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Seedling responses of three Australian tree species to toxic concentrations of zinc in solution culture

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Abstract

A frequently desired outcome when rehabilitating Zn toxic sites in Australia is to establish a self-sustaining native ecosystem. Hence, it is important to understand the tolerance of Australian native plants to high concentrations of Zn. Very little is known about the responses of Australian native plants, and trees in particular, to toxic concentrations of Zn. *Acacia holosericea*, *Eucalyptus camaldulensis* and *Melaleuca leucadendra* plants were grown in dilute solution culture for 10 weeks. The seedlings (42 days old) were exposed to six Zn treatments viz., 0.5, 5, 10, 25, 50 and 100 μM. The order of tolerance to toxic concentrations of Zn was *E. camaldulensis*> *A. holosericea*> *M. leucadendra*, the critical external concentrations being approximately 20, 12 and 1.5 μM, respectively. Tissue Zn concentrations increased as solution Zn increased for all species. Root tissue concentrations were higher than shoot tissue concentrations at all solution Zn concentrations. The critical tissue Zn concentrations were approximately 85 and 110 μg g^{-1} DM for *M. leucadendra*, 115 and 155 μg g^{-1} DM for *A. holosericea* and 415 and 370 μg g^{-1} DM for *E. camaldulensis* for the youngest fully expanded leaf and total shoots, respectively. The results from this paper provide the first comprehensive combination of growth responses, critical external concentrations, critical tissue concentrations and plant toxicity symptoms for three important Australian genera, viz., *Eucalyptus*, *Acacia* and *Melaleuca*, for use in the rehabilitation of potentially Zn toxic sites.

Introduction

Zinc is a natural constituent of the earth's biogeochemical cycles and is required by plants in trace amounts. As a result of human activities such as metal mining, the use of agrochemicals, and the agricultural use of sewage sludge, Zn can be present in soils and growth media in excessive amounts, raising issues of plant tolerance to high levels of Zn (Kabata-Pendias and Pendias, 1992; Kieken, 1995).

A substantial amount of research has been conducted on the tolerance of crops (Chino and Baba, 1981; Cox, 1990; Fontes and Cox, 1995; Parker et al., 1990) and pasture species (Brookes et al., 1981) to Zn toxicity. Some research has been undertaken also on European and American tree species (Brown and Wilkins, 1985; Godbold and Huttermann, 1985). However, very little is known about the tolerances of Australian plant species to Zn toxicity.

In many cases, rehabilitating toxic sites with crop, pasture or introduced species may be the desired end-land-use. However, in some cases, including many mine sites in Australia, the desired end-product is a self-sustaining native ecosystem. Therefore, for the effective rehabilitation of these Zn-toxic sites an understanding is needed of the tolerance of Australian native plants to Zn. This paper examines the tolerance of three species which have a wide distribution across the continent and are commonly used in rehabilitation work viz., *Acacia holosericea*, *Eucalyptus camaldulensis* and *Melaleuca leucadendra*, to Zn toxicity in solution culture.

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Materials and methods

General methodology

The responses of *Acacia holosericea, Eucalyptus camaldulensis* and *Melaleuca leucadendra* to Zn concentration was studied in a glasshouse (typical temperature range during the experiment was 20 – 45 °C). Seed of the three species was germinated in sterilised University of California potting mix B with fertiliser combination II (Asher, 1978) in a controlled temperature glasshouse (23 °C day/18 °C night). When seedlings reached the four to six-leaf stage (42 days from sowing) they were gently removed from the potting mix with tap water ready for immediate transfer to solution culture.

The experiment was conducted in 22 L black polythene drums that had been painted on the outside with aluminium paint to reduce heating of the nutrient solutions. The drums were half-filled with deionised water, the aliquots of nutrients added and the solutions made up to volume. The drums were covered with removable aluminium-coloured polythene lids. The nutrient solutions were continuously aerated and stirred with filtered oil-free air from a rotary compressor. Extra deionised water was added as required throughout the experiment.

Seedlings were supported in the drum lids in plant-support baskets made by wedging a piece of plastic shade cloth between two polystyrene cups with the bases removed. The seedlings were gently pushed through the shade cloth so that the shoots were above and the roots below the mesh. Black polythene beads (approximately 2 mm diameter) were placed in the baskets to a depth of approximately 1 cm to provide support for the seedling, and to exclude light from the nutrient solution below. The baskets were placed in evenly spaced holes around the lid of each drum. There was one seedling per basket with four baskets per drum, except for *A. holosericea* where there were three seedlings per drum.

Seedlings were allowed to establish for 24 h before the Zn treatments were imposed. After 2 weeks, the seedlings were thinned leaving two plants per drum and one plant per drum for *A. holosericea*.

