Toxic effects of Pb^{2+} on the growth and mineral nutrition of signal grass (*Brachiaria decumbens*) and Rhodes grass (*Chloris gayana*)

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Abstract

Although grasses are commonly used to revegetate sites contaminated with lead (Pb), little is known regarding the Pb-tolerance of many of these species. Using dilute solution culture to mimic the soil solution, the growth of signal grass (*Brachiaria decumbens* Stapf cv. Basilisk) and Rhodes grass (*Chloris gayana* Kunth cv. Pioneer) was related to the mean activity of Pb^{2+} $\{Pb^{2+}\}$ in solution. There was a 50% reduction in fresh mass of signal grass shoots at 5 µM $\{Pb^{2+}\}$ and at 3 µM $\{Pb^{2+}\}$ for the roots. Rhodes grass was considerably more sensitive to Pb in solution, with shoot and root fresh mass being reduced by 50% at 0.5 µM $\{Pb^{2+}\}$. The higher tolerance of signal grass to Pb appeared to result from the internal detoxification of Pb, rather than from the exclusion of Pb from the root. At toxic $\{Pb^{2+}\}$, an interveinal chlorosis developed in the shoots of signal grass (possibly a Pb-induced Mn deficiency), whilst in Rhodes grass, Pb^{2+} caused a bending of the root tips and the formation of a swelling immediately behind some of the root apices. Root hair growth did not appear to be reduced by Pb^{2+} in solution, being prolific at all $\{Pb^{2+}\}$ in both species.

Abbreviations: AMF – arbuscular mycorrhizal fungi, DAP – days after planting, EC - electrical conductivity, FIA - flow injection analysis, ICP-MS/OES - inductively coupled plasma-mass spectrometry/optical emission spectrometry, XPS - X-ray photoelectron spectroscopy

Introduction

Lead is a major environmental pollutant of world-wide concern. Although grasses are commonly used for the revegetation of contaminated sites, little information is available regarding the tolerance of many of these species to Pb or to other heavy metals. Two perennial grass species, signal grass (*Brachiaria decumbens* Stapf.) and Rhodes grass (*Chloris gayana* Kunth), were selected for study. Rhodes grass is widely grown in the tropics and sub-tropics of eastern and southern Africa, Australia and Central America, and is known to be highly tolerant of soil salinity and sodicity (Deifel et al. 2006; Shaw 1999). Signal grass, one of the more commonly sown tropical pasture species, is widespread throughout tropical America, south-eastern Asia and the Pacific, and is well adapted to highly acidic soils (Wenzl et al. 2001). However, signal grass is sensitive to salinity in contrast to Rhodes grass and many other perennial grass species (Deifel et al. 2006).

Few phytotoxicity studies have reported measurements of the soil solution Pb concentration. Badawy et al. (2002) measured Pb and calculated the soil solution Pb^{2+} activity $\{Pb^{2+}\}$ in 11 soils, many of which were near to highways and urban areas where Pb pollution was likely to have occurred. These authors reported that one soil, which had received sewage effluent for 90 years, had a soil solution Pb^{2+} activity of 15 μM, but all the others had $\{Pb^{2+}\}$ between 0.1 and 1 μM. Similarly, Degryse et al. (2007) reported soil solution Pb concentrations of up to 1.1 μM Pb, Sauve et al. (2003) reported Pb concentrations of up to 0.2 μM in the organic horizons of a forest soil, and Weng et al. (2001) reported a Pb concentration of 5 μM in a soil collected from a shooting range. In contrast, numerous solution culture experiments examining the phytotoxicity of Pb have utilized initial Pb concentrations orders of magnitude greater than these soil solution concentrations (for

example, Verma and Dubey (2003) and Yang et al. (2001)). In very few of such studies, however, have actual Pb concentrations been measured during the course of the experiment.

When considering plant growth in contaminated soils, it is important to consider the influence of arbuscular mycorrhizal fungi (AMF) on the growth of the plants. Arbuscular mycorrhizal fungi form a symbiotic relationship with approximately 80 % of vascular plants (Smith and Gianinazzi-Pearson 1988), and may be important in assisting plant growth in sites contaminated by Pb (Marschner et al. 1996). Mycorrhiza may improve plant growth in contaminated sites by influencing metal uptake and/or sequestration (Khan et al. 2000) and by improving plant nutrition (particularly in regards to P) (Andrade et al. 2004). However, the size of the AMF population is typically lower in contaminated soils than in non-contaminated soils (Khan et al. 2000). For example, in the study of Andrade et al. (2004), AMF were found to be more sensitive to increasing Pb than was the growth of soybean (*Glycine max* L. Merr.). Indeed, the AMF colonization of soybean plants growing in a Pb-contaminated soils was substantially lower than those in a non-contaminated soil (Andrade et al. 2004). Thus, (1) given that AMF colonization may potentially be low in some Pb-contaminated sites, and (2) to allow identification of the underlying mechanisms which confer Pb-tolerance to plants, there is a need to study plant growth under Pb-stress both with and without AMF. Therefore, although both Rhodes grass and signal grass form associations with AMF, this study will focus on the influence of Pb^{2+} on the growth of non-inoculated plants.

