Ali et al. 2002; Taylor et al. 1992; Wheeler and Power 1995). This lack of response in shoot trace metal concentration to soil availability typically has resulted in a poor prediction of shoot uptake from soil tests for both Cu (Badilla-Ohlbaum et al. 2001; Pedersen et al. 2000) and Pb (Sistani et al. 1995; Taylor et al. 1992). However, plant species appear to differ markedly in this respect. For example, Walker et al. (2003) showed that radish tissue Cu content was correlated with 0.1 M $CaCl_2$ Cu (R^2 = 0.56), while in the same

soils, tissue Cu of *Brassica juncea* (L.) Czern. was not correlated with extractable Cu (R^2 = 0.001). Hence, although soil testing has been reported to be correlated with shoot concentration by some authors (e.g. DTPA by Cajuste et al. (2000); EDTA by Gupta and Aten (1993); Mehlich 1 by Borkert et al. (1998); neutral salts by Gupta and Aten (1993)), reports of poor prediction of Cu toxicity are more common (Brun et al. 1998; Faust and Christians 1999; Jarvis and Whitehead 1981). Assessed across the range of soils and species reported in the literature, no relationship between plant shoot Cu concentration and extractable Cu could be determined for any extractant (data not presented).

While the assessment of the root trace metal concentration has been suggested as an alternative to the shoot concentration (Chaignon and Hinsinger 2003; Rooney et al. 1999), one clear difficulty with this approach is cleaning soil material from the roots. In the study of Rooney et al. (1999) the plant roots contained less Pb than the soil, making contamination of plant tissue by soil a concern unless rigorous cleaning is performed. Thus, for metals which accumulate in the roots (in which the primary concern is a reduction in plant growth, rather than the consumption of plant shoots by grazing animals), it is considered preferable to relate the concentration of extractable trace metal to the relative yield of the plant (Figures 5 and 6). However, few studies in the literature have published such data for Cu and Pb, and hence only limited datasets were established for these trace metals.

Relationship between trace metals in soil-extracts and plant tissue

Data are presented below for commonly used extractants across a range of soil types and experimental conditions. Ideally, an effective extractant should be able to assess the phytoavailability of the trace metal under a variety of conditions. Thus, where a relationship is found between the soil-extractant concentration and the plant tissue concentration, the data may be used to predict the likelihood that the trace metal will either reduce plant growth or accumulate within the shoots to levels which are likely to be detrimental to animals which consume the plants. However, comparatively few data available for some extractants (particularly the neutral-salt extractants). Thus, care must be taken when utilising this data, bearing in mind that datasets are more commonly provided to establish that an extractant is effective in the prediction of phytoavailability (and hence poor relationships are developed largely through comparison of multiple studies).

Total elemental concentration

The total concentration of trace metal in the soil generally provided a poor indication of plant phytoavailability compared to other extractants with $R^2 < 0.50$ for Cd (Figure 2), Zn (Figure 3), and Pb (Figure 6), although a slight correlation was found for Cu (R^2 = 0.609) (Figure 5). The data for Zn presented in Figure 3 is primarily derived from agricultural soils which have been contaminated with Zn through sewage sludge applications, or through the addition of metal salts. It would be expected that, under these conditions, the metal would be held primarily in forms other than as a constituent of mineral phase (for example, chelated to organic matter, adsorbed to mineral surfaces or present as recently formed precipitates), and hence would be relatively

plant available (c.f. mineral Zn). Even so, the observed relationship for Zn was poor, indicating that the total metal content is a poor predictor of availability.

The observation that the total soil trace metal content is a poor indicator of phytoavailability is not unexpected, seeing as free trace metal ions are more toxic to growth than mineral-phase or strongly complexed trace metals. Indeed, several authors have reported that the phytoavailability of trace metals is more strongly correlated to the free metal ion activity in the soil solution than to total metal content of the soils (Sauve et al. 1996; 1998). Similarly, under the experimental conditions of Murray et al. (2000), it was concluded that the level of metals in plant tissue was not influenced by the total soil concentration of Cd, Cu, Ni, Pb and Zn.

Complexing reagents – DTPA and EDTA

The DTPA extractant has been widely used to assess the phytoavailability of many trace metals, and concentrations of DTPA-extractable trace metals have been reported to correlate well with plant uptake for Cd, Zn, and Ni (Cajuste et al. 2000; L'Huillier and Edighoffer 1996; Schwab et al. 1991; Simmons and Pongsakul 2004). However, when compared across a wide range of soil types in the current study, although DTPA was generally better than the total soil content, both DTPA and EDTA provided a poor prediction of phytoavailability for all five trace metals (see Figures 2 to 6); only in one instance was $R^2 \geq 0.50$. These results are similar to those reported by other authors who also concluded that complexing reagents give poor correlation to plant uptake (Baxter et al. 1983; Cajuste et al. 2000; Miner et al. 1997; Sistani et al. 1995). In a comparison of the effectiveness of seven different extractants for the prediction of Cd availability Krishnamurti et al. (2000) found that the two extractants based on EDTA provided the poorest prediction of Cd availability. This apparent conflict in the reported effectiveness of complexing extractants may be due, at least partially, to differences between studies in the soils organic matter content; soil pH; the amount, source and form of the metal contaminant; and the ‘age’ of contaminant.

It is also noted that, in studies which report good correlations, often only one or two soils are used; hence, correlations are likely to be high, and the relationships developed to have little general applicability (see McLaughlin et al. (2000) and references therein). Method alterations (modification of extractant chemical composition, soil:solution ratio etc), pH considerations (incorrect pH of extracting solution or soil), metal loading (metal levels far in excess of the critical level), and use of the DTPA method for metals other than Fe, Zn, Mn and Cu without detailed study may also contribute to these conflicting results (Sims and Johnson 1991). The high pH associated with the DTPA extractant is often a poor representation of the true soil pH, hence resulting in changes to the soils characteristics and trace metal speciation.

Both DTPA and EDTA extractants use organic ligands capable of forming a strong complex with metals as the basis for the extraction process. This approach was developed as a chemical representation of the phytosiderophore release strategy used by metal deficient plants, and was intended to be used for testing trace metal availability (particularly Zn, Cu, Fe, and Mn) in near neutral and calcareous soils (Lindsay and Norvell 1978). However, this extractant has now been used for widely varying soils and to estimate non-essential metal (Cd, Cr, Ni, Pb) availability (Sims and Johnson 1991).

For Cd, Zn and Ni, DTPA has been reported to remove approximately 11% of the total soil metal (Sims et al. 1991), a concentration greatly in excess of that which would be removed by plants over many years and decades (McBride et al. 2003). Furthermore, O'Connor (1988) reported that the concentration of DTPA-extractable metal may be more directly correlated to the total soil metal than to the plant-available metal.

Another important consideration which is often overlooked is the soil:solution ratio and the ligand concentration. Lindsay and Norvell (1978) calculated that the capacity of 0.005 M DTPA at pH 7.3 and a 1:2 soil:solution ratio to extract Zn, Fe, Mn and Cu ranged from 550 – 650 mg/kg soil, and that during 2 h extractions of 77 Colorado soils only 3.5% of the DTPA complexation capacity was occupied collectively by the four micronutrient cations. In contrast, in some heavily contaminated soils, the ligand may become saturated. Clayton and Tiller (1979) reported that 20 mL of 0.005 M DTPA added to a soil sample has only 1/25 of the complexation capacity of the 25 mL aliquot of 0.1 M EDTA, and suggested 0.1 M EDTA may better evaluate metal availability in heavily contaminated soils. Thus, in order to avoid ligand saturation when extracting trace metals, the ligand concentration and/or the soil:solution ratio should be considered.

Acid extractants

Acid extractants such as 0.1 M HCl and Mehlich extractants tended to provide poor prediction of availability for Zn (Figure 3), Ni (Figure 4) and Cu (Figure 5). Poor prediction of Zn availability has been demonstrated for these extractants in a number of studies (McBride et al. 2003; Miner et al. 1997; Sistani et al. 1995), although under certain circumstances, they have also been reported to provide effective prediction of metal availability (Borkert et al. 1998).

Extractants such as 0.1 M HCl aim to remove metals chelated by organic matter. However, results from such extractants have been found to relate well to the total soil content (c.f. the phytoavailable fraction) (Tucker and Kurtz 1955); indeed, the Mehlich buffers have been reported to extract up to 32% of the total soil metal content (Sims et al. 1991).