Nutrient solution composition

Sufficient $ZnSO_4.7H_2O$ solution was added to the drums to give initial Zn concentrations of 0.5, 5, 10, 25, 50 or 100 μM. The Zn treatments were replicated four times. The nutrient solutions were sampled and analysed weekly for Zn (by atomic absorption spectroscopy (AAS) to week 6, and by inductively coupled plasma emission spectroscopy (ICP-AES) thereafter). Zinc was added when necessary to restore the initial concentrations.

The initial concentrations of basal nutrients were (μM): N 850, K 250, Ca 250, Mg 100, S 100, P 5, B 3, Fe (as FeEDTA) 2, Mn 1, Cu 0.1, and Mo 0.02, as used in the flowing solution culture experiment of Asher and Loneragan (1967). The pH of the solution was adjusted daily to 5.0 using 0.1 *M* HCl or NaOH. To assist in pH control, 15% of the N was added as NH_4^+ until day 42 and then 10% as NH_4^+ until the end of the experiment. To help maintain saturation of the FeEDTA with Fe, a small piece of metallic Fe (acid-washed 75 mm Fe nail) was placed in each drum and no further Fe added throughout the experiment.

The NUTRADD computer simulation program (Asher and Blamey, 1987) was used to predict nutrient demand, and hence the likely time-course of nutrient withdrawal by healthy plants of each species. For these simulations, growth curves and mineral composition data were obtained from healthy plants in an earlier experiment (data not presented). The output of NUTRADD was used to calculate the size and frequency of addition of basal nutrients necessary to maintain adequate but not excessive supplies of elements other than Fe. Basal nutrient additions were made on days 0, 42, 49, 56 and 63, except for P, where more frequent, but smaller, additions were made to prevent precipitation of Zn. Solution basal nutrient composition of was analysed on days 42, 49, 56 and 63 to verify the NUTRADD predictions and where necessary, to adjust nutrient inputs in the Zn-toxic treatments. In these cases, the scheduled basal nutrient addition was multiplied by the ratio of actual uptake in the previous time interval to the predicted uptake to determine the adjusted basal nutrient addition.

Ion speciation calculations with GEOCHEM (Sposito and Mattigod, 1980) indicated that there would be no precipitation of Zn by phosphate, and little complexation with EDTA. Thus, Zn was predominantly (>90%) present as the free ion in all treatments.

Harvesting and chemical analysis

Plants were harvested 70 days after transplanting into the solution cultures. Plants were rinsed four times in deionised water. Roots, shoots and youngest fully expanded leaves (YFEL) were harvested separately. In

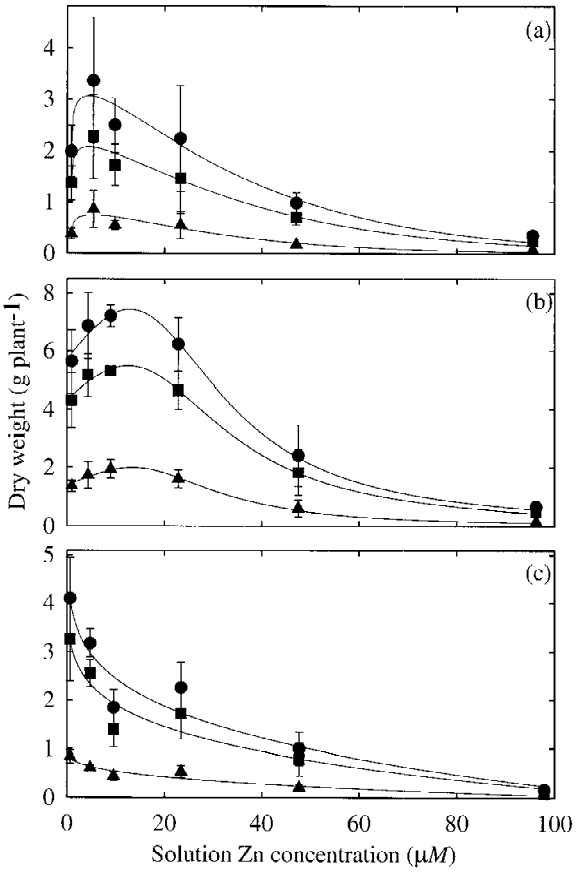

Figure 1. Effects of mean concentration of Zn in solution on the dry weights of roots (▲), shoots (■) and whole plants (●) of (a) *Acacia holosericea*, (b) *Eucalyptus camaldulensis*, and (c) *Melaleuca leucadendra*. Values are means ± standard errors.

the case of *E. camaldulensis* and *M. leucadendra*, the two plants in each drum were considered as one unit for harvesting purposes.

