REGULAR ARTICLE

Toxicity of Cd to signal grass (*Brachiaria decumbens* Stapf.) and Rhodes grass (*Chloris gayana* Kunth.)

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Abstract Given that Cd accumulates within plant tissues to levels that are toxic to animals, it is necessary to understand the role of plants in highly Cd-contaminated systems and their subsequent impact on the health of animals. A solution culture experiment was conducted to elucidate the effects of increasing Cd^{2+} activity ($\{Cd^{2+}\}$) on growth of Rhodes grass (*Chloris gayana* Kunth.) and signal grass (*Brachiaria decumbens* Stapf.). The shoot and root fresh mass of both Rhodes grass and signal grass was reduced by 50% at ca. 0.5 μM $\{Cd^{2+}\}$. Elevated $\{Cd^{2+}\}$ resulted in a significant decrease in the tissue Mn concentration for both the shoots and roots, and caused a chlorosis of the veins in the shoots. Root hair growth was prolific even at high $\{Cd^{2+}\}$, thus root hair growth appeared to be less sensitive to elevated Cd than was root growth *per se*. The critical shoot tissue concentrations (50% reduction in growth), 230 μg g^{-1} for Rhodes grass and 80 μg g^{-1} for signal grass, exceeded the maximum level of Cd tolerated in the diet of animals (ca. 5 μg g^{-1}). When assessing the risk associated with the revegetation of Cd-contaminated sites with Rhodes grass or signal grass, careful consideration must be given, therefore, to the transfer of toxic concentrations of Cd to grazing animals and through the wider food chain.

Keywords Cadmium · Phytotoxicity · Revegetation · Root and shoot growth · Symptoms

Abbreviations

EC	electrical conductivity
ICP-	inductively coupled plasma-optical emis-
OES	sion spectroscopy
SEM	scanning electron microscope

Introduction

The anthropogenic release of Cd into the landscape poses a serious environmental problem, and may occur through activities including metal refining, coal combustion, disposal of mine wastes, pigment production, and the disposal of sewage sludges. Further, contamination from the use of P fertilizers has added an estimated >6000 t of Cd to Australian soils in the last 90 years (Mann et al. 2002; McLaughlin et al. 1996). Elevated levels of trace metals are particularly troublesome because of their long residence times in

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soils, and the largely irreversible nature of the contamination (Bowen 1979). Generally, Cd contamination is of concern due to its potential to accumulate to levels within the plant tissues that are toxic to animals (including humans) but not toxic to the plant (Dudka and Miller 1999). For animals, Cd may be adsorbed in toxicologically relevant amounts by either oral or inhalation exposure (National Research Council (U.S.) 2005). Generally, the accumulation of toxic levels of Cd in animals is thought to disrupt the cellular redox state due to similarities between Cd and Zn, Ca, and other divalent cations (Pinot et al. 2000).

Whilst the phytotoxicity of Cd is generally not of direct relevance, it is still necessary to understand the role of plants in Cd-contaminated systems and their subsequent impact on the health of animals. Ideally, the composition of a nutrient solution should mimic that of a soil solution (Parker and Norvell 1999). However, commonly used nutrient solutions often contain 100 to 1,000 μM P (see Parker and Norvell (1999)) while soil solutions typically contain <10 μM P (even in fertilized agricultural soils) (Gillman and Bell 1978; Kovar and Barber 1988; Menzies and Bell 1988; Reisenauer 1966). In a recent review of studies examining trace metal phytotoxicity, Kopittke et al. (2009b) identified 27 studies examining Cd toxicity which met their selection criteria relating to the quality of the data. The median Cd concentration for toxicity was 5 μM ($n=32$), but ranged from 0.5 to 700 μM. Interestingly, of the 26 studies using compete nutrient solutions, only six used <100 μM P and none ≤10 μM P (surprisingly, one study used 8270 μM P). The use of solutions with high concentrations of nutrient salts may influence the apparent toxicity of metals (for example, see Lock et al. (2007)). Thus, there is a need for well-controlled studies utilizing environmentally relevant experimental conditions. However, consideration should also be given to the general limitations of nutrient solution systems, particularly that nutrient solutions are an imperfect simulation of plant growth in soil. In particular, compared to soil, nutrient solutions lack mechanical support and provide an altered chemical environment in the rhizosphere (in which the fluxes of O_2, water, and nutrients differ from those in soil) (for reviews, see Parker and Norvell (1999) and Asher and Edwards (1983)).

