Evaluation of extractants for estimation of the phytoavailable trace metals in soils

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Abstract

Despite its environmental (and financial) importance, there is no agreement in the literature as to which extractant most accurately estimates the phytoavailability of trace metals in soils. A large dataset was taken from the literature, and the effectiveness of various extractants to predict the phytoavailability of Cd, Zn, Ni, Cu, and Pb 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 1 extractant) are only poorly correlated to plant phytoavailability. Whilst there is no consensus, it would appear that neutral salt extractants (such as 0.01 M CaCl₂ and 0.1 M NaNO₃) provide the most useful indication of metal phytoavailability across a range of metals of interest, although further research is required.

Capsule: Neutral salt extractants appear to provide the most useful prediction of trace metal phytoavailability.
Introduction
Trace metals, which include metals (e.g. Zn, Cu, Cd) and metalloids (e.g. As), are natural components of the geosphere, hydrosphere, biosphere and the atmosphere, and are present in varying amounts in the environmental media surrounding plants, animals and humans (Thornton, 1995). Several of the trace metals (viz. Mn, Cu, Zn, Ni) are essential elements for the growth of higher plants, while these four elements together with Cr and Se are essential for animals (Underwood, 1975). The remediation of contaminated land (particularly that resulting from human activities) is driven largely by public concern and the common assumption that all trace metals are toxic, regardless of the concentrations and form in which they exist. The accurate estimation of metal phytoavailability in soils and solid wastes is becoming more important as risk assessments and remediation efforts acknowledge that total metal concentrations may not be the best predictors of metal phytoavailability. Unfortunately, the term “phytoavailability” has been defined inadequately and used ambiguously in the literature, with the concept on which it is based being unclear. The confusion stems from the fact that “phytoavailability” is not always considered as a dynamic process. A complex variety of abiotic and biotic processes affects metal phytoavailability; these include adsorption onto and desorption from mineral surfaces, precipitation, release through the dissolution of minerals, and interactions with soil, plants and microbes (Basta et al., 2005). Therefore, the accurate definition of phytoavailability
requires an understanding of the processes operating, not only in the soil, but also at the soil-plant interface (rhizosphere).

The readily soluble fraction of metals is generally considered to be phytoavailable, but there is a growing awareness that current methods of assessment of “soluble” and “phytoavailable fractions” need re-evaluation due to their variability both in space and time. These variations could arise from climate fluctuations or management practices (Adriano, 2001). Ideally, a soil test used to assess the plant-accumulation of trace metals is one which extracts trace metals under conditions similar to those exerted by the plant in the rhizosphere (Alloway and Jackson, 1991). However, metal phytoavailability has generally been estimated by various chemical extractants such as neutral salts, mild acids, organic extractants, and resin based techniques, all with only limited/varying success (McLaughlin et al., 2000). Older testing methods for trace elements are frequently chemically aggressive by design because earlier analytical methods were generally too insensitive to detect low levels of elements in the extracts (McBride et al., 2003). 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.

The objective of the work presented in this study was to examine the effectiveness of a variety of extracts used for predicting the phytoavailability of trace metals. This study was specifically targeted to the rehabilitation of contaminated mine and industrial sites where a revegetation community consisting of a range of species is to be established. A dataset consisting of 4500 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 each of the extractants.

**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 Geobase, GeoRef, Biological Abstracts, and Web of Science), with the final date of searching being July 2004. 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. Foremost, it was required that 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). Furthermore, the minimum dataset required for inclusion was species, plant tissue or yield data, and extractable concentration. However, where available, the plant species, plant tissue metal concentration, relative yield (typically defined as the relative shoot mass), concentration of metal extractable from the soil, and soil pH were recorded.

Unpublished data (from the authors) was also collected and included in the analysis. Although database searching initially retrieved approximately 500 studies, the majority of these studies varied from the standard extraction procedures (predominately due to a change in the extractant concentration) or the dataset presented was inadequate for this study. A total of 104 studies (approximately 4500 individual data points) met the selection criteria and were included in the dataset.

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*), tomato (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*) and soybean (*Glycine max*) for the dicots, and maize (*Zea mays*), wheat (*Triticum aestivum*), oats (*Avena sativa*) and barley (*Hordeum vulgare*) for the monocots.
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 1. Similarly, although data was collected for both monocots and dicots, only the data for monocots are presented herein (except for Fig. 1). Therefore, of the 4500 initially collected, approximately 2000 data points are presented in this manuscript.

The approach described here offers the advantages of providing a very diverse set of test samples. However, datasets in publications are more commonly provided 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.