Plant parts were placed in paper bags and dehydrated at 60 °C for 36 h. Plant dry weights were measured. Plant parts were then digested in 5 parts nitric acid:1 part perchloric acid and analysed for Zn by ICP-AES.

Data analysis

The curve fitting section of SigmaPlot 4.0 (SPSS, 1997) was used to perform regression analyses on the data. MINITAB (Minitab, 1995) was used for descriptive statistics such as standard errors and box-plots for outlier analysis. Three outliers were found in the plant chemical data and removed prior to further analysis of the data (Table 1).

Results

Toxicity symptoms

Although *Acacia holosericea* showed growth reductions in the higher Zn treatments there were few foliar symptoms. From approximately 5 weeks into the experiment, in the 5–25 μM treatments, some plants showed a general chlorosis of the phyllodes. In some of the more extreme cases, the chlorosis progressed to necrosis of the phyllode tip. However, foliar symptoms did not appear on all the plants in any treatment, and there was considerable variation in the amount of symptom development within any treatment. In the 50 and 100 μM treatments little chlorosis was observed, and the foliage appeared to be considerably darker in these treatments than in the controls, especially throughout the last few weeks of the experiment.

In *Eucalyptus camaldulensis* plants, Zn toxicity became apparent as a slight chlorosis plus some reddening of the main vein of younger leaves from the 10 μM treatment, which progressed to the lateral veins in the leaves of some plants. In the 25 μM treatment and higher, these symptoms progressed with time to the middle leaves of the plant. More severe Zn toxicity was apparent, in the 25 μM and higher treatments, as necrotic spotting along leaf veins, and in some cases, a loss of apical dominance due to necrosis of the growing shoot tip.

In *Melaleuca leucadendra*, from the 10 μM treatment and higher, the most conspicuous and widespread symptom of toxicity was bronzing of affected leaves accompanied by tip necrosis and inward curling, starting with the younger leaves but progressing down the plant with time. Some of these leaves eventually became entirely necrotic and dropped off, leaving plants with leafless sections of stem. Plants in the 100 μM treatment did not appear to express these symptoms. Instead, growth was poor, with bright reddening of older leaves in some plants and necrotic lesions or total necrosis of some leaves, eventually leading to the death of some plants. Similar symptoms were also present in some plants in the 50 μM treatment.

Throughout the experiment, roots of *A. holosericea* plants in the 25 μM and higher Zn treatments became increasingly blackened and stubby, with little fine root growth. This type of growth has been described as 'corraloid' (Foy et al., 1978). In the 100 μM treatment, roots were severely stunted, and in many of the plants, little root growth occurred throughout the course of the experiment. At the termination of

Table 1. List of outliers removed from data before analysis

Species	Treatment (μM)	Replicate	Measurement	Value ($\mu g\ g^{-1}$)
A. holosericea	50	4	Root tissue Zn	17 281
M. leucadendra	100	1	Shoot tissue Zn	3708
M. leucadendra	100	1	YFEL tissue Zn	17 624

the experiment, some of the plants in the 10 μM treatment were beginning to display these symptoms. *Eucalyptus camaldulensis* and *M. leucadendra* root growth declined with increasing Zn concentration but the roots did not become stubby as in *A. holosericea*. Lateral and fine root growth were not strongly reduced in any treatment for these two species, except in the 100 μM treatment for *M. leucadendra*.

Effects of Zn supply on dry matter yields

Growth of *A. holosericea* was somewhat reduced in the 0.5 μM treatment, indicating a slight Zn deficiency (Figure 1a and Appendix). However, maximum dry weight was produced with the first increment of Zn, and regression analysis suggesting that maximal above ground dry weight would be produced at a Zn concentration of about 4.5 μM. Using the external concentration at 90% of the computed maximum shoot dry weight (Smith and Loneragan, 1997), the critical solution Zn concentration for toxicity was approx. 12 μM.

As with *A. holosericea*, growth of *E. camaldulensis* was depressed in the lowest treatment (Figure 1b and Appendix), the dry weight in roots and shoots increasing until after the 10 μM treatment. From the fitted curve, it appears that maximum dry weight would be produced at about 12 μM with the critical value for toxicity being about 20 μM.

Maximum growth of *M. leucadendra* occurred in the 0.5 μM Zn treatment, yields declining as the Zn concentration in the nutrient solution increased (Figure 1c and Appendix). The critical solution Zn concentration for toxicity was found to be approx. 1.5 μM.