Perennial grasses, including Rhodes grass (*Chloris gayana* Kunth.) and signal grass (*Brachiaria*

decumbens Stapf.), are frequently used for the revegetation of mine sites and other contaminated sites located in low-rainfall areas. Signal grass is widespread throughout tropical America, south-eastern Asia and the Pacific, is well adapted to highly acidic soils (Wenzl et al. 2001), but is comparatively sensitive to salinity (Deifel et al. 2006). Rhodes grass is widely grown in the tropics and sub-tropics of eastern and southern Africa, Australia and Central America, and is highly tolerant of soil salinity and sodicity (Deifel et al. 2006). However, comparatively little information is available regarding the toxic effects of Cd on grasses. The objective of the current work was to examine the toxic effects of Cd^{2+} on the growth of Rhodes grass and signal grass. The growth of the grasses was related to (i) the activity of the free Cd^{2+} ion ($\{Cd^{2+}\}$) calculated from measured Cd concentrations and (ii) alterations to the external morphology of the roots (examined by microscopy).

Materials and methods

Plants were grown in semi-controlled glasshouse conditions at The University of Queensland, St Lucia, Australia (27° 29′ 43″ S, 153° 0′ 34″ E). High-pressure sodium lamps were used to supplement natural sunlight, providing 16 h of light per day. Photosynthetically active radiation at plant height generally reached a maximum of ca. 1,500 μmol m^{-2} s^{-1} (measured at midday), with the lamps ensuring a minimum value of 400 μmol m^{-2} s^{-1}. Temperature was maintained at approximately 28°C during the light period, and approximately 25°C during the dark. Relative humidity was not controlled, but a soaker hose was used to keep the floor wet to increase humidity. During the day, humidity generally ranged between 40 and 60%, increasing to 70 to 90% during the night. Average daily pan evaporation was 2.7 mm.

Experimental design and plant growth

Rhodes grass and signal grass seedlings were grown in 22 L polypropylene containers (Kopittke et al. 2007) filled with 21 L of the basal nutrient solution containing (μM): 680 NO_3^--N, 120 NH_4^+-N, 700 Ca, 500 S, 300 K, 100 Mg, 10 Fe, 3 B, 2 P, 2 Mn, 1 Zn, 0.2 Cu, and 0.02 Mo. The Fe was supplied as CDTA to minimize complexation (see Kopittke and Menzies

(2006) for details). There were 22 containers in which 11 Cd treatments and two grass species were arranged in a completely randomized block design with two replicates over time. Calculations performed using GEOCHEM v2.0 (Parker et al. 1995) predicted that, under the current experimental conditions, <0.1% of the Cd was associated with CDTA. The pH of each nutrient solution was lowered to 4.75 using approximately 4 mL of 0.1 M HCl. An air-driven water pump was used to circulate the solutions and provide aeration. Using appropriate volumes of a 10 mM $CdCl_2$ stock solution, 22 treatments were established (at rates determined from preliminary experiments); 0, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5, 7.5, and 10 μM Cd for signal grass, and 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 1.5, 2.5, 5, and 10 μM Cd for Rhodes grass. Each of the 22 containers was connected to a separate pH titration unit (TPS, miniCHEM-pH) and peristaltic pump (Masterflex 5 RPM with Masterflex Tygon tubing, L/S 17) which was used to maintain the pH at 4.75 for the duration of the experiment by the addition of 2 mM $Ca(OH)_2$.

Seeds of Rhodes grass and signal grass were germinated as described by Kopittke et al. (2007). After 7 d growth, four seedlings of each species were transplanted into each of the 22 L containers, with the average of these four plants representing one replicate. Plants were grown for 15 d after transplanting, with nutrient uptake from the solution offset by the addition of pre-calculated quantities of a delivery solution containing (mM) NO_3^--N 680, NH_4^+-N 120, Ca 150, S 20, K 400, Mg 75, P 20, Cl 234, Fe 5, B 1, Mn 0.2, Zn 0.6, Cu 0.4, and Mo 0.02. Additional deionized water was supplied to the containers throughout the growth period to maintain solution volume.