Neutral salt solutions

Of all the extractant types examined, neutral salt solutions tended to provide a better relationship between soil-extractable trace metal and plant tissue accumulation. However, even for these extractants, trace metal concentrations were also generally only poorly correlated to plant phytoavailability. Further, the use of neutral salt solutions for the extraction of trace metals is a comparatively new technique, and only a limited number of studies reporting such data were found in the literature. As a result, the number of studies contributing to each of these datasets is generally low, compared to those contributing to the datasets of the more traditional extractants such as DTPA (typically 10 – 15). Considering that datasets are more commonly provided to establish that an extractant is effective in the prediction of phytoavailability (and hence poor relationships are developed largely through comparison of multiple studies), the better relationships observed in this study for neutral salt solutions are due (at least in part) to the limited datasets available.

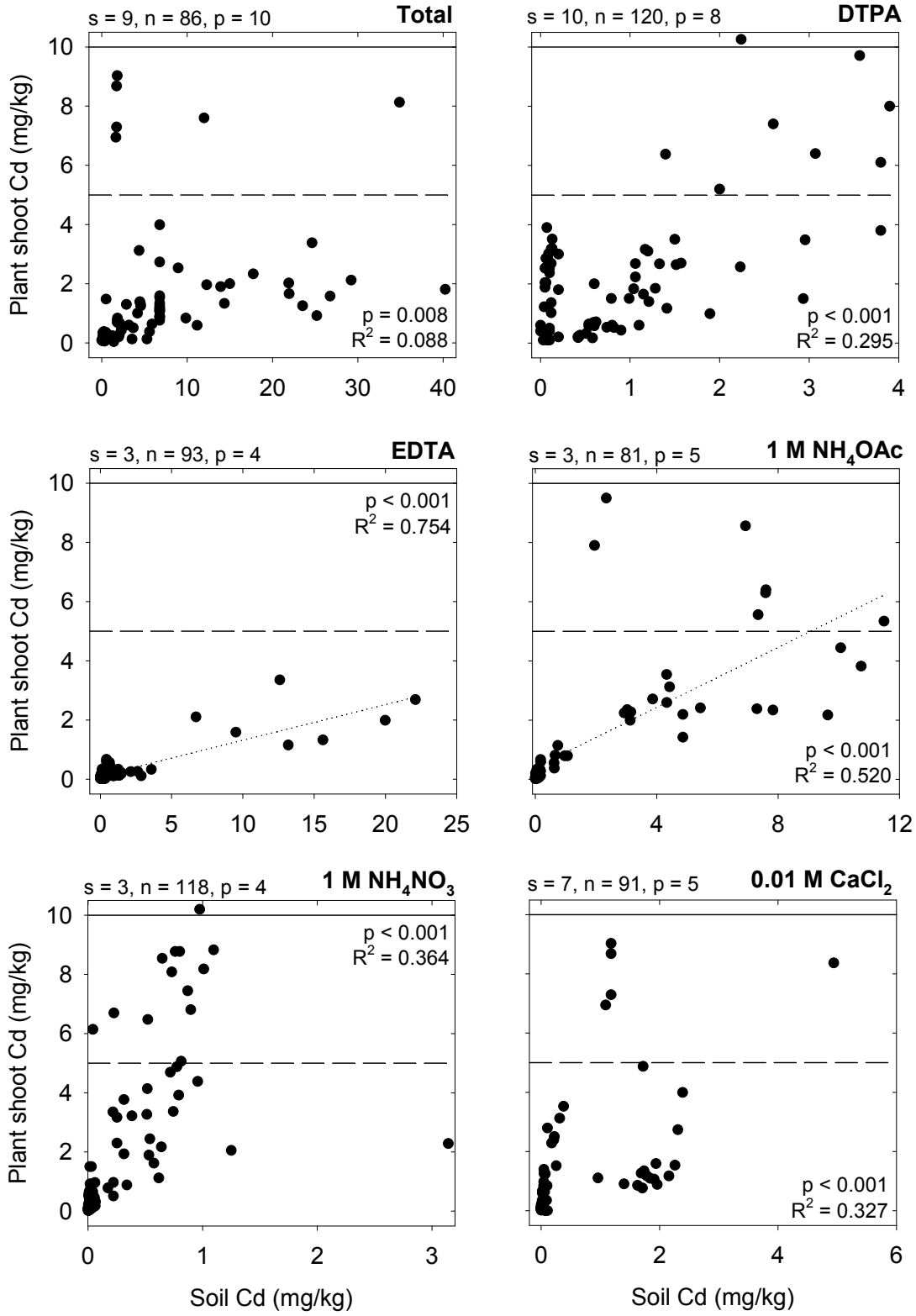

Figure 2. Relationship between soil extractable Cd and shoot Cd concentration of a range of monocots using various extractants.

Note: The horizontal dashed line represents the indicative toxicity threshold level for Cd in animal diets (5 mg/kg), while the solid horizontal line represents the indicative level for plant toxicity (10 mg/kg). For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given. A linear regression (dotted line) is fitted where $R^2 \geq 0.50$ - only data where the plant tissue Cd concentration ≤ 10.0 mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.

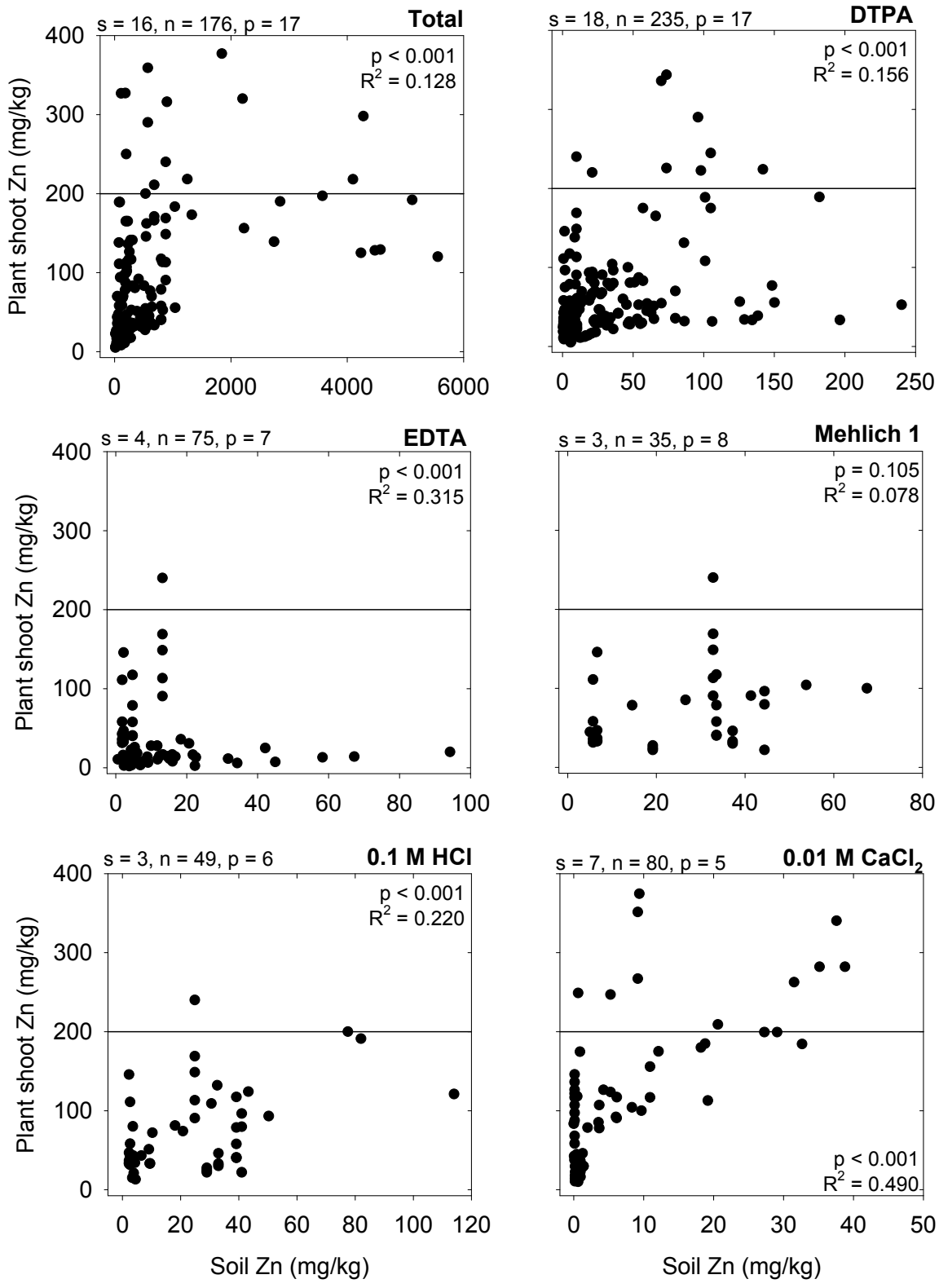

Figure 3. Relationship between soil extractable Zn and shoot Zn concentration of a range of monocots using various extractants.