In each species, concentrations of Zn in the tissues increased with increasing Zn concentrations in solution (Figure 2 and Appendix). Root Zn concentrations at all external Zn concentrations and for all species were considerably higher than concentrations in the shoot or the YFEL. At low external Zn concentrations, the concentrations of Zn in whole shoots and YFEL were similar in the three species. However, in *E. camaldulensis* and *M. leucadendra*, Zn concentrations in whole shoots were higher than in the YFEL in the higher Zn treatments (Figure 2d,f).

Relationship between tissue Zn concentration and relative dry matter yield

It was established for *A. holosericea* using the relationship between relative shoot dry weight and tissue Zn concentrations, that the critical concentration for Zn toxicity was approx. 115 $\mu g\ g^{-1}$ for the YFEL and 155 $\mu g\ g^{-1}$ for whole shoots (Figure 3a,b and Appendix). The corresponding critical values for deficiency were approx. 40 $\mu g\ g^{-1}$ for the YFEL and 65 $\mu g\ g^{-1}$ for whole shoots. *Eucalyptus camaldulensis* critical concentrations for toxicity were approx. 415 $\mu g\ g^{-1}$ Zn in the YFEL and 370 $\mu g\ g^{-1}$ Zn in the shoots (Figure 3c,d and Appendix). The critical concentrations for deficiency were approx. 175 $\mu g\ g^{-1}$ for the YFEL and 145 μg^{-1} for the shoots. *Melaleuca leucadendra* critical concentrations for toxicity were about 70 $\mu g\ g^{-1}$ in the YFEL and 110 $\mu g\ g^{-1}$in the shoots (Figure 3d,f and Appendix).

Discussion

Effect of Zn supply on dry matter yields

On the basis of critical external concentrations for Zn toxicity (Figure 1), *M. leucadendra* was the least Zn tolerant species, followed by *A. holosericea*, and then *E. camaldulensis*. *Melaleuca leucadendra* also had the highest number of plant morbidity by the end of the experiment in the 100 μM Zn treatment (3 out of 8 plants being dead compared with 1 out of 8 for *E. camaldulensis* and 0 out of 4 for *A. holosericea*). However, at 100 μM Zn, the concentration of Zn in the shoots ranked *A. holosericea*<*M. leucadendra*<*E. camaldulensis*. This indicates that it is not ability to restrict uptake of Zn into the shoots which defines relative tolerance in these species. While root tissue Zn