The objective of the current work was to determine the critical $\{Pb^{2+}\}$ associated with reductions in the growth of signal grass and Rhodes grass. Dilute nutrient solutions were used to simulate soil solutions, with plant growth related to the $\{Pb^{2+}\}$ calculated from concentrations of Pb measured in solution.

Materials and Methods

Preliminary investigations

Lead is known to form highly insoluble salts with P, and hence there is the potential for both Pb and P to be lost by precipitation from the nutrient solution, particularly in concentrated nutrient solutions containing high concentrations of P. However, in previous research on Pb toxicity in cowpea (*Vigna unguiculata* L.) in a dilute, low-P (2 μM) nutrient solution containing 0 to 2.5 μM Pb (Kopittke et al. 2007a), no evidence of such losses was obtained. Indeed, uptake by the plants accounted for the losses of Pb from solution, which averaged 11 % across all treatments during the 13 d experiment. A preliminary experiment with signal grass and Rhodes grass (data not presented) showed that signal grass in particular was much more tolerant of Pb and hence Pb concentrations higher than 2.5 μM would be required. Thus, the ranges selected for the present experiment were 0 to 20 μM Pb for signal grass and 0 to 5.5 μM for Rhodes grass. Computer modeling with PhreeqcI 2.11 (Parkhurst 2006) (using the Minteq database) showed that a dilute nutrient solution (ionic strength = 2.2 mM) containing 2 μM P, and maintained at pH 4.75, became supersaturated with respect to chloropyromorphite ($Pb_5(PO_4)_3Cl$) at Pb concentrations $\geq$ 0.5 μM. For the purposes of this simulation, we used the updated stability constant of Ma et al. (1994) for chloropyromorphite viz. log K = -83.7. To check for potential losses of Pb and P due to precipitation, a test nutrient solution was established with 20 μM Pb, 2 μM P and pH 4.75, with aeration but no plants, and samples were withdrawn for analysis at intervals over a 28 d period. All samples were filtered (0.22 μm Millipore GSWP) and analyzed for Pb by inductively coupled plasma-mass spectrometry (ICP-MS), and for P by flow injection analysis (FIA). No losses of either Pb or P were detected over the 28 d period. Hence, it was concluded that, if these nutrient solutions are indeed supersaturated with respect to chloropyromorphite, they are nevertheless quite stable and hence can be used in plant growth experiments.

Experimental design and establishment

Experiments were carried out in semi-controlled glasshouse conditions at The University of Queensland, St Lucia, Australia. Three high pressure sodium lamps were used to supplement natural sunlight and to provide 16 h of light per day (minimum photosynthetically active radiation = 400 μmol m^{-2} s^{-1} at plant height). Temperature was maintained at 28 °C during the light period and 25 °C during the dark. Relative humidity was not controlled, but a soaker hose was used to keep the floor wet so as to increase humidity. During the day, relative humidity generally ranged between 50 and 80 %, and reached a maximum of 90 to 95 % during the night. Average net daily pan evaporation throughout the experimental period was 1.4 mm.

Twenty four polypropylene containers (22 L; 265 mm diameter by 400 mm deep) were arranged in a completely randomized block design with a total of 24 treatments (two grass species, each with 12 Pb levels) replicated twice over time. Each of the 24 containers was filled with 21 L of a basal nutrient solution containing (μM): 680 NO_3^--N, 120 NH_4^+-N, 700 Ca, 550 S, 300 K, 240 Cl, 100 Mg, 10 Fe, 3 B, 2 P, 2 Mn, 1 Zn, 0.2 Cu, and 0.02 Mo. To minimize Pb complexation, Fe was supplied as CDTA (see Kopittke et al. (2007a) for details). Based on calculations in GEOCHEM v2.0 (Parker et al. 1995), over the range of Pb concentrations of interest and at pH 4.75, the amount of total solution Pb complexed by CDTA was calculated as between 0.2 % (at 0.01 μM Pb) and 0.02 % (at 20 μM Pb).