Note: The horizontal dashed line represents the indicative toxicity threshold level for Zn in animal diets (500 mg/kg), while the solid horizontal line represents the indicative level for plant toxicity (200 mg/kg). For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given. A linear regression (dotted line) is fitted where $R^2 \geq 0.50$ - only data where the plant tissue Zn concentration ≤ 400 mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.

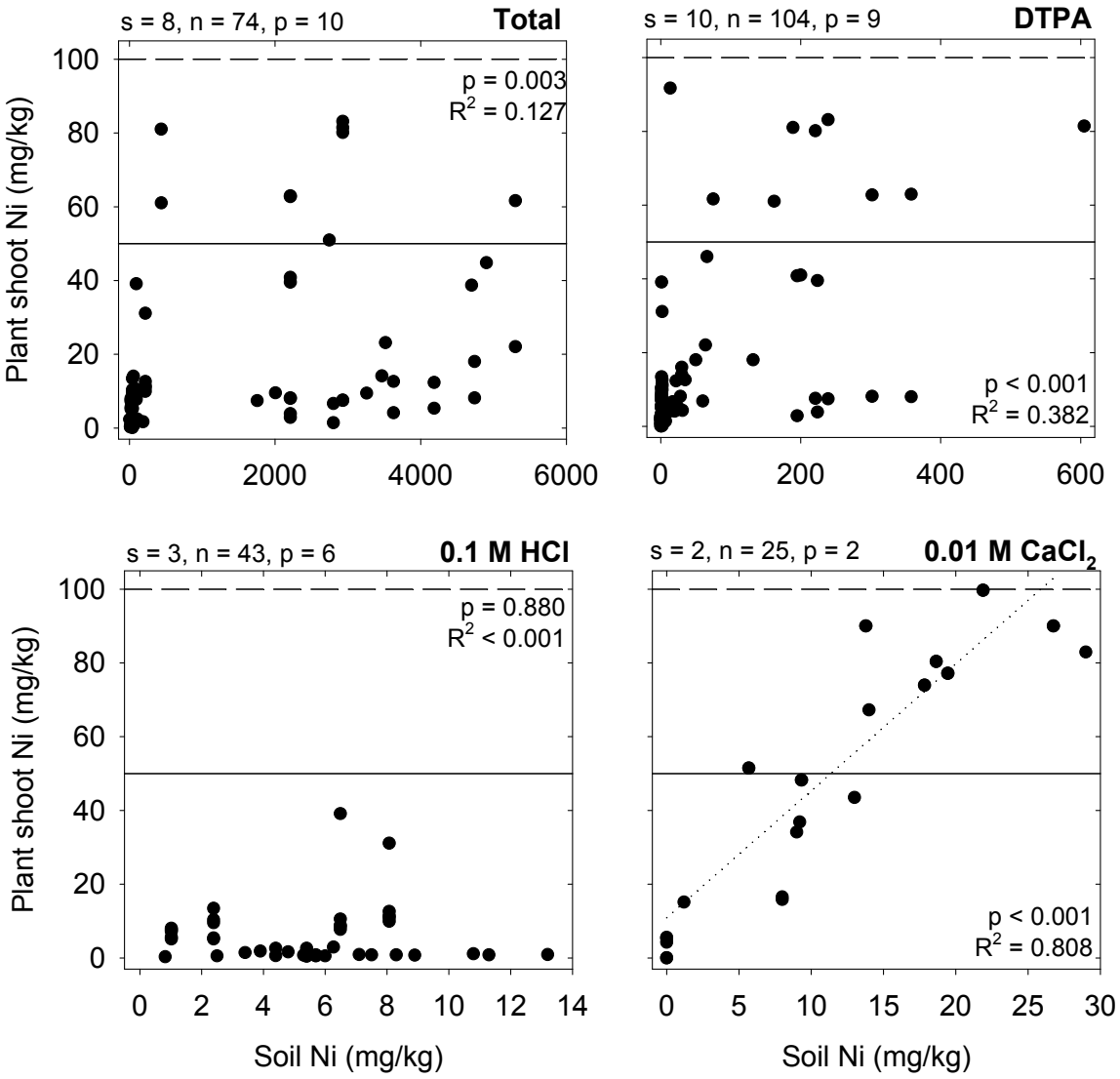

Figure 4. Relationship between soil extractable Ni and shoot Ni concentration of a range of monocots using various extractants.

Note: The horizontal dashed line represents the indicative toxicity threshold level for Ni in animal diets (100 mg/kg), while the horizontal solid line represents the indicative level for plant toxicity (50 mg/kg). For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given. A linear regression (dotted line) is fitted where $R^2 \geq 0.50$ - only data where the plant tissue Ni concentration ≤ 100 mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.

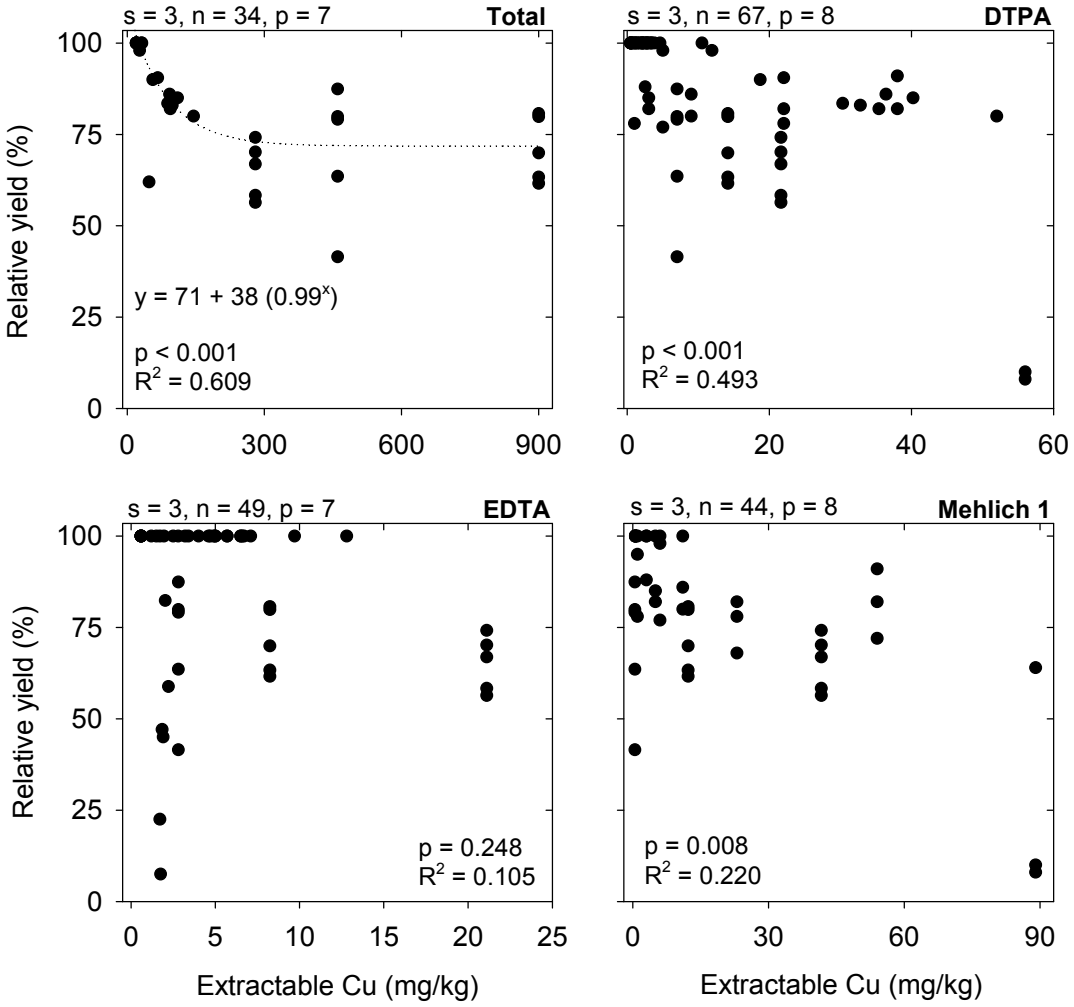

Figure 5. Relationship between soil extractable Cu and shoot Cu concentration of a range of monocots using various extractants.

Note: A Mitscherlich (exponential) model (dotted line) is fitted where $R^2 \geq 0.50$. For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given.