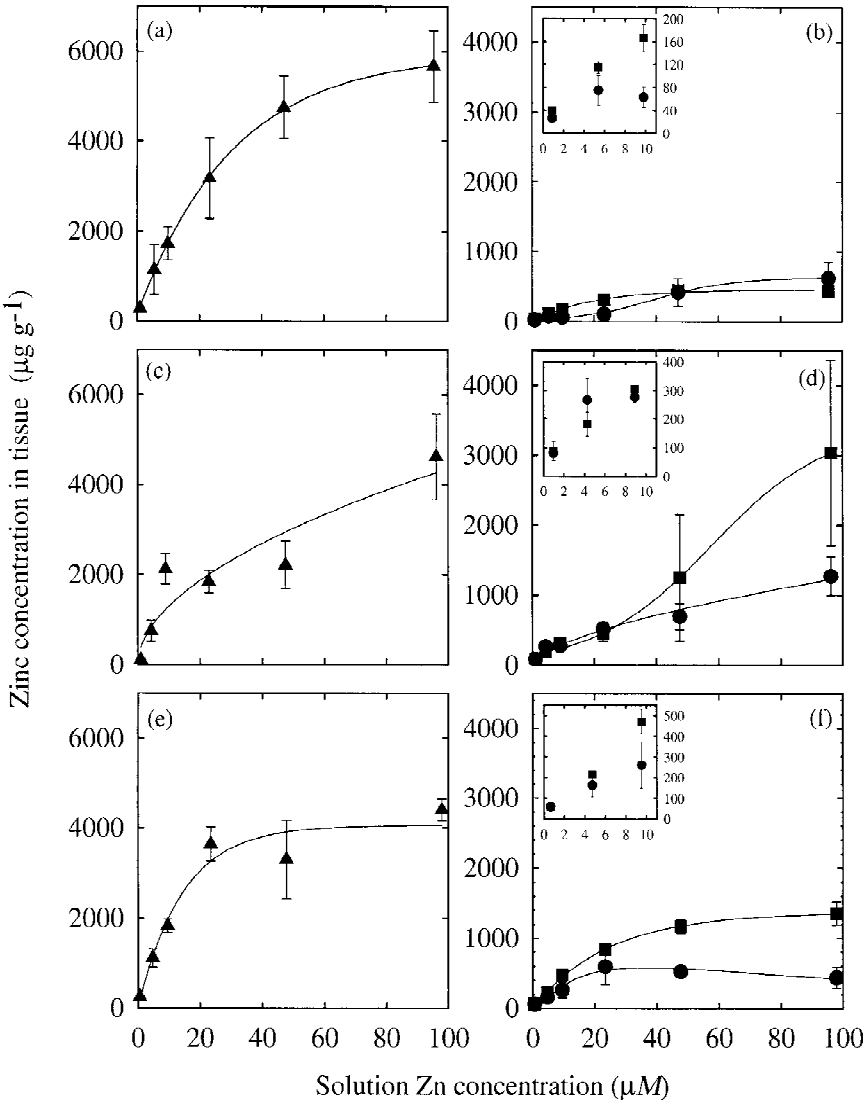

Figure 2. Effects of mean solution Zn concentration on Zn in root (▲), shoot (■) and YFEL (●) tissues of (a, b) *Acacia holosericea*, (c, d) *Eucalyptus camaldulensis* and, (e, f) *Melaleuca leucadendra*. Values are means ± standard errors.

did not follow the trend of whole-plant sensitivity, *A. holosericea* had the highest concentration of Zn in its roots in the region of the critical external concentration for toxicity (Figure 2) and was also the species with the most sensitive root system as evidenced by visible symptoms.

In each species tested, significant declines in biomass (decrease in dry weight of 10%) occurred at similar solution Zn concentrations for both the roots and shoots. Therefore, there are three possible scenarios with respect to relative tolerance of the above and below ground parts of the plants. Either roots and shoots have a similar threshold for Zn toxicity in the species tested, or either roots or shoots are more sensitive but the feedback mechanisms (e.g. reduction in supply of photo assimilates to roots or nutrients to shoots) are so tightly interconnected that it appears as though the sensitivities are similar between roots and shoots. No research has been conducted on the tolerance mechanisms of Australian species to excess Zn. Results with grafting different soybean (*Glycine max*) cultivars have shown that relative tolerances to Zn for cultivars within this species is conferred by the shoot (White et al., 1979). This would suggest that dif-

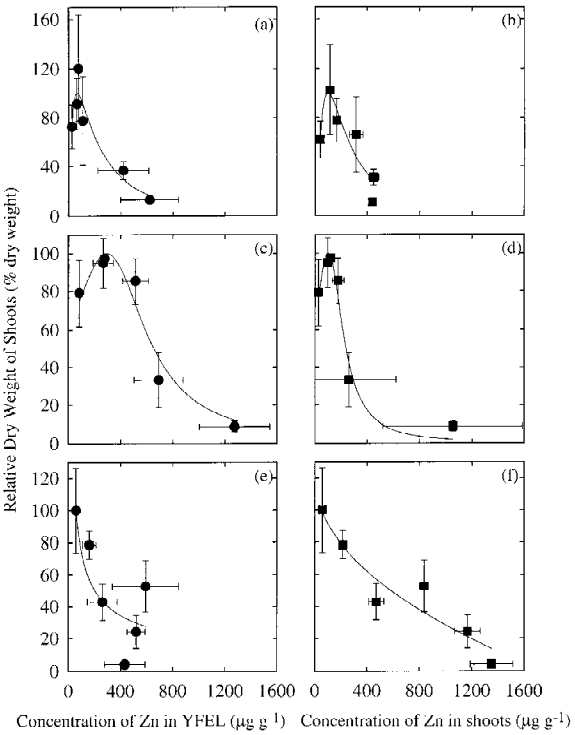

Figure 3. Relationship between relative dry weight of shoots and concentration of Zn in plant tissue (a,c,e YFEL and b,d,f shoots) for (a,b) *Acacia holosericea*, (c,d) *Eucalyptus camaldulensis*, and (e,f) *Melaleuca leucadendra*. Values are means ± standard errors.

ference in tolerance of above and below ground plant parts is genotype specific.

All three species used in this study were more sensitive to Zn than wheat (*Triticum aestivum*) which had a threshold toxicity (concentration at which growth decline first occurred) of 45 μM (Taylor et al., 1991). Broad-leaved cattail (*Typha latifolia*) was found to have a significant reduction in growth at 15 μM Zn in solution compared to a control treatment (Ye et al., 1998), an outcome comparable to the results for *A. holosericea* and *E. camaldulensis* in the present study. Using root elongation to measure toxicity, Godbold and Hutterman (1985) found that the induction of toxicity in Norway spruce (*Picea abies*) commenced between 15 and 30 μM which is again comparable with *E. camaldulensis*. In comparison, four herbaceous legumes had reductions in growth at 1.25 μM Zn in nutrient solution (Carroll and Loneragan, 1968) which is more comparable to the pattern observed for *M. leucadendra*.