After an initial 24 h aeration of the basal nutrient solution, each of the 24 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 by the addition of 2 mM $Ca(OH)_2$. The percentage of N supplied as NH_4^+ (15 %) had been shown in preliminary experiments to cause a slow acidification of the nutrient solution by these species over time.

Aliquots of 10.5 mM $Pb(NO_3)_2$ stock solution were added to the basal nutrient solutions to result in final concentrations (μM) of: 0, 0.10, 0.35, 1.0, 1.75, 2.5, 3.5, 5.0, 7.5, 10, 17.5, and 20 for signal grass (cv. Basilisk), and 0, 0.010, 0.025, 0.050, 0.10, 0.25, 0.35, 0.75, 1.25, 1.75, 3.5, and 5.5 for Rhodes grass (cv. Pioneer). 'Basilisk' and 'Pioneer' represent the most widely used cultivars of the two grasses.

Signal grass seeds were dried in a desiccator for 2 d, immersed in concentrated H_2SO_4 for 10 min (Humphreys 1987), rinsed thoroughly with DI water, and then soaked in aerated DI water for 6 h. Seeds of both species were then placed on separate moistened paper towels at 30 °C for 18 h before being transferred to washed river sand to grow for 7 d. Water content in the sand was maintained at a constant level through the use of a capillary watering bench, with a thin layer of polypropylene beads spread across the sand surface to limit evaporative losses. During this initial 7 d period, nutrients were supplied through the application of 'Flowfeed EX7' (a soluble fertiliser, Grow Force, Australia). Following this period of 7 d, the sand was placed in a large container of water so that the sand fell away from the roots and the seedlings floated to the surface. Each seedling was placed in a polystyrene cup with the roots protruding through a mesh bottom, before being transferred to the nutrient solutions. Four plants (in four cups) were placed in each of the 24 containers, with the average of these four plants forming one replicate.

Maintenance of the experiment

Nutrient removal by the plants was offset by frequent small additions (< 1 mL per container) 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. Plant growth, the probable nutrient removal, and hence the quantity of delivery solution required, were calculated using Nutradd v2.2

(Asher and Blamey 1986). The rate of nutrient addition was decreased in the higher-Pb treatments in which there was an evident reduction in growth rate. The delivery solution was added at the calculated rates three times during the first 7 d, then daily thereafter until harvest at 14 days after planting (DAP). Additional DI water was supplied to the containers throughout the growth period to maintain solution volume.

Samples of the nutrient solutions were taken 0, 6, 10, and 14 DAP and measured for electrical conductivity (EC) and pH (to check the accuracy of the pH titration units). In addition, 10 mL of each sample was filtered (0.22 μm), acidified to pH < 2.0 using 20 μL of concentrated HCl, and refrigerated (3.5 ºC) before analysis by inductively coupled plasma optical emission spectrometry (ICPOES) for Ca, Cu, Fe, K, Mg, Mn, Na, S, and Zn, ICPMS for Pb, and FIA for P.

Harvesting and light microscopy

One day prior to the completion of the experiment (i.e. 13 DAP), one of the four plants from each container was harvested. The roots were placed in a 70 mL container filled with the corresponding nutrient solution and taken for immediate observation under a dissecting microscope. Two stains were used for the observation of the roots under the dissecting microscope; 0.5 % crystal violet (a non-Pb-specific stain) and rhodizonate (a Pb-specific stain which forms dark red precipitates in the presence of Pb (Jurkiewicz et al. 2001; Tung and Temple 1996)). The rhodizonate stain was prepared immediately prior to its use by dissolving 2 g of sodium rhodizonate per liter in a tartaric acid-sodium bitartrate buffer (0.1 M tartaric acid and 0.1 M sodium bitartrate). A precipitate was visible on the outside of the roots and root hairs of the signal grass growing in high-Pb solutions. Some of this precipitate was collected from the signal grass roots prior to harvest on 14 DAP by filling a 50 mL centrifuge tube with the appropriate nutrient solution, removing a plant from the 22 L container, placing the roots in the 50 mL tube, and gently shaking the plant. The 50 mL tubes

were then centrifuged for 15 min, the supernatant decanted, and the precipitate stored at 3.5 °C until analysis using X-ray photoelectron spectroscopy (XPS) (Kratos, Axis Ultra).

Upon completion of the experiment (14 DAP), the fresh mass of the roots and shoots of the remaining three plants was determined. The roots were thoroughly rinsed in DI water, and the elemental concentrations of Pb and nine other elements in the roots and shoots determined using ICPOES after drying for 7 d at 65 ºC and acid digestion as described by Martinie and Schilt (1976). For the roots, rinsing with DI water does not remove adsorbed Pb, and hence all root tissue Pb measurements reported in this study include both the Pb taken up by the plant and the Pb adsorbed to the root surface.