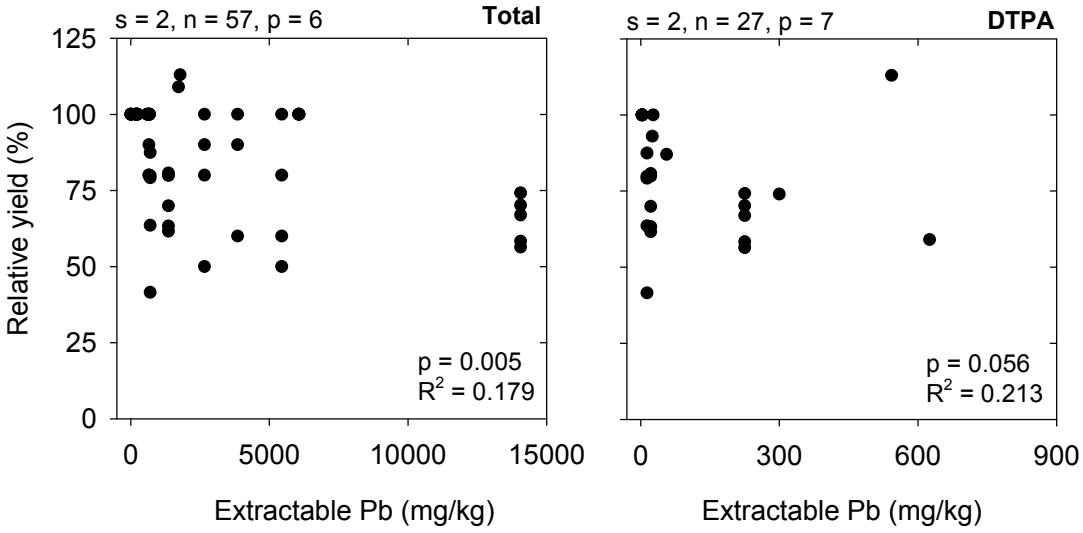

Figure 6. Relationship between soil extractable Pb and shoot Pb concentration of a range of monocots using various extractants.

Note: A Mitscherlich (exponential) model (dotted line) is fitted where $R^2 \geq 0.50$. For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given.

The use of neutral salt solutions as extractants is advocated on the assumption that phytoavailable trace metals are mostly located on mineral surfaces and can be displaced by other cations. Unlike chelating extractants (such as DTPA), neutral salts remove the metal from the soil solid phase by swamping the soil with the desorbing cation (McLaughlin et al. 2000). A variety of neutral salt extractants have been proposed for the measurement of trace metals in soils, including 1.0 M NH_4Cl or NH_4NO_3 (Krishnamurti et al. 1995; Symeonides and McRae 1977), 1.0 M NH_4OAc (Sanka and Dolezal 1992), 0.1 M $NaNO_3$ (Gupta and Aten 1993), 0.01/0.1 M $CaCl_2$ or $Ca(NO_3)_2$ (Andrewes et al. 1996; Krishnamurti et al. 1995; Whitten and Ritchie 1991), and 1.0 M $Mg(NO_3)_2$ or $MgCl_2$ (Krishnamurti et al. 1995; Shuman 1979). For metals that form complexes with the Cl ion, the use of the Cl (rather than NO_3) salt encourages desorption due to complexation of the free ion by Cl (McLaughlin et al. 2000).

While other studies have also reported neutral salt solutions to be more effective in estimating plant availability than the more aggressive tests such as DTPA (Gupta and Aten 1993; Lebourg et al. 1996), there tends to be no general agreement as to which neutral salt solution is the most effective. Although the use of $CaCl_2$ has been advocated in Europe (Houba et al. 2000; Jackson and Alloway 1991), USA (McBride et al. 2004), New Zealand (Andrewes et al. 1996), and Australia (Whitten and Ritchie 1991), it has also been reported that other neutral salt extractants such 1 M NH_4Cl (Krishnamurti et al. 1995; Krishnamurti et al. 2000) and 0.1 M $NaNO_3$ (Gupta and Aten 1993) provide a substantially better indication of plant available concentrations than does $CaCl_2$. In fact, each of the different extractants have been reported to provide various benefits when compared to the others (e.g. 0.01 M $CaCl_2$ in Houba et al. (1990), 0.1 M $NaNO_3$ in Gupta and Aten (1993), 1.0 M NH_4NO_3 in Gupta and Aten (1993)). However, although Gupta and Aten (1993) recommend the use of 0.1 M $NaNO_3$, examination of their dataset suggests that other extractants (such as 0.01 M $CaCl_2$) performed equally well or better. Thus, based on the datasets analysed in this study, and in view of the effectiveness of 0.01 M $CaCl_2$ for a number of other metals (e.g. Al and Mn (Menzies 2003)), it is likely that this extractant may also be suitable for trace metals such as Cd, Zn, Ni, and Cu.

In conclusion, after examination of a large dataset taken from published concentrations for Cd, Zn, Ni, Cu, and Pb measured using a range of extractants, we suggest that the total soil heavy metal concentration should be used only to establish threshold values to undertake further detailed investigations. Similarly, heavy metal concentrations determined by extraction using complexing agents (such as the widely used DTPA and EDTA extractants) or acid extractants (such as 0.1 M HCl) were generally poorly correlated to plant uptake. Whilst it would appear that neutral salt extractants (such as 0.01 M $CaCl_2$, 0.1 M $NaNO_3$, and 1.0 M NH_4OAc) provide a better indication of metal bioavailability across a range of metals of interest, trace metal concentrations determined by these neutral salt extractants was also only poorly correlated to plant phytoavailability in most instances. Thus, the data presented here indicate that none of the commonly used chemical extractants can be used to consistently predict availability of trace metals to plants.

APPENDIX B.

Review of trace metal toxicity in solution

Summary

A review of the literature suggests that, across a range of plant species and experimental conditions, the phytotoxicity of the trace metals in solution 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, 1.8 Cu, 5.0 Cd, 12 As, 17 Co, 20 Ni, 23 Zn, and 47 Mn. The reported toxic concentrations of the nine trace metals varied by five orders of magnitude due to differences among the trace metals in their toxicity, plant genotypes in their sensitivity to trace metal stress, and experimental procedures. The data presented here will assist in assessing the risk associated with sites contaminated by trace metals and, in particular, with relating the soil-extractant concentration to the plant tissue concentration.

Introduction

While it is important to understand the relationship between the concentration of the trace metal in the soil and its concentration within the plant tissue (Appendix 2), examination of phytotoxicity in solution culture allows the concentration of the metal which induces toxic effects to be determined, and allows comparison of the tolerance of various species to the metals. There is a need, therefore, to summarise the data that have examined the influence of trace metals on plant growth in solution culture. However, while the phytotoxicity of trace metals has been studied for over a century (e.g. Jensen (1907)), there remains considerable variation within the literature as to the concentrations of trace metals which are used to induce toxic effects. An initial examination of the literature relating to trace metal toxicities in solution culture revealed that the concentrations used to induce toxic effects vary by at least eight orders of magnitude, from 1 nM (Godbold 1991) to 400 mM (Chacon et al. 1998). This variation results from differences in the toxicity of the various trace metals, tolerances among plant species, and the experimental techniques used in the various studies.

Although it is these first two points (i.e. the toxicity of trace metals and the tolerance of plants to them) which form the basis of many phytotoxicity studies, it appears that differences in experimental conditions confound ‘true’ toxic effects. For example, Taylor and Foy (1985) reported that ca. 30 μM Cu is required to reduce growth of wheat (*Triticum aestivum* L.) by 50%, a result achieved by Wheeler et al. (1993) with only 0.5 μM Cu. It would appear unlikely that this large discrepancy could be solely attributed 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, Cd, Co, Cu, Hg, Mn, Ni, Pb, and Zn) 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; rare trace metals (such as Ga, Gd, and Sc) were also excluded. Additionally, Fe toxicity is confined to waterlogged soils, and may be of particular interest under paddy conditions. Given the wide range of concentrations which have been reported to be toxic, selection criteria (Section 3.2)

were first established to minimise the influence of experimental conditions on apparent 'toxicity' of the nine trace metals. This review of the literature includes results of only those studies meeting the criteria.

Methods

Dataset

An extensive data set was collected from the literature for solution culture studies examining the phytotoxicity of As, Cd, Co, Cu, Hg, Mn, Ni, Pb, and Zn. Two databases (ISI Web of Science; Google Scholar) were searched from 1975 onwards; the final date of searching was July 2009. Furthermore, using ISI Web of Science, all articles citing the retrieved references and all articles cited in the retrieved references were searched for further relevant publications. The following parameters were recorded for each study entered into the database:

- publication details
- trace metal stressor
- solution pH
- total number of treatments per stressor
- duration of exposure
- plant species and cultivar, where available
- P concentration in solution
- concentration (or activity) of stressor determined as being toxic
- growth reduction caused by the stressor at that concentration
- plant growth variable measured, and
- the ionic strength of the nutrient solution as determined by modelling using Phreeqcl 2.15.0 (Parkhurst 2009) based upon the reported solution composition.