Samples of nutrient solutions were taken after 0 and 15 d, and electrical conductivity (EC) and pH measured (to check the accuracy of the pH titration units). In addition, 10 mL of each sample was filtered (0.22 μm Millipore GSWP), acidified to pH<2.0 using 20 μL of concentrated HCl, and refrigerated (3.5ºC) before analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) for Cd. Calculations of ionic strength and ion activities were performed using PhreeqcI 2.13.04 with the Minteq database (Parkhurst 2007). Approximately 88% of the solution Cd was Cd^{2+}, with approximately 10% as the uncharged $CdSO_4^0$ ion-pair and 2% as

$CdCl^+$ (Smolders and McLaughlin 1996). The activity of Cd was calculated for each treatment, being (μM): 0, 0.028, 0.070, 0.17, 0.37, 0.56, 0.75, 2.0, 3.9, 6.0, and 7.8 μM $\{Cd^{2+}\}$ for signal grass, and 0, 0.029, 0.064, 0.15, 0.29, 0.61, 0.74, 1.2, 2.0, 4.0, and 7.8 μM $\{Cd^{2+}\}$ for Rhodes grass.

Upon completion of the 15 d growth period, roots from one of the four plants were harvested for microscopic examination (see below). The fresh mass of the remaining three roots and shoots was determined, the roots thoroughly rinsed in DI water, and the elemental concentrations of both the roots and shoots determined using ICP-OES (for B, Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn) after drying for 7 d at 65ºC and acid digestion as described by Martinie and Schilt (1976).

Data analysis

A grouped regression analysis (fitting exponential curves) was performed on relative shoot and root mass, using GenStat 7 (GenStat 2003). Grouped regression analysis was also used to examine the relationship between the mean nutrient solution $\{Cd^{2+}\}$ (based on the mean measured Cd concentrations in the nutrient solution after 0 and 15 d) and the root and shoot Cd concentrations at harvest (exponential regression) (GenStat 2003).

Microscopy

Upon completion of the experiment, roots were taken for examination using a dissecting light microscope and scanning electron microscope (SEM). The dissecting light microscope was used to examine roots in all treatments after storage at 3.5ºC in 70 mL of the appropriate nutrient solution and staining with 0.5% crystal violet. Examination occurred within 6 h of harvest. The SEM was used to provide high resolution images with good depth-of-field of roots from the 0, 0.1, 0.25, and 0.50 μM Cd treatments for signal grass (corresponding to 0, 0.070, 0.17, and 0.37 μM $\{Cd^{2+}\}$) and from the 0, 0.4, 0.8, and 1.0 μM treatments from Rhodes grass (corresponding to 0, 0.29, 0.61, and 0.75 μM $\{Cd^{2+}\}$). Root tips (approximately 20 mm) were freeze-substituted according to Wharton (1991) (see Kopittke et al. (2008a) for details) and examined using a SEM (JEOL JSM 6400F) at 10 kV.

Results

Solution and tissue composition

Initial solution Cd concentrations were generally 5 to 10% higher than expected, although concentrations subsequently decreased during the 14 d experimental period (Table 1). Decreases in solution Cd were greatest in the low-Cd treatments (being approximately 50% at 0.05 μM Cd), whilst concentrations changed by <5% in all treatments with initial nominal concentrations ≥1 μM (Table 1). For treatments corresponding to approximately to a 50% reduction in growth, Cd concentrations decreased by ca. 15% (Table 1). Solution EC (mean value of 0.25 dS m^{-1}) remained relatively constant, increasing an average of 13% during the 15 d experimental period (data not presented).

The concentration of Cd in shoots (Fig. 1a) and roots (Fig. 1b) increased as the solution $\{Cd^{2+}\}$ increased. With ≤1 μM $\{Cd^{2+}\}$ in solution, the roots of both species had a similar concentration of Cd; above this, the roots of signal grass had almost twice that of Rhodes grass at the highest $\{Cd^{2+}\}$ (1,300 versus 720 μg g^{-1}) (Fig. 1b). The Cd concentration in the shoots was less than that in the roots, with that of Rhodes grass increasing markedly to 340 μg g^{-1} with up to 2 μM $\{Cd^{2+}\}$ but with no further increase above this level (Fig. 1a). In contrast, the Cd concentration in signal grass shoots continued to increase up to 600 μg g^{-1} at 8 μM $\{Cd^{2+}\}$. The critical tissue concentrations (associated with a 50% reduction in fresh mass) was calculated from the fitted curves as 230 μg g^{-1} for shoots of Rhodes grass and 80 μg g^{-1} for shoots of signal grass; corresponding values for

roots were 530 μg g^{-1} for Rhodes grass roots and 520 μg g^{-1} for signal grass (Fig. 1).