Zhang et al. (1998) reported a critical concentration of 9 μM for *A. auriculaeformis*, a value quite similar to the 12 μM found for *A. holosericea* in the present study (Figure 1). However, at higher solution Zn concentrations, the growth of the two species was quite different. Thus, Zhang et al. (1998) found only a 50% decline in shoot growth at a Zn concentration of 18.3 mg.L^{-1} (i.e. 280 μM), whereas, in the highest treatment in the present study (100 μM), shoot growth of *A. holosericea* was reduced by 92% (Figure 1). This species difference could be a result of *A. auriculaeformis* having a greater tolerance of high Zn concentrations, but it may also be an expression of differences in the composition of the nutrient solutions. Zhang et al. (1998) did not state the pH of their nutrient solutions but at pH 5, as used in this current study, GEOCHEM (Sposito and Mattigod, 1980) predicts that for the 2 mM treatment of Zhang et al. (1998), in which plants were still surviving, 74% (or 1.5 mM) of the Zn was in the free-ion form, and therefore available to plants, and 23% would have been present as insoluble Zn-phosphate. However, if the pH was that used for the Rhizobium medium in the Zhang et al. (1998) paper, i.e. 6.7–6.8, then GEOCHEM predicts that only 9% (or 174 μM) of the Zn would be present and available to the plants as the free-ion, while 34% of the Zn would be present as solid Zn-phosphate. Hence, the differences between the results of Zhang et al. (1998) for *A. auriculaeformis* and the results for *A. holosericea* in the present study may not be as great as they at first appear. The ambiguity presented by this comparison also highlights the need to report solution pH as well as elemental composition of nutrient solutions.

While dilute solution culture systems provide a good means of approximating the soil solution in many situations (Asher and Edwards, 1978; Parker and Norvell, 1999), important differences between the systems should be recognised. The roots of plants growing in solution culture are continuously presented with nutrient-filled water, thus the effect of a reduction in root mass on nutrient and water supply to the plant may be diminished. This situation contrasts to that of a plant growing in soil, where plant roots must continually explore the soil to gain nutrients and water. Hence, it is likely that in soil any reduction in root growth induced by Zn toxicity would have a larger effect on overall plant biomass than would be the case in solution culture. This is particularly important when considering the small root biomass of plants in the 50 and 100 μM treatments. In a soil, the roots of these plants would only have explored the top few centimetres of soil, making it likely that they would have

been subjected to extreme nutrient and water stress under field conditions. Therefore, it could be expected that the species tested would be more sensitive in soil than in solution culture once there was a significant reduction of root growth.

Zn toxicity and plant tissue Zn

As the concentration of Zn in solution increased, the tissue Zn increased for all species studied and for all tissue types. For both the *Eucalyptus* and the *Melaleuca*, whole shoot tissue Zn started at similar concentrations to those in the YFEL but became higher than the YFEL as solution Zn increased. In contrast, in the *Acacia*, shoot tissue Zn remained similar to YFEL Zn at all concentrations of Zn in solution. The reason for this remains unclear but it may have been influenced by the fact that Acacia 'leaves' are not true leaves but rather phyllodes (modified stems). Few other Zn toxicity studies have differentiated the YFEL from the remainder of the shoot and so comparisons with the present study are difficult. However, both Wallace (1989) and Fontes and Cox (1995) found that the younger trifoliate leaves in legumes start with lower Zn than older leaves (although as external Zn supply increased the Zn concentrations between different types of leaves becomes more similar). It appears that there is no consistent pattern among species, although the pattern as described for *E. camaldulensis* and *M. leucadendra*, with greater cumulative uptake as plant tissues age, is one that would be expected for a relatively phloem immobile element like Zn.

Critical tissue concentrations for Zn toxicity in the tree species studied in this experiment were comparable with those found for other species. Thus, Van Assche and Clijsters (1986) determined a threshold value for Zn toxicity of 226 μg g^{-1} in the leaves of *Phaseolus vulgaris* (bean), and *Typha latifolia* at 15 μM Zn had a shoot tissue concentration of 782 μg g^{-1} and a root tissue concentration of 1296 μg g^{-1} (Ye et al., 1998). *Acacia auriculaeformis* had a critical toxicity concentration in the shoots of 143 μg g^{-1} (Zhang et al., 1998) making it comparable with the Australian native species tested in this paper (Figure 3).

The results from this paper provide the first comprehensive combination of growth responses, critical external concentrations, critical tissue concentrations and plant toxicity symptoms for three important Australian genera, viz., *Eucalyptus*, *Acacia* and *Melaleuca*, for use in the rehabilitation of potentially Zn toxic sites.