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} to EC_{50} (i.e. 25 – 50% growth reduction) were selected from studies where the growth response had been modelled (e.g. regression analysis). Some studies reported the toxicity of the stressor as the activity of the free ion (for example, the activity of Cu^{2+}); this was noted in the database, 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 typically simply report the nominal (added) concentrations. A comparison with studies on trace metal toxicity to aquatic organisms indicates greater awareness in some instances of trace metal loss from solution. Slade and Pegg (1993) and Bianchini and Wood (2008), for example, found that ≥ 50% of Ag was lost from solution. Such findings led Lee et al. (2005) to conclude from a literature search that the 'compilation is of limited value since the EC_{50} values were all reported as nominal total silver concentrations, with no consideration of silver speciation or of silver loss from the exposure media during the toxicity tests.'

Finally, values were recorded for each plant species studied, and the lowest (most toxic) value was recorded in those studies that investigated the influence of experimental conditions on toxicity (for example, pH or nutrient solution composition). A total of 132 studies were entered into the database, including 29 for Cu, 27 for Cd, 20 for Mn, 16 for Ni, 15 for Zn, 9 for As, 8 for Hg, 5 for Co, and 3 for Pb. There was an overall total of 183 data points; some studies investigated a number of plant species. The most commonly investigated species was wheat (*Triticum aestivum* L.), which was included in 18 studies. The median number of trace metal treatments was 6 (ranging from 4 to 58).

Assessment criteria for solution

Acceptance criteria

Acceptance criteria were developed to exclude studies where the test results were incompatible with the purpose to determine the range in concentrations over which trace metals have been reported to exert phytotoxic effects in solution culture (Table 5).

Table 5. Acceptance criteria used to assess studies for examining the phytotoxic effects of trace metals in solution culture.

No.	Acceptance criteria
1	The study must be the primary source of the data
2	The test medium is solution culture
3	Only a single stressor is used (or, if multiple stressors were examined, data must be provided for the stressors individually in addition to their combined effects)
4	A direct measurement of plant growth is provided, such as biomass, or elongation of the root or shoot
5	The study must examine the growth of intact plants
6	A control must be included, which either contains no added metal or a basal (non-toxic) concentration in the case of essential trace metals
7	The study must utilise a minimum of four levels (inclusive of the control) with reported nominal or measured concentrations
8	The duration of exposure (and any non-exposure periods, for example, during germination or early seedling growth) must be stated
9	The study must utilise metal concentrations sufficient to cause a significant decrease in growth
10	The study must investigate the toxicity of the free, ionic metal (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).

Evaluation criteria

Given that the experimental conditions can influence the apparent toxicity of trace metals in solution (see earlier), a set of evaluation criteria were applied to the study to ensure that the data were of sufficient quality to include in the phytotoxicity dataset (Table 6). Each of the three evaluation criteria are discussed in detail in the following sections.

Table 6. Evaluation criteria used to assess studies for examining the phytotoxic effects of trace metals in solution culture.

No.	Evaluation criteria
1	*Nutrient solution composition.* The plants must be grown in a complete nutrient solution, or at a minimum, a solution containing Ca (i.e. studies in which plants were grown in deionised water were excluded).
2	*Solution pH and trace metal speciation.* The pH of the nutrient solution must be reported. In instances where thermodynamic modelling (for example, with Phreeqcl 2.15.0 (Parkhurst 2009)) indicates the solution to be supersaturated with respect to the metal of interest, it is necessary for the solution to have been sampled, filtered and the soluble metal concentration measured.
3	*Time of exposure to metals.* If plant growth is assessed using a 'bulk' parameter, a minimum of 50% of the growth-time must be in the metal-containing solution.

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, there is a need to pay particular attention to the composition of the nutrient solution.

As 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. Because Ca does not move towards the root tip, it must be present in the test solution to maintain structural and functional integrity. Further, it has been noted that root growth is reduced rapidly when placed in solutions lacking Ca (Burstrom 1953; 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. 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 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 deionised 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 deionised water for 4 d.

The composition of a nutrient solution should ideally mimic that of a soil solution (Table 7) (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 to 3 orders of magnitude higher than those commonly found in soil solutions (Table 7). This is particularly so for P, which is typically present in soil solution at low concentration relative to those used in many nutrient solution culture studies. Soil solution P concentration is often < 2 µM in unfertilised forest soils and in highly weathered soils (Gillman and Bell 1978; Menzies and Bell 1988) (Table 7).

Table 7. 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.

	Hoagland's No. 2 solution[1] (µM)	Dilute nutrient solution[2] (µM)	Soil solution[3] (unfertilised) (µM)	Soil solution[4] (0 kg P ha^{-1} y^{-1}) (µM)	Soil solution[4] (80 kg P ha^{-1} y^{-1}) (µM)
Ionic strength	26,000	2700	4900	-	-
NO_3^--N	14,000	450	1740	-	-
NH_4^+-N	1000	150	320	-	-
K	6000	300	850	250	240
Ca	4000	500	520	370	450
S	2000	600	310	89	91
Mg	2000	100	700	150	150
P	1000	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

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

[1] See Hoagland and Arnon (1950) or Parker and Norvell (1999).

[2] Taken from Wheeler et al. (1993).

[3] Surface soil of a highly weathered Krasnozem (Oxisol) from Queensland, Australia (Menzies and Bell 1988).

[4] Average values of soil solutions collected from eight surface soils (0 to 50 mm) from New Zealand receiving P fertiliser at either 0 or 80 kg P ha^{-1} y^{-1} for 4 y (Wheeler and Edmeades 1995).

In agricultural soils, soil solution P is increased by fertiliser 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 samples in a study of 33 soils by Kovar and Barber (1988), fell below 10 µM P (Table 7). It is only in soils which have recently received P fertiliser that soil solution P concentration of ca. 100 µM is evident (Adams et al. 1980; Wheeler and Edmeades 1995; Wiklander and Andersson 1974). However, toxicities of trace metals such as Pb would not occur in these highly fertile (high-P) soils due to precipitation of metal-phosphates. Indeed, P-fertilisation is one method of reducing soluble Pb concentrations when remediating contaminated sites (for example, see Zhu et al. (2004)). Yet, in the solution culture studies reviewed, the median P concentration was 100 µM (ranging from 0 to 8300 µM) (Table 7).

In the studies reviewed, the median ionic strength was found to be 4.7 mM (ranging from 0.29 to 46 mM), with soil solutions typically having an ionic strength of ca. 0.5 to 10 mM (Agbenin 2003; Bruce et al. 1989; Edmeades et al. 1985; Menzies and Bell 1988). High ionic strength solutions often affect trace metal toxicity, as the concentration of other nutrients has an influence the toxicity of the metal. For example, Lock et al. (2007b) 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 to 105 µM) as the solution Mg concentration increased from 0.05 to 3.9 mM. Similarly, a study using the technique

of Kopittke et al. (2008b) 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 ca. 0.24 to 0.59 µM (Figure 7). This cation amelioration of cation toxicity likely 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 Figure 7 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 (Figure 7A) or at the root-cell plasma membrane surface (Figure 7B)). 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 (for example, differences in sensitivity among species). The effects of specific ions should be considered also. 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. Interestingly, Zn toxicity was alleviated in wheat and radish (*Raphanus sativus* L.) by as little as 1 to 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 Figure 7).

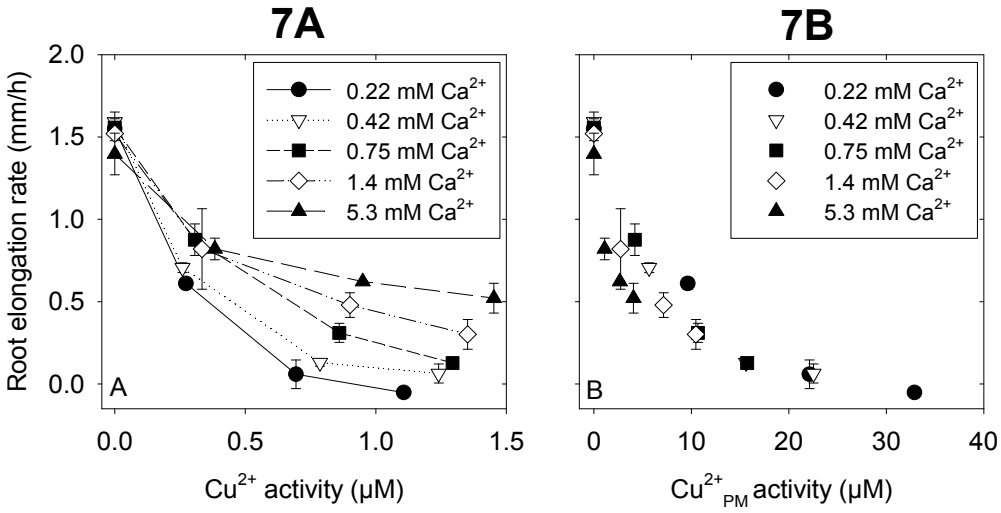

Figure 7. Effects of the activities of Cu^{2+} (in either the bulk solution (A) or at the plasma membrane surface (B)) and Ca^{2+}, and on the average root elongation rate (0 to 24 h) of 3-d-old cowpea seedlings grown in a solution containing 5 µM H_3BO_3 at pH 5.3.