It is noteworthy that Cd in solution had no significant effects on nutrient concentrations in the shoots and roots (data not presented) other than Mn which decreased significantly with an increase in $\{Cd^{2+}\}$. There is little information on critical concentrations for nutrient deficiency in Rhodes grass and signal grass, but shoot tissue nutrient concentrations generally appeared to be within the range for healthy plant tissue (Marschner 1995; Pinkerton et al. 1997) (data not presented). The Mn concentration in the roots decreased markedly from ca. 700 μg g^{-1} in the low-Cd treatments to ca. 20 μg g^{-1} at 1 μM $\{Cd^{2+}\}$; there was no further decrease at higher $\{Cd^{2+}\}$ (Fig. 2). The decrease in Mn concentration in the shoots was more moderate, declining from ca. 200 to 60 μg g^{-1} from low to high $\{Cd^{2+}\}$. Even at the highest $\{Cd^{2+}\}$, the Mn concentration was similar to that in healthy plant shoots (50 μg g^{-1}) (Marschner 1995)).
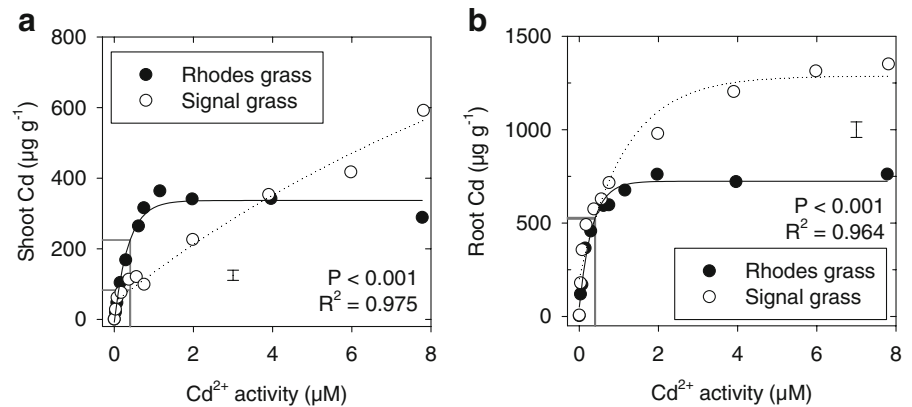
Plant growth and symptoms of toxicity

Good plant growth was evident in the low Cd^{2+} treatments, with a fresh mass of approximately 5 g of shoots and 4 g of roots in Rhodes grass. The fresh mass of signal grass was slightly lower at 3 g of shoots and 3 g of roots. However, growth of both the roots and shoots decreased markedly with an increase in Cd in solution (Fig. 3). Grouped regression analysis indicated that there was no significant difference in response to $\{Cd^{2+}\}$ between the shoots and roots for either signal grass (P=0.496) or Rhodes grass (P=0.237). Thus, a single curve was used to describe the response of both the shoots and roots to

Table 1 Concentrations of Cd measured in nutrient solutions for the lowest and highest Cd-containing treatments and the treatment closest to the Cd^{2+} activity causing a 50% reduction in the shoot and root fresh mass (Fig. 3)

Species	Cd treatment (μM)	Measured Cd (0 d) (μM)	Measured Cd (14 d) (μM)	Decrease (%)	Calculated Cd^{2+} activity (μM)
Signal grass	0.05	0.053	0.028	47	0.028
	0.5	0.56	0.48	14	0.37
	10	11	11	0	7.8
Rhodes grass	0.05	0.054	0.028	48	0.029
	0.4	0.44	0.38	13	0.288
	10	11	11	0	7.8

increasing $\{Cd^{2+}\}$ for each species (Fig. 3). Although there was a difference between the two species in response to elevated Cd, there was a 50% reduction in shoot and root fresh mass of both Rhodes grass and signal grass with 0.4 μM $\{Cd^{2+}\}$ in solution (Fig. 3).

A veinal chlorosis was evident in the shoots of Rhodes grass when grown in solutions containing ≥0.6 μM $\{Cd^{2+}\}$ and in those of signal grass at the highest $\{Cd^{2+}\}$ (7.8 μM). The foliar application of Fe (as 0.1% Fe-EDDHA), Zn (as 0.5% $ZnCl_2.4H_2O$), and Cu (as 0.5% $CuSO_4.5H_2O$) did not alleviate the symptoms. In contrast with the effects of many trace metals (for example, Al and Cu), elevated Cd in solution did not cause damage to roots (Fig. 4a, b) despite the marked detrimental effect on root growth. Also, root hair growth was prolific in all treatments (Fig. 4), but root hairs did develop close to the apex suggesting some disruption to normal root development (Fig. 4c).