Data analysis

Using PhreeqcI, Pb^{2+} was calculated to account for approximately 88 % of the total soluble Pb, the remainder being mostly the uncharged $PbSO_4^0$ ion-pair, and Pb-CDTA < 0.2 %. The $\{Pb^{2+}\}$ was calculated from the means of the measured Pb concentrations.

A grouped regression analysis was used to examine the relationship between the relative shoot and root mass and the $\{Pb^{2+}\}$ in the nutrient solution, exponential curves being fitted using GenStat 7 (GenStat 2003). Regression analysis was also used to examine the relationship between the nutrient solution $\{Pb^{2+}\}$ and the Pb concentrations in the root (linear regression model) and shoot (exponential regression model) tissues at the time of harvest (GenStat 2003).

Results

Composition of nutrient solutions

Solution EC was relatively constant during the 14 d experimental period, increasing by no more than 8 % (average = 5 %) in any treatment (data not presented). Concentrations of measured nutrients also remained relatively constant, typically varying by no more than 15 % from the initial value (data not presented). However, in treatments in which plant growth was reduced by > 50 %, the concentrations of some nutrients, particularly P increased above the initial values (data not presented), the average P concentration reaching 5.1 μM in the highest Pb treatments at 14 DAP.

Solution Pb concentrations (measured 0 and 14 DAP) were relatively constant between replicates, with < 15 % difference between the replicates for most treatments (data not presented). The variation in Pb concentrations between replicates tended to be greater at 14 than at 0 DAP. Within each replicate, Pb concentrations remained relatively steady over the 14 d experimental period, with decreases in Pb generally corresponding well with the calculated quantities taken up (and adsorbed) by the plants, except for the 17.5 and 20 μM Pb treatments in signal grass (data not presented). Excluding the two highest signal grass treatments, solution Pb concentrations decreased by an average of 7 % for Rhodes grass and 3 % for signal grass between 0 and 14 DAP (data not presented). In the two highest signal grass treatments, it was calculated that the uptake of Pb should have lowered the solution Pb concentration by approximately 0.3 μM, but by the end of the experiment (14 DAP) the Pb concentrations had fallen to 11 μM in the 17.5 μM treatment and to 13 μM in the 20 μM treatment. In both of these treatments, there were clearly visible deposits of a Pb-containing precipitate on the roots (see below).

Using the mean measured Pb concentrations in the nutrient solutions (two replicates, each with four sampling times), PhreeqcI was used to calculate the mean $\{Pb^{2+}\}$ for each treatment, *viz*: 0, 0.09, 0.16, 0.51, 0.96, 1.5, 2.1, 3.3, 4.8, 6.5, 9.9, and 10 μM for signal grass, and 0, 0.01, 0.02, 0.03, 0.06, 0.18, 0.19, 0.40, 0.59, 1.1, 2.2, and 3.4 μM for Rhodes grass.

Effects of $\{Pb^{2+}\}$ on growth

Plant growth for 14 d in the low-Pb treatments was good, with the fresh mass being approximately 1.5 g for shoots and 1.5 g for roots of signal grass, and 1.5 g for shoots and 1.0 g for roots of Rhodes grass. However, growth of both signal grass and Rhodes grass decreased markedly as $\{Pb^{2+}\}$ increased (Figure 1). With signal grass, the roots were more sensitive to Pb than the shoots ($P = 0.008$). Indeed, a 50 % reduction in relative fresh mass occurred at a $\{Pb^{2+}\}$ of 5 μM for the shoots and at 3 μM for the roots (Figure 1a). However, Rhodes grass was much more sensitive to Pb than signal grass (Figure 1b), with the roots and shoots equally sensitive to Pb the difference between separately fitted curves being non-significant ($P = 0.764$). Hence, a single curve was fitted to the two data sets (Figure 1b). In this species a $\{Pb^{2+}\}$ of only 0.5 μM was found to correspond to 50 % reduction in relative shoot and root mass.

Symptoms of $\{Pb^{2+}\}$ toxicity

Signal grass leaves displayed an interveinal chlorosis in solutions containing ≥ 1.5 μM $\{Pb^{2+}\}$, the severity of chlorosis increased as $\{Pb^{2+}\}$ increased. Foliar applications of Zn (as 0.5 % $ZnSO_4.7H_2O$ plus 0.25 % $Ca(OH)_2$) and Fe (as Sequestrene, a commercial Fe chelate) failed to correct the condition, suggesting that the symptoms were not due to Pb-induced deficiencies of either Zn or Fe, a conclusion supported by the plant analysis data (see below).