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Appendix 1. Models fitted to data in Figures 2, 3 and 4

Figure	Parameters	Model	R^2
Figure 1a	x = Zn in solution (μM), y = Root dry weight (g plant^{-1})	$y = 0.96*(\text{abs}(((x-5.38)/28.14)+0.16))^{0.14}*\exp(-(\text{abs}(((x-5.38)/28.14)+0.16))^{1.14}+0.12)$	0.90
Figure 1a	x = Zn in solution (μM), y = Shoot dry weight (g plant^{-1})	$y = 2.56*(\text{abs}(((x-4.40)/34.37)+0.10))^{0.09}*\exp(-(\text{abs}(((x-4.40)/34.37)+0.10))^{1.09}+0.08)$	0.96
Figure 1a	x = Zn in solution (μM), y = Total dry weight (g plant^{-1})	$y = 3.78*(\text{abs}(((x-4.51)/34.20)+0.11))^{0.09}*\exp(-(\text{abs}(((x-4.51)/34.20)+0.10))^{1.09}+0.09)$	0.95
Figure 1b	x = Zn in solution (μM), y = Root dry weight (g plant^{-1})	$y = 1.99/(1+((x-13.41)/20.25)^2)$	0.99
Figure 1b	x = Zn in solution (μM), y = Shoot dry weight (g plant^{-1})	$y = 5.48/(1+((x-12.53)/24.39)^2)$	1.00
Figure 1b	x = Zn in solution (μM), y = Total dry weight (g plant^{-1})	$y = 7.44/(1+((x-12.84)/23.37)^2)$	0.99
Figure 1c	x = Zn in solution (μM), y = Root dry weight (g plant^{-1})	$y = 1.00-0.23*x^{0.32}$	0.92
Figure 1c	x = Zn in solution (μM), y = Shoot dry weight (g plant^{-1})	$y = 5.53-2.45*x^{0.17}$	0.93
Figure 1c	x = Zn in solution (μM), y = Total dry weight (g plant^{-1})	$y = 6.33-2.47*x^{0.20}$	0.93
Figure 2a	x = Zn in solution (μM), y = Root Zn (μg g^{-1})	$y = 139.98+5808.19*(1-\exp(-0.03*x))$	1.00
Figure 2b	x = Zn in solution (μM), y = Shoot Zn (μg g^{-1})	$y = 461.61*(1-\exp(-0.05*x))$	0.99
Figure 2b	x = Zn in solution (μM), y = YFEL Zn (μg g^{-1})	$y = 630.57/(1+\exp(-1*(x-39.33)/12.48))$	0.99
Figure 2c	x = Zn in solution (μM), y = Root Zn (μg g^{-1})	$y = 383.75*x^{0.53}$	0.87
Figure 2d	x = Zn in solution (μM), y = Shoot Zn (μg g^{-1})	$y = 3426.03/(1+\exp(-1*(x-57.84)/18.74))$	1.00
Figure 2d	x = Zn in solution (μM), y = YFEL Zn (μg g^{-1})	$y = 72.36*x^{0.62}$	0.98
Figure 2e	x = Zn in solution (μM), y = Root Zn (μg g^{-1})	$y = 4051.18*(1-\exp(-0.07*x))$	0.95
Figure 2f	x = Zn in solution (μM), y = Shoot Zn (μg g^{-1})	$y = 1372.73*(1-\exp(-0.04*x))$	1.00
Figure 2f	x = Zn in solution (μM), y = YFEL Zn (μg g^{-1})	$y = 577.6473*\exp(-0.5*\ln(x/44.4073)^2)$	0.96
Figure 3a	x = YFEL Zn (μg g^{-1}), y = Relative shoot dry weight (%)	$y = 100*\exp(-0.5*(\ln(x/78.23)^2)$	0.90
Figure 3b	x = Shoot Zn (μg g^{-1}), y = Relative shoot dry weight (%)	$y = 100*\exp(-0.5*(\ln(x/94.50))^2)$	0.86
Figure 3c	x = YFEL Zn (μ g g^{-1}) y = Relative shoot dry weight (%)	$y = 100/(1+((x-293.95)/361.48)^2)$	0.94
Figure 3d	x = Shoot Zn (μ g g^{-1}), y = Relative shoot dry weight (%)	$y = 100/(1+((x-257.67)/340.11)^2)$	0.96
Figure 3e	x = YFEL Zn (μg g^{-1}), y = Relative shoot dry weight (%)	$y = 966.34*x^{-0.56}$	0.67
Figure 3f	x = Shoot Zn (μg g^{-1}), y = Relative shoot dry weight (%)	$y = 121.76-3.07*x^{0.49}$	0.91

References

Asher C J 1978 Natural and synthetic culture media for spermatophytes. *In* CRC Handbook Series in Nutrition and Food. Section G: Diets, Culture Media, Food Supplements. Volume III Culture Media for Microorganisms and Plants. Ed. M. Rechcigl, Jnr. pp 575–609. CRC Press, Cleveland.