Note: All bulk solution Ca^{2+} and Cu^{2+} activities were calculated using Phreeqcl from measured concentrations (see Kopittke et al. (2008b) 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 $CuCl_2.2H_2O$.

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, ca. one third of studies did not list the pH utilised, including recently published studies (e.g. Israr et al. (2006); Krantev et al. (2008); Sahi et al. (2007)). As 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 4.0 to 7.7). 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 (Figure 8) among the trace metals examined in the current review.

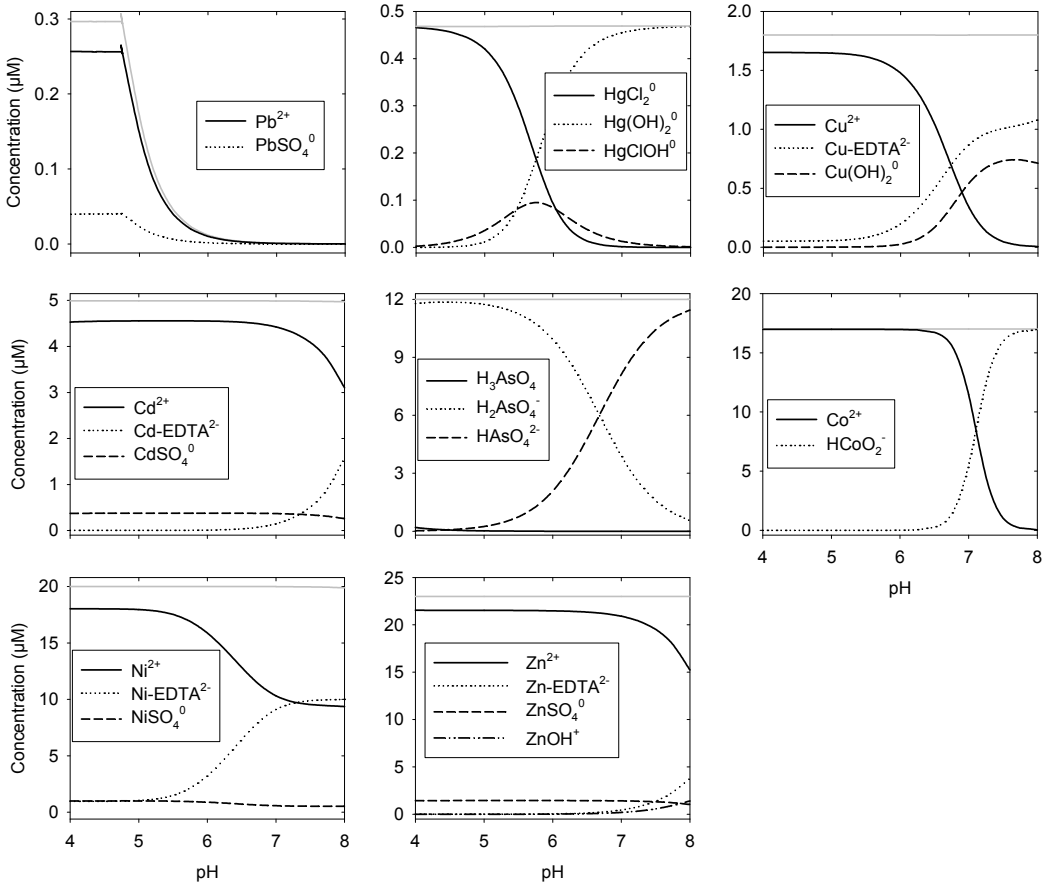

Figure 8. Speciation of nine trace metals in a dilute nutrient solution containing a total of (µM): 0.30 Pb, 0.47 Hg, 1.8 Cu, 5.0 Cd, 12 As, 17 Co, 20 Ni, 23 Zn, or 47 Mn (the median toxic concentrations listed in Figure 10).

Note: The solid gray 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 Phreeqcl 2.15.0 (Parkhurst 2009), with the Minteq database (other than for Co), using a dilute nutrient solution containing (µM): 680 NO_3^--N, 120 NH_4^+-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. 2008a) 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 Phreeqcl 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).

Lead-phosphates are highly insoluble (Kopittke et al. 2008a), 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 to 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, had 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 $Ca(NO_3)_2$ 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 Phreeqcl that < 1 µM remained in solution. Similarly, <1 µM of the 256 µM Fe (assuming Fe^{2+} was oxidised 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 likely to have also 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), Larbi et al. (2002), and Wong and Bradshaw (1982) may not have reached equilibrium. This further emphasises the need to measure the soluble trace metal concentrations in solution, 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 $HgCl_2^0$ or $Hg(OH)_2^0$ (Figure 8). The influence of Fe-chelators (such as EDTA) on solution speciation should also be considered, particularly in solutions at pH ≥ ca. 5.5 (Figure 8). 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. (2007a) 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 to 23 µM with a decrease from pH 7.0 to 4.0. Similarly, a short-term study similar to that of Kopittke et al. (2008b) showed that poor root elongation rate (0.2 mm/h) was evident at pH 4.0 irrespective of Cu concentration. At higher pH, however, the EC_{50} for Cu^{2+} toxicity in cowpea increased from 0.52 to 0.87 µM Cu^{2+} as the pH decreased from 5.3 to 4.6 (Figure 9A). As with the effect of Ca (Figure 7B), 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 (Figure 9B).

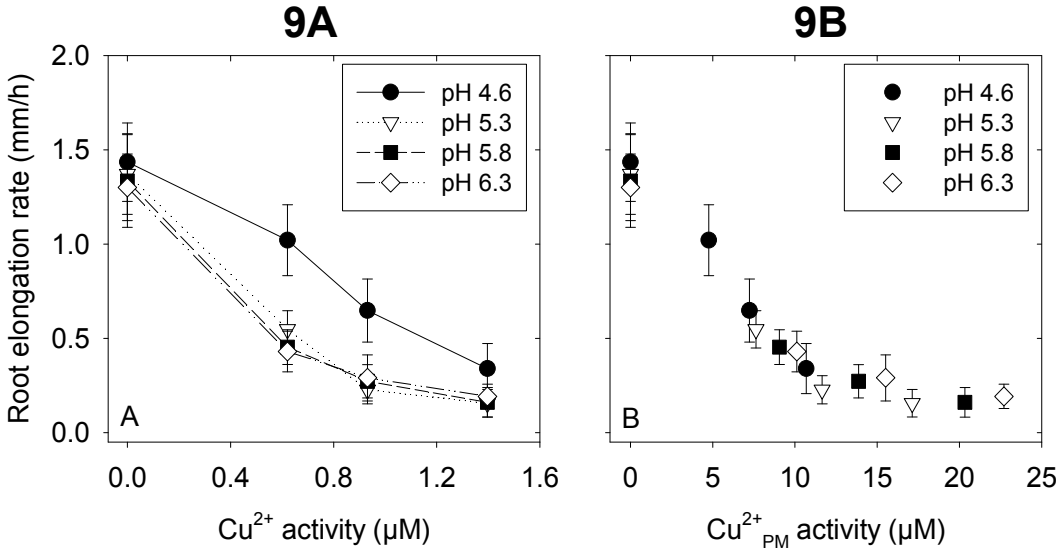

Figure 9. Effects of solution pH and the activity of Cu^{2+} (in either the bulk solution (A) or at the plasma membrane surface (B)) on root elongation rate (0 to 26 h) of 3-d-old cowpea seedlings grown in solution containing 1000 µM Ca and 5 µM H_3BO_3.

Note: All bulk solution Cu^{2+} activities were calculated using Phreeqcl from measured concentrations (see Kopittke et al. (2008b) 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 $CuCl_2.2H_2O$.

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 (Blamey et al. 2004; Kopittke et al. 2008b; Kopittke et al. 2009c; Rengel 1996), 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 including roots 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 Figure 10), but because most of the biomass had been produced in the toxicant-free environment with insufficient time allowed for differences to develop between treatments.