Critical concentrations in plants and animals

To provide an indication of the impact of metal contamination on the overall ecosystem, two factors were considered, (i) the approximate concentration in plant tissue at which a yield decrease occurs, and (ii) the concentration of metal that can be tolerated in an animal’s diet. Where there was the potential for plant tissues to accumulate more than the threshold level for animal toxicosis (for example, Cd), this threshold level was used in addition to, or rather than, the threshold for a plant yield response. Approximate plant-toxic tissue concentrations were determined from a
wide-range of published literature values, whilst the indicative animal toxicosis value was determined from guidelines published by the US National Research Council (2005) (Table 2).

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 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 (i) exclude data greatly in excess of the thresholds which may have high leverage and thus skew the statistical analysis, and (ii) 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.

**Results and Discussion**

The usefulness of any soil extractant to predict the phytoavailability of trace metals is dependant 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.

An immediate difficulty 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*), spinach (*Spinacia oleracea*), and raddish tops (*Raphanus sativa*)) tend to accumulate more Cd and Zn than monocots and dicots (Fig. 1). Identification of this source of variability is important when considering the revegetation of contaminated lands and wastes. So as to reduce the variability caused by having multiple species types (Fig. 1), all data presented in this study (other than for Fig. 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 be different from that presented in this study.
**Accumulation in the roots or shoots**

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 (Fig. 1 to Fig. 4). However, for trace metals which accumulate in the roots, plant shoot concentrations are typically low (<25-50 mg/kg) (Godbold and Kettner, 1991; Kopittke and Menzies, 2006) and do not present a toxicity risk to grazing animals (Table 2). Furthermore, shoot concentrations of these root-accumulating trace metals may not necessarily reflect the supply (and phyto-toxicity) of that trace metal (see below). Thus, in order to assess the effectiveness of the various extractants at predicting the phyto-toxicity 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 ($R^2 = 0.56$), whilst in the same soils, tissue Cu of Brassica juncea was not correlated with extractable Cu ($R^2 = 0.001$). Hence, whilst 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), and 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).

Whilst 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 (Fig. 5 and Fig. 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.

The effectiveness of the various extractants at predicting the phytoavailability of trace metals in plants is considered below.

**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 (Fig. 2), Zn (Fig. 3), and Pb (Fig. 6), although a slight correlation was found for Cu ($R^2 = 0.609$) (Fig. 5). The data for Zn presented in Fig. 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 that 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, given that 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; Sauve et al., 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 Fig. 2 to 6); in no 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). Indeed, 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 also the “age” of contaminant. It is also noted that studies in which good correlations are reported often use only one or two soils and 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 pH associated with the DTPA extractant (pH 7.3) 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 1 tended to provide poor prediction of availability for both Zn (Fig. 3) and Cu (Fig. 5). Poor prediction of Zn availability has been demonstrated for these extractants in a number of studies (McBride et al.,
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 the best relationship between soil-extractable trace metal and plant tissue accumulation. Of the six relationships examined for neutral salts (1 M NH₄OAc for Cd, 1 M NH₄NO₃ for Cd, 0.1 M NaNO₃ for Zn, and 0.01 M CaCl₂ for Cd, Zn, and Ni), all had $R^2$ values ≥ 0.50 other than the 1 M NH₄NO₃ for Cd ($R^2 = 0.412$) (Fig. 2). However, 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 six datasets is low (< 5) 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 good relationships observed in this study for neutral salt solutions are due at least in part to the limited datasets available.

It is also noteworthy that preliminary evidence indicates that 0.01 M CaCl$_2$ may also be effective at predicting Cu bioavailability. For Cu, the 0.01 M CaCl$_2$ extractable concentration has been shown to correlate well to that in the soil solution (Lock and Janssen, 2003), and the use of 0.01 M CaCl$_2$ extraction for Cu has provided good prediction of plant uptake in some studies (Brun et al., 1998; McBride and Evans, 2002; McBride et al., 2003). However, the use of dilute CaCl$_2$ extraction has not been universally successful (Pedersen et al., 2000), and use of analytical methods to discriminate against organically-complexed Cu may also be required in dilute salt extracts as they are in soil solutions (McBride and Martinez, 2000).

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$_4$Cl or NH$_4$NO$_3$ (Krishnamurti et al., 1995; Symeonides and McRae, 1977), 1.0 M NH$_4$OAc (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; Sauerbeck and Styperek, 1985; 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 NO₃) 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; Sauerbeck and Styperek, 1985), there tends to be no general agreement as to which neutral salt solution is the most effective. Although the use of CaCl₂ 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 NH₄Cl (Krishnamurti et al., 1995; Krishnamurti et al., 2000) and 0.1 M NaNO₃ (Gupta and Aten, 1993) provide a substantially better indication of plant available concentrations than does CaCl₂. In fact, each of the different extractants have been reported to provide various benefits when compared to the others (for example, 0.01 M CaCl₂ in Houba et al. (1990), 0.1 M NaNO₃ in Gupta and Aten (1993), 1.0 M NH₄NO₃ in Gupta and Aten (1993)). However, although Gupta and Aten (1993) recommend the use of 0.1 M NaNO₃, examination of their dataset suggests that other extractants (such as 0.01 M CaCl₂) performed equally well or better. Based on the datasets analysed in this study, and in view of the effectiveness of 0.01 M CaCl₂ for a number of other metals (for example, see Menzies (2003) and Sauerbeck and Styperek (1985)), it is possible that this extractant may also be suitable for trace metals such as Cd, Zn, Ni, and Cu.