Discussion

Fresh mass of both Rhodes grass and signal grass was reduced by 50% by 0.5 μM $\{Cd^{2+}\}$ (Fig. 3). However, the concentration of Cd in the shoot tissue associated with this 50% reduction in growth (230 μg g^{-1} for Rhodes grass and 80 μg g^{-1} for signal grass) exceeds the maximum tolerable level for Cd of ca. 5 μg g^{-1} (*i.e.* the dietary intake level, that when fed for a defined period of time, will not impair animal performance or health) (National Research Council (U.S.) 2005). For both grasses, solution $\{Cd^{2+}\}$ should be <0.1 μM to avoid accumulation of Cd in the shoot tissue to concentrations which exceed the maximum level tolerated by animals. Thus, for the growth of both Rhodes grass and signal grass on Cd-contaminated sites, accumulation of Cd within the shoots to levels detrimental to animal health is of greater concern than is phytotoxicity.
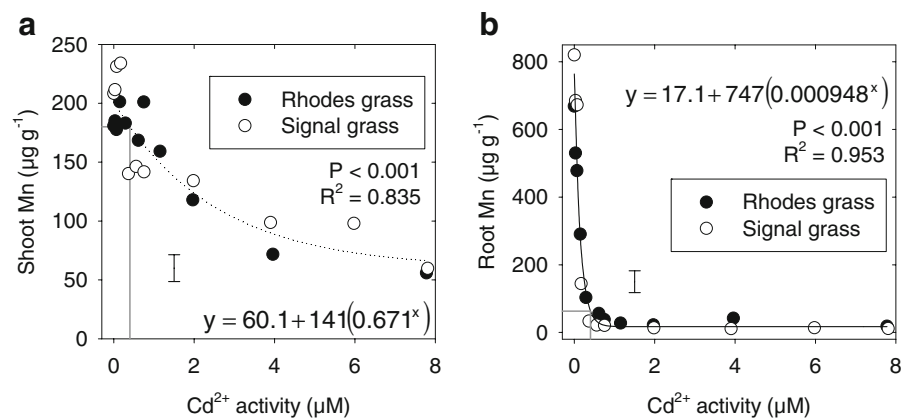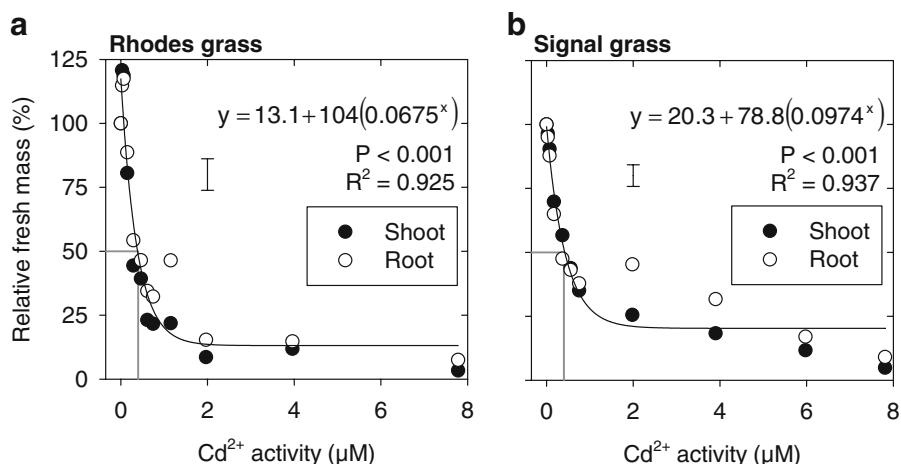
Fig. 3 Effect of solution Cd^{2+} activity on the relative fresh mass of shoots and roots of Rhodes grass **a** and signal grass **b** grown for 15 d in a dilute nutrient solution at pH 4.75. The solid gray line represents the Cd^{2+} activity which caused a 50% reduction in relative fresh mass, whilst the vertical bar represents the standard error of the observations

The critical solution concentration for the two grasses examined in the current study (0.5 µM $\{Cd^{2+}\}$, Fig. 3) is similar to values reported for other species. In a review of 132 studies, Kopittke et al. (2009b) reported that the median toxic value for Cd in solution culture was 5 µM. Solution Cd concentrations toxic to other monocotyledon species have been reported as 0.02 µM for wheat (*Triticum aestivum* L.)