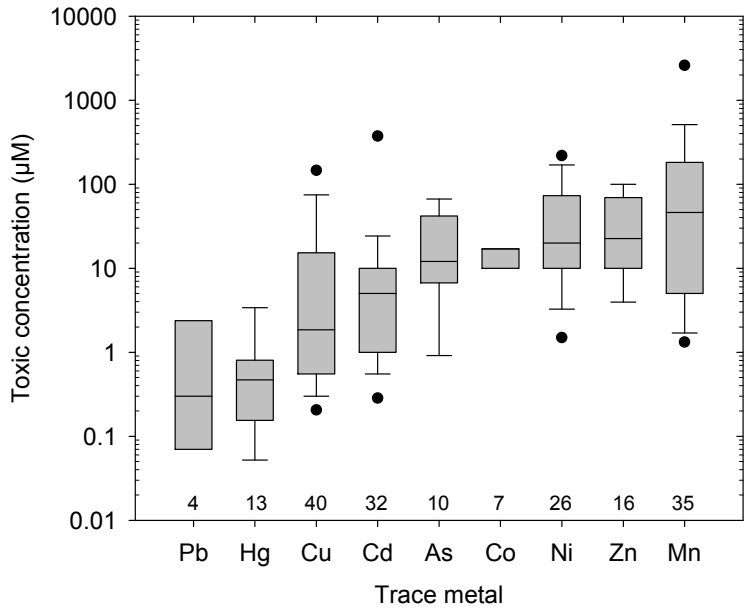

Figure 10. The concentrations of nine trace metals that reduce growth of plants in solution culture obtained from a review of literature from 1975 to 2009 (n = 183).

Note: For each trace metal, the Tukey box plot represents the 25th and 75th percentile (with the median contained therein). The whiskers represent the largest and smallest values which are not outliers, and the dots represent the largest and smallest outliers (in cases where there are at least nine data points). The numbers above each trace metal on the x-axis indicate the number of data points for that metal.

Results and discussion

Phytotoxicity of trace metals in solution

A review of scientific literature from 1975 to 2009 showed that the phytotoxicity of the trace metals followed the general trend (from most toxic to least toxic): Pb ≈ Hg > Cu ≈

Cd > As ≈ Co ≈ Ni ≈ Zn > Mn (Figure 10). The median toxic concentration varied by ca. two orders of magnitude among the nine metals, being (μM): 0.30 Pb, 0.47 Hg, 1.8 Cu, 5.0 Cd, 12 As, 17 Co, 20 Ni, 23 Zn, and 47 Mn (Figure 10). This tends to follow the general order reported in individual studies investigating the toxicity of a range of metals in one 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 in wheat (Al toxicity was also studied).

These median trace metal concentrations that are toxic are all < 100 μM (ca. 1 μM for Hg and Pb to 47 μM for Mn). The review of literature, however, identified numerous studies which utilised concentrations of trace metals which are up to four orders of magnitude higher. For example, Chacon et al. (1998) utilised solutions with up to 400 mM Mn to investigate toxicity in *Clusia multiflora* HBK. Interestingly, the ionic strength of a 400 mM $MnSO_4$ solution is 0.9 M (i.e. greater than that of seawater of ca. 0.7 M). Similarly, Zeid (2001) used to 50 mM Co in a sand culture study on common bean (*Phaseolus vulgaris* L.); Sahi et al. (2007) used up to 4.7 mM Cu in solution culture when investigating rattlebush (*Sesbania drummondii* (Rydb.) Cory), and 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.). In contrast to these (and other) trace metal concentrations used in solution culture, soil solutions toxic to plant growth have been found to contain up to ca. 1 μM Pb (Degryse et al. 2007; Weng et al. 2001), 5 μM Cu (Aguirre-Gomez et al. 2006; Luo et al. 2006), or 50 μM Ni (Anderson et al. 1973; Proctor et al. 1981). Therefore, the results of studies using high concentrations are of little value since the experimental conditions are not representative of the field situation. Although for each trace metal, the 25th and 75th percentile varied by ca. one order of magnitude (for example, ranging from 0.99 to 10 μM for Cd), this variation in concentration required to induce toxic effects is not unexpected. Rather, there are several factors which 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 inter-species differences in tolerance (for example, to aid in the identification of tolerance mechanisms, or simply to select tolerant species for revegetation of contaminated lands). Certainly, there is a large variation in the tolerance of different plants to trace metals; under the same experimental conditions, different species (or even populations within the one species) may vary by up to two orders of magnitude in their tolerance to a trace metal. For example, de Vos et al. (1991) reported that the EC_{50} for root elongation of *Silene cucubalus* Wib. was 4.0 μM Cu in a sensitive population, but only 150 μM Cu in a tolerant population collected from a Cu-contaminated site. Similarly, in 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 at 330 μM Mn for *Eucalyptus camaldulensis* Dehnh. Whilst comparisons in a specific experiment are possible, comparing metal toxicity between studies is often difficult due to different experimental conditions which markedly affect the concentration of metal which is toxic. As part of the quality assessment in the current study, several criteria were developed to include those studies where it is possible to compare results. We propose that these criteria should form the basis of all experiments investigating the phytotoxicity of trace metals.

APPENDIX C.

Definitions and data interpretation

Animal threshold: The maximum solution concentration of the trace metal for the production of shoots safe for consumption by fauna.

EC_{50} (50% effective concentration): The concentration of the trace metal in the growth media resulting in a 50% decrease in shoot growth.

MTL (Maximum tolerable level): The maximum tolerable level of a substance in the diet which, when fed for a defined period of time, will not impair animal health and/or performance (National Research Council (U.S.) 2005).

PT_{50} (50% phytotoxicity threshold): The shoot tissue concentration of the trace metal corresponding to a 50% decrease in growth.

Table 8. Use and interpretation of the 'PT_{50}' (50% phytotoxicity threshold) and 'MTL' (maximum tolerable level) for the selection of plants for the phytostabilisation of contaminated sites.

Criterion	Interpretation
PT_{50} < MTL	Lower risk of transfer of the contaminant through the food chain - the metal is likely to be more toxic to plants than to animals which consume the plant shoots. Plant growth is likely to be reduced at contaminant concentrations lower than that which would result in the accumulation of the metal within the shoots at concentrations of concern to animals. The greater the magnitude of the difference between the **PT_{50}** and the **MTL**, the lower the risk.
PT_{50} ≈ MTL	Moderate risk of transfer of the contaminant through the food chain - the metal is likely to be approximately equally toxic to plants and the animals consuming the plant shoots. Plant growth is likely to be reduced at contaminant concentrations approximately equal to those which result in the accumulation of the metal within the shoots at concentrations of concern to animals.
PT_{50} > MTL	Higher risk of transfer of the contaminant through the food chain - the metal is likely to be more toxic to animals consuming the plants than to the plants themselves. The plants are likely to continue growing and producing substantial biomass, even when the contaminant is accumulating within the shoots at levels which of concern to animals consuming the plant. The greater the magnitude of the difference between the **PT_{50}** and the **MTL**, the greater the risk.

Table 9. Approximate maximum tolerable level (MTL) and general 'PT' (phytotoxicity threshold) for a range of trace metals.

Parameter	Desired value	Explanation
EC_{50}	high	Higher values indicate a higher tolerance to the contaminant.
Animal threshold	high	A plant with a high 'animal threshold' is able to grow on a more highly contaminated site than is a plant with a low 'animal threshold' whilst still producing shoots which are safe for consumption by animals.
PT_{50} and MTL	$PT_{50} \ll$ MTL	The metal is likely to be more toxic to plants than to animals which consume the plant shoots (i.e. lower risk of transfer of the contaminant through the food chain).

Copper (Cu)

Grasses

Table 10. MTL_{Cu} = 40 µg g^{-1} for cattle (National Research Council (U.S.) 2005).

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Cu)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold[1] (µM Cu)
Decreasing Cu tolerance →	Sabi grass	*Urochloa mosambicensis* (Hack.) Dandy cv. Saraji	10	19	NA[1]
	Kangaroo grass	*Themeda australis* Forssk.	5.7	16	NA
	Hume wallaby grass	*Austrodanthonia richardsonii* cv. Hume	4.5	16	NA
	Rhodes grass	*Chloris gayana* (Kunth) cv. Pioneer	4.4	10	NA
	Signal grass	*Brachiaria decumbens* (Stapf.) cv. Basilisk	2.8	-	NA
	Curly Mitchell grass	*Astrebla lappacea* (Lindl.) Domin	2.4	12	NA
	Queensland blue grass	*Dichanthium sericeum* (R. Br.) A. Camus	1.7	18	NA

[1]*NA – the 'animal threshold' is much less than the $EC_{50\text{-shoot}}$, and could not be calculated because the plant was either dead or producing negligible biomass by the time the shoot tissue concentration would have exceeded the MTL. Data taken from Kopittke et al. (2009b)*

Table 11. MTL_{Cu} = 40 µg g^{-1} for cattle (National Research Council (U.S.) 2005).