**Conclusions**
After examination of datasets for Cd, Zn, Ni, Cu, and Pb, we suggest that the total soil trace metal concentration should be used only to establish threshold values to undertake further detailed investigations, with generally no correlation between the total trace metal content and the phytoavailable concentration. Similarly, trace 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 there is no consensus in the literature, it would appear that neutral salt extractants (such as 0.01 M CaCl$_2$, 0.1 M NaNO$_3$, and 1.0 M NH$_4$OAc) provide the most useful indication of metal phytoavailability across a range of metals of interest. However, it is considered that the apparent success of these neutral salt extractants is due in part to the limited number of datasets available in the literature for examination. Thus, further research is required to investigate the effectiveness of these neutral salt extractants.

**Acknowledgments**

The authors wish to acknowledge the support provided for this study by the Australian mining industry through the Australian Centre for Minerals Extension and Research (ACMER) and for the support of the Australian Research Council.

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Table 1. Extractants previously used (and reported in the current study) to predict the phytoavailability of trace metals.

<table>
<thead>
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<th>Extractant/method</th>
<th>Reference</th>
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<tr>
<td>HF with heating (total elemental concentration)</td>
<td>Hossner (1996)</td>
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<tr>
<td>DTPA (0.005 M)</td>
<td>Lindsay and Norvell (1978)</td>
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<tr>
<td>EDTA (0.05 M)</td>
<td>Quevauviller et al. (1997)</td>
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<td>HCl (0.1 M)</td>
<td>Baker and Amacher (1982)</td>
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<td>CaCl$_2$ (0.01 M)</td>
<td>Novozamsky et al. (1993)</td>
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<tr>
<td>NH$_4$OAc (1 M)</td>
<td>Sanka and Dolezal (1992)</td>
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<tr>
<td>NH$_4$NO$_3$ (1 M)</td>
<td>Symeonides and McRae (1977)</td>
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<tr>
<td>NaNO$_3$ (0.1 M)</td>
<td>Sanka and Dolezal (1992)</td>
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<td>Mehlich 1</td>
<td>Korcak and Fanning (1978)</td>
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Table 2. Approximate indicative threshold concentration in the dietary intake for the onset of metal toxicosis in animals, and an indication of the plant tissue concentrations at which yield decrease occurs. All values are on a dry weight basis.

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<th>Indicative threshold</th>
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<tr>
<td></td>
<td>- animal toxicity^a</td>
<td>- plant toxicity</td>
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<td></td>
<td>(mg/kg)</td>
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<tr>
<td>Cd</td>
<td>5</td>
<td>20</td>
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<tr>
<td>Cu</td>
<td>250</td>
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<td>Ni</td>
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<td>Zn</td>
<td>1000</td>
<td>400</td>
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^aNational Research Council (U.S.) (2005)
Fig. 1. Relationship between the soil DTPA extractable concentration and the plant shoot concentration for both Cd and Zn for a range of monocots (hollow circle), leafy vegetables (solid square), and dicots (grey triangle). The horizontal dashed line represents the indicative toxicity threshold level in animal diets, whilst 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.
Fig. 2. Relationship between soil extractable Cd and shoot Cd concentration of a range of monocots using various extractants. The horizontal dashed line represents the indicative toxicity threshold level for Cd in animal diets (5 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 $\leq 10.0$ mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.

**Fig. 3.** Relationship between soil extractable Zn and shoot Zn concentration of a range of monocots using various extractants. The horizontal dashed line represents the indicative toxicity threshold level for Zn in animal diets (1000 mg/kg), whilst the solid horizontal line represents the indicative level for plant toxicity (400 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 $\leq 800$ mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.
Fig. 4. Relationship between soil extractable Ni and shoot Ni concentration of a range of monocots using various extractants. The horizontal solid line represents the indicative level for both plant and animal toxicity (100 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 $\leq 200$ mg/kg (i.e. double the lower of the two indicative toxicity threshold levels) is included in the linear regression.
Fig. 5. Relationship between soil extractable Cu and relative yield of a range of monocots using various extractants. 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.
Fig. 6. Relationship between soil extractable Pb and relative yield of a range of monocots using various extractants. For each extractant, the number of studies (s), data points (n), and plant species (p) comprising that dataset is given.