Fig. 4 Scanning electron micrographs of roots of Rhodes grass and signal grass grown for 15 d in a dilute nutrient solution at pH 4.75 containing Cd^{2+} at the activities indicated. Images are presented only for the control (0 µM) treatment, 0.29 and 0.37 µM $\{Cd^{2+}\}$ (ca. 50% relative shoot and root mass), and 0.75 µM $\{Cd^{2+}\}$
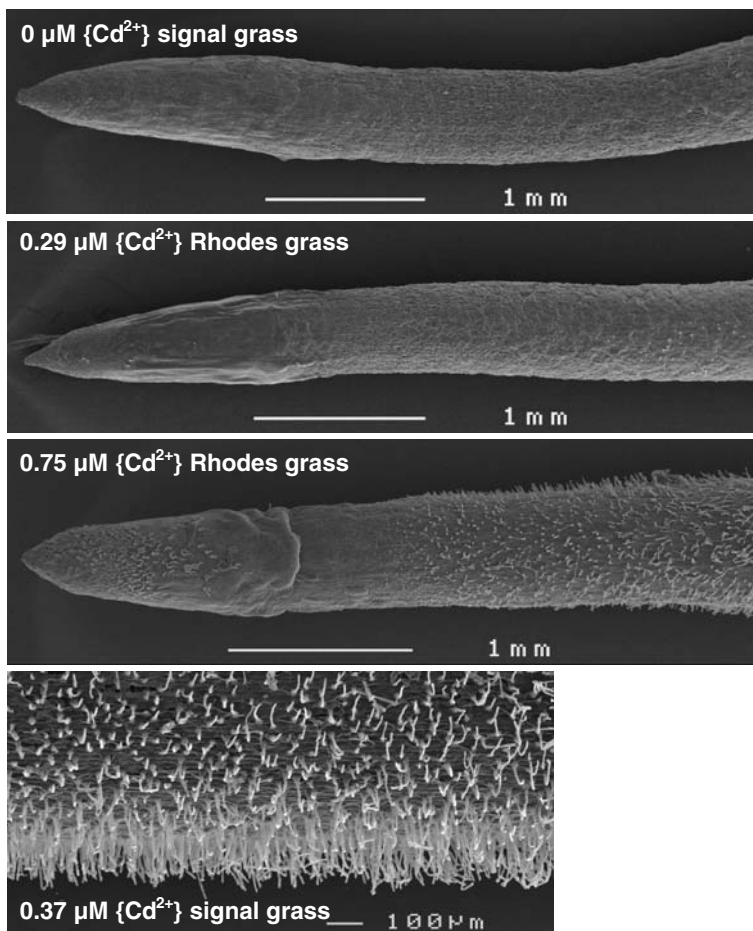
(Taylor et al. 1991), 1 to 5 µM for rice (*Oryza sativa* L.) (Hassan et al. 2005), 8 µM for perennial ryegrass (*Lolium perenne* L.) and maize (*Zea mays* L.) (Yang et al. 1996), and 0.5 µM for barley (*Hordeum vulgare* L.) (Cabrera et al. 1988). Similarly, the critical shoot tissue concentrations (50% reduction in fresh mass), being 230 µg g^{-1} for Rhodes grass and 80 µg g^{-1} for signal grass (Fig. 1), are comparable to values reported for other species. For example, reductions in growth have been reported to be associated with Cd concentrations of 90 µg g^{-1} in shoots of *Phragmites australis* (Cav.) Trin. ex Steud. (Ali et al. 2004), 67 µg g^{-1} in leaves of cotton (*Gossypium hirsutum* L.) (Wu et al. 2004), and 17 µg g^{-1} in shoots of barley (Cabrera et al. 1988). Thus, it would appear that the high P concentrations used by many authors (see Introduction) has not resulted in incorrect answers being obtained. This is in contrast to other trace metals where the P concentration influences the solution composition (for example, see Kopittke et al. (2008b)).