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (DTPA-Cu) (mg/kg)	$PT_{50\text{-shoot}}$ (µg/g)[1]	Animal threshold (DTPA-Cu) (mg/kg)
Decreasing Cu tolerance →	Pearl millet	*Pennisetum americanum* (L.) Leeke	83	18	NA[2]
	Sabi grass	*Urochloa mosambicensis* (Hack.) Dandy cv. Nixon	68	18	NA
	Rhodes grass	*Chloris gayana* (Kunth) cv. Pioneer	67	18	NA
	Indian couch	*Bothriocloa pertusa* (L.) A. Camus cv. Bowen	64	18	NA
	Buffel grass	*Cenchrus ciliaris* (L.) cv. Biloela	56	18	NA
	Green couch	*Cynodon dactylon* (L.) Pers.	52	18	NA
	Setaria	*Setaria sphacelata* (Schumach.) cv. Kazungula	51	18	NA
	African lovegrass	*Eragrostis curvula* (Schrad.) Nees	50	18	NA
	Signal grass	*Brachiaria decumbens* (Stapf.) cv. Basilisk	49	18	NA
	Green panic	*Panicum maximum* Jacq. var. trichoglume cv. Petrie	48	18	NA
	Makarikari grass	*Panicum coloratum*\ (L.) var. makarikariense cv. Bambatsi	48	18	NA
	Black speargrass	*Heteropogon contortus*	35	18	NA

[1]*Critical values were calculated for all species combined, rather than for individual species.*

[2]*NA – the 'animal threshold' is much less than the $EC_{50\text{-shoot}}$, and could not be calculated because the plant was either dead or producing negligible biomass by the time the shoot tissue concentration would have exceeded the MTL. Data taken from Plenderleith and Bell (1990).*

Trees

Table 12. MTL_{Cu} = 40 µg g^{-1} for cattle (National Research Council (U.S.) 2005).

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Cu)	$PT_{50\text{-shoot}}$ (µg/g)[1]	Animal threshold (µM Cu)
Decreasing Cu tolerance	Candlebra wattle	*Acacia holosericea* (Atherton Tableland)	2.2	90	1.1
	Narrow-leaved ironbark	*Eucalyptus crebra* (Rockhampton)	1.0	22	NA[2]
	River red gum	*Eucalyptus camaldulensis* (Parish of Hollymount)	1.0	14	NA
	Weeping tea tree	*Melaleuca leucadendra* (Fitzroy River Pink Lily)	0.8	18	NA

[1] *Data taken from Reichman et al. (2006) and Reichman (2001); calculated using the data of Reichman et al. (2006).*

[2] *NA – the 'animal threshold' is much less than the $EC_{50\text{-shoot}}$, and could not be calculated because the plant was either dead or producing negligible biomass by the time the shoot tissue concentration would have exceeded the MTL.*

Manganese (Mn)

Grasses

Table 13. MTL_{Mn} = 2000 µg g^{-1} for cattle (National Research Council (U.S.) 2005).

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Mn)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold[1] (µM Mn)
Decreasing Mn tolerance →	Setaria	*Setaria sphacelata* (Schumach.) var. *anceps* cv. Narok	> 2800	N/A	1500
	Paspalum	*Paspalum dilatatum* Poir.	> 2800	N/A	380
	Green panic	*Panicum maximum* Jacq. var. *trichoglume* Robyns cv. Petrie	> 2800	N/A	880
	Rhodes grass	*Chloris gayana* Kunth cv. Pioneer	2400	3200	970
	Buffel grass	*Cenchrus ciliaris* L. cv. Biloela	1900	1900	2500
	Sabi grass	*Urochloa mosambicensis* (Hack.) Dandy cv. Nixon	800	1200	1500

[1] *Data taken from Smith (1979); calculated from exponential regressions fitted to the data of Smith (1979).*

Trees

Table 14. MTL_{Mn} = 2000 µg g^{-1} for cattle (National Research Council (U.S.) 2005) *Data from Reichman et al. (2004) and Reichman (2001).*

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Mn)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold (µM Mn)
Decreasing Mn tolerance →	River red gum	*Eucalyptus camaldulensis* (Parish of Hollymount)	> 2100	12,000	56
	Narrow-leaved ironbark	*Eucalyptus crebra* (Rockhampton)	210	5400	77
	Candlebra wattle	*Acacia holosericea* (Atherton Tableland)	130	1600	160
	Weeping tea tree	*Melaleuca leucadendra* (Fitzroy River Pink Lily)	130	2400	71

Nickel (Ni)

Grasses

Table 15. MTL_{Ni} = 100 µg g^{-1} for cattle (National Research Council (U.S.) 2005)

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Ni)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold (µM Ni)
Decreasing Ni tolerance →	Sabi grass	*Urochloa mosambicensis* (Hack.) Dandy cv. Saraji	82	120	66
	Buffel grass	*Cenchrus ciliaris* (L.) cv. Biloela	58	110	59
	Signal grass	*Brachiaria decumbens* Stapf. cv. Basilisk	57	69	77
	Queensland blue grass	*Dichanthium sericeum* (R. Br.) A. Camus	51	170	45
	Tall windmill grass	*Chloris ventricosa* (R. Br.)	46	52	70
	Rhodes grass	*Chloris gayana* Kunth cv. Pioneer	27	35	53
	Curly Mitchell grass	*Astrebla lappacea* (Lindl.) Domin	20	19	56

Data from Kopittke et al. (2009a).

Zinc (Zn)

Grasses *(Sand culture experiment)*

Table 16. MTL_{Zn} = 500 µg g^{-1} for cattle (National Research Council (U.S.) 2005)

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Zn)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold (µM Zn)
Decreasing Zn tolerance →	Hume wallaby grass	*Austrodanthonia richardsonii* cv. Hume	90	970	< 2.4
	Buffel grass	*Cenchrus ciliaris* (L.) cv. Biloela	43	570	45
	Signal grass	*Brachiaria decumbens* Stapf. cv. Basilisk	42	1500	7.3
	Rhodes grass	*Chloris gayana* Kunth cv. Pioneer	32	1200	9.3
	Curly Mitchell grass	*Astrebla lappacea* (Lindl.) Domin	18	720	9.9
	Tall windmill grass	*Chloris ventricosa* (R. Br.)	14	210	29
	Redgrass	*Bothriochloa macra* (Steud.) S.T.Blake	7.6	450	9.5
	Paspalum	*Paspalum dilatatum* Poir.	7.1	480	6.5

Note: Data from Plenderleith (1984).

Table 17. MTL_{Zn} = 500 µg g^{-1} for cattle (National Research Council (U.S.) 2005)

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (DTPA-Zn) mg/kg)	$PT_{50\text{-shoot}}$ (µg/g)[1]	Animal threshold[1] (DTPA-Zn) (mg/kg)
Decreasing Zn tolerance →	Makarikari grass	*Panicum coloratum* (L.) var. makarikariense cv. Bambatsi	199	780	250
	Signal grass	*Brachiaria decumbens* (Stapf.) cv. Basilisk	199	1300	150
	Green couch	*Cynodon dactylon* (L.) Pers.	182	1100	170
	Rhodes grass	*Chloris gayana* (Kunth) cv. Pioneer	180	1400	140
	Setaria	*Setaria sphacelata* (Schumach.) cv. Kazungula	180	790	230
	African lovegrass	*Eragrostis curvula* (Schrad.) Nees	160	680	230
	Sabi grass	*Urochloa mosambicensis* (Hack.) Dandy cv. Nixon	124	560	250
	Indian couch	*Bothriocloa pertusa* (L.) A. Camus cv. Bowen	96	680	130
	Pearl millet	*Pennisetum americanum* (L.) Leeke	90	590	160
	Green panic	*Panicum maximum* Jacq. var. trichoglume cv. Petrie	89	640	130
	Black speargrass	*Heteropogon contortus*	87	620	140
	Buffel grass	*Cenchrus ciliaris* (L.) cv. Biloela	62	270	273

[1]*Calculated using the data of Plenderleith (1984).*

Table 18. MTL_{Zn} = 500 µg g^{-1} for cattle (National Research Council (U.S.) 2005)

	Common name	Scientific name	$EC_{50\text{-shoot}}$ (µM Zn)	$PT_{50\text{-shoot}}$ (µg/g)	Animal threshold (µM Zn)
Decreasing Zn tolerance	River red gum	*Eucalyptus camaldulensis* (Parish of Hollymount)	37	600	41
	Candlebra wattle	*Acacia holosericea* (Atherton Tableland)	35	310	N/A
	Narrow-leaved ironbark	*Eucalyptus crebra* (Rockhampton)	27	600	40
	Weeping tea tree	*Melaleuca leucadendra* (Fitzroy River Pink Lily)	15	585	33

Note: Data from Reichman et al. (2001) and Reichman (2001).

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