Asher C J and Blamey F P C 1987 Experimental control of plant nutrient status using programmed nutrient addition. J. Plant Nutr. 10, 1371–1380.

Asher C J and Edwards D G 1978 Relevance of dilute solution culture studies to problems of low fertility tropical soils. *In* Mineral Nutrition of Legumes in Tropical and Subtropical Soils. Eds. CS Andrew and EJ Kamprath. pp 131–152. CSIRO, Brisbane.

Asher C J and Loneragan J F 1967 Response of plants to phosphate concentration in solution culture: 1. Growth and phosphorus content. Soil Sci. 103, 225–233.

Brookes A, Collins J C and Thurman D A 1981 The mechanism of zinc tolerance in grasses. J. Plant Nutr. 3, 695–705.

Brown M T and Wilkins D A 1985 Zinc tolerance in *Betula*. New Phytol. 99, 91–100.

Carroll M D and Loneragan J F 1968 The relevance of solution culture studies to the absorption of zinc from soils. 9th International Congress of Soil Science, pp 191–202. Sydney, International Society of Soil Science and Angus and Robertson.

Chino M and Baba A 1981 The effects of some environmental factors on the partitioning of zinc and cadmium between roots and tops of rice plants. J. Plant Nutr. 3, 203–214.

Cox F R 1990 A note on the effect of soil reaction and zinc concentration on peanut tissue zinc. Peanut Sci. 17, 15–17.

Fontes R L F and Cox F R 1995 Effects of sulfur supply on soybean plants exposed to zinc toxicity. J. Plant Nutr. 18, 1893–1906.

Foy C D, Chaney R L and White M C 1978 The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29, 511–566.

Godbold D L and Huttermann A 1985 Effect of zinc, cadmium and mercury on root elongation of *Picea abies* (Karst.) seedlings, and the significance of these metals to forest die-back. Environ. Pollut. Ser. A Ecol. Biol. 38, 375–381.

Kabata-Pendias A and Pendias H 1992 Trace Elements in Soils and Plants. CRC Press, Boca Raton.

Kieken L 1995 Zinc. *In* Heavy Metals in Soils. Ed. BJ Alloway. pp 284–305. Blackie Academic and Professional, London.

Minitab 1995 MINITAB. Minitab, Inc, State College, USA.

Parker D R and Norvell W A 1999 Advances in solution culture methods for plant mineral nutrition research. Adv. Agron. 65, 151–213.

Parker M B, Gaines T P, Walker M E, Plank C O and Davis-Carter J G 1990 Soil zinc and pH effects on leaf zinc and the interaction of leaf calcium and zinc on zinc toxicity of peanuts. Commun. Soil Sci. Plant Anal. 21, 2319–2332.

Smith F W and Loneragan J F 1997 Interpretation of plant analysis: concepts and principles. *In* Plant Analysis An Interpretation Manual. Eds. DJ Reuter, JB Robinson and C Dutkiewicz. pp 1–33. CSIRO Publishing, Collingwood.

Sposito G and Mattigod S V 1980 GEOCHEM: A computer program for the calculation of chemical equilibria in soil solutions and other natural water systems. University of California, Riverside, California.

SPSS 1997 SIGMAPLOT for windows version 4.00. SPSS Inc, Chicago.

Taylor G J, Stadt K J and Dale M R T 1991 Modelling of phytotoxicity of aluminium, cadmium, copper, manganese, nickel and zinc using the Weibull frequency distribution. Can. J. Bot. 69, 359–367.

Van Assche F and Clijsters H 1986 Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc: Effect on ribulose-1,5-biphosphate carboxylase/oxygenase. J. Plant Physiol. 125, 355–360.

Wallace A 1989 Effects of zinc when manganese was also varied for bush beans grown in solution culture. Soil Sci. 147, 444–445.

White M C, Chaney R L and Decker A M 1979 Role of roots and shoots of soybean in tolerance to excess soil zinc. Crop Sci. 19, 126–128.

Ye Z, Baker A J M, Wong M H and Willis A J 1998 Zinc, lead and cadmium accumulation and tolerance in *Typha latifolia* as affected by iron plaque on the root surface. Aquat. Bot. 61, 55–67.

Zhang Z Q, Wong M H, Nie X P and Lan C Y 1998 Effects of zinc (zinc sulfate) on rhizobia-earleaf acacia (*Acacia auriculaeformis*) symbiotic association. Bioresour. Technol. 64, 97–104.