Whilst tissue concentrations of Cd were higher in the roots than the shoots, the magnitude of the difference (ca. 2-fold, Fig. 1) is less than that observed for other metals. For example, concentrations of Ni in tissues of these two grasses were 5 to 50 times higher in the roots than the shoots (Kopittke et al. 2008a), concentrations of Pb were 10 times higher in the roots (Kopittke et al. 2007), whilst in Sabi grass (*Urochloa mosambicensis* (Hack.) Dandy), concentrations of Cu were 10 to 100 times in the root (Kopittke et al. 2009a). Similarly, root Pb concentrations were 9 to 130 times higher than shoot concentrations in a range of species (Wierzbicka 1999), and 100 to 300 times higher in Norway spruce (*Picea abies* L.) (Godbold and Kettner 1991).

Elevated {Cd^{2+}} had no apparent influence on concentrations of nutrients in the shoot and root tissue other than Mn (data not presented). The decrease in shoot and root Mn at elevated {Cd^{2+}} (Fig. 2) is similar to that observed by Wallace et al. (1977) for *Phaseolus vulgaris* L., who reported that 1 µM Cd reduced leaf Mn concentrations from 81 to 45 µg g^{-1} and root Mn concentrations from 370 to 19 µg g^{-1}. Also studying *Phaseolus vulgaris* L., Barceló et al. (1985) reported that elevated Cd concentrations resulted in a decrease in the Mn concentrations of the shoot tissues (stems, primary leaves, and trifoliate leaves). However, in contrast to the current study (and

that of Wallace et al. (1977)), Barceló et al. (1985) reported that elevated Cd concentrations resulted in an increase in the Mn concentration of the root tissue. Further, the observed decrease in tissue Mn in the current study is also in contrast to that reported by Ramos et al. (2002) for lettuce (*Lactuca sativa* L.), where toxic levels of Cd resulted in an increase in shoot Mn concentrations. Presumably, a Cd-induced decrease in tissue Mn results from antagonism between Cd and Mn; a hypothesis supported by the observation that Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} inhibit absorption and translocation of Cd in soybean (*Glycine max* L. Merr.) (Cataldo et al. 1983). Although shoot tissue Mn concentrations (ca. 60 µg g^{-1}) were similar to the average concentration in healthy plant shoots (50 µg g^{-1}, Marschner (1995)), it is proposed that the chlorosis of the veins observed in the shoots of both species was a Cd-induced Mn deficiency (the symptoms were not alleviated by the foliar application of Fe, Zn, or Cu). It is noteworthy, however, that Mn typically causes an interveinal chlorosis (*c.f.* the chlorosis of the veins observed in the current study).

For both Rhodes grass and signal grass, although the distance between the root apex and the root hair zone was decreased, root hair growth did not appear to be inhibited by elevated {Cd^{2+}} (Fig. 4). The observation that root hair growth appears to be less sensitive to Cd than is root growth *per se* is similar to that reported for Ni and Pb toxicity in these two grasses (Kopittke et al. 2007, 2008a) and for Cu toxicity in Sabi grass (Kopittke et al. 2009a). However, this observation is in contrast to previous reports on Cu toxicity in Rhodes grass (Sheldon and Menzies 2005) and cowpea (*Vigna unguiculata* L. Walp.) (Kopittke and Menzies 2006) and for Al toxicity in a range of plant species (Brady et al. 1993; Hecht-Buchholz et al. 1990).

In conclusion, the shoot and root fresh mass of both Rhodes grass and signal grass was reduced by 50% at ca. 0.5 µM {Cd^{2+}}. Although shoot tissue Cd concentrations were ca. two times higher than those in the root tissue, the critical shoot tissue concentrations (associated with a 50% reduction in growth, being 230 µg g^{-1} for Rhodes grass and 80 µg g^{-1} for signal grass) exceeded the maximum level of Cd tolerated in the diet of animals (ca. 5 µg g^{-1}). Thus, for Cd-contaminated sites, Cd accumulates to levels which are detrimental to animal health before inducing

phytotoxic effects.. When assessing the risk associated with the use of Rhodes grass or signal grass for the revegetation of Cd-contaminated sites, careful consideration must be given, therefore, to the potential transfer of toxic concentrations of Cd to grazing animals and through the wider food chain. Elevated $\{Cd^{2+}\}$ resulted in a significant decrease in the tissue Mn concentration for both the shoots and roots, and possibly reduced growth due to a Cd-induced Mn deficiency. Examination of the roots by microscopy revealed that root hair growth appears to be less sensitive to Cd than is root growth *per se*, with root hair growth prolific in all treatments.

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