Despite root growth reductions in signal grass at $\{Pb^{2+}\}$ as low as 1 μM (Figure 1), visible symptoms on the roots appeared only in the two highest Pb treatments (9.9 and 10 μM). These symptoms included slightly bent root tips and slight swellings behind some root apices (Figure 2c,d). Root hair growth was prolific in all treatments, the root hair zone extending to within 1 mm of the root apex in some of the higher Pb treatments (Figure 2d).

A crystalline white precipitate was observed to form around the roots of signal grass when grown in solutions with $\{Pb^{2+}\} \geq 4.8$ μM, being present in much greater abundance at the two highest treatments (9.9 and 10 μM Pb^{2+}) (Figure 2d,e). This precipitate was particularly prevalent on the seminal (primary) roots and the older parts of the adventitious (secondary) roots. When stained with rhodizonate, the white precipitate became dark red (Figure 2e), indicating the presence of Pb. However, apart from these superficial precipitates, the root surface of signal grass remained white when stained with rhodizonate (Figure 2e). From computer modelling of the nutrient solution, it seems probable that the precipitate was chloropyromorphite ($Pb_5(PO_4)_3Cl$), in which Pb, P, O, and Cl would be expected to occur in the ratio 5 : 3 : 12 : 1. Although the sample of precipitate was small (and likely contaminated by small root fragments), analysis by XPS confirmed the presence of Pb, P, O, and Cl in the precipitate, at ratios of 5 : 2.5 : 12 : 0.7 roughly approximating those expected for chloropyromorphite.

Although Rhodes grass was much more sensitive to Pb toxicity than signal grass (Figure 1), no leaf symptoms were observed in any of the Pb treatments for Rhodes grass. Symptoms of root tip bending were observed at $\{Pb^{2+}\} > 0.4$ μM (Figure 2i), and some root tips showed severe swelling behind the root apex at $\{Pb^{2+}\} > 1.1$ μM (Figure 2j). As with signal grass, root hair growth was prolific in all treatments, and in some cases, the root hair zone extended to within 1 mm of the root apex (Figure 2i,k). With Rhodes grass, small amounts of the Pb-containing precipitate were observed on the roots at $\{Pb^{2+}\}$ 3.4 μM.

Effects on shoot and root chemical composition

Concentrations of Pb in the shoots of both species increased rapidly with the first increments in $\{Pb^{2+}\}$ before reaching a plateau (Figure 3a); the concentrations reached in signal grass (0.29 mg

g^{-1}) being substantially higher than in Rhodes grass (0.19 mg g^{-1}). The total root Pb concentrations were much higher than the shoot Pb concentrations (note the difference in the scales of the y-axes in Figure 3). Root Pb concentrations of both species increased linearly with increasing $\{Pb^{2+}\}$ (Figure 3b), the rates of increase being similar up to the highest $\{Pb^{2+}\}$ at which Rhodes grass was grown (3.4 μM). The critical tissue Pb concentrations (corresponding to a 50 % reduction in relative fresh mass) were calculated to be 0.29 mg g^{-1} for shoots and 3.9 mg g^{-1} for roots in signal grass, compared with 0.16 mg g^{-1} for shoots and 0.82 mg g^{-1} for roots in Rhodes grass.

Elevated $\{Pb^{2+}\}$ decreased the concentrations of Ca, P, Mg, Cu, Zn and Mn in the shoots of both species, as well as decreasing K and S in Rhodes grass (Table 1). The relatively high concentrations of Fe and Zn in signal grass shoots are consistent with the conclusion that the interveinal chlorosis observed at higher $\{Pb^{2+}\}$ were not due to deficiencies of these elements, but possibly to a Pb-induced Mn deficiency (Table 1).

Discussion

Critical $\{Pb^{2+}\}$ for growth on contaminated lands

When monitoring the restoration of Pb-contaminated land, it is important to consider the planned end-use of the site and hence the role of the vegetation which is established on the contaminated site. Often, 50 % maximum growth is considered satisfactory for the revegetation of contaminated sites. Results of the present study (Table 2) show that for this end-use, signal grass was able to tolerate 10 times more $\{Pb^{2+}\}$ in the root environment than was Rhodes grass. For greater productivity (*viz*. a 10 % growth reduction), signal grass tolerated a $\{Pb^{2+}\}$ approximately five times greater than did Rhodes grass (Table 2). If animals are to consume the grasses, however, consideration should be given to the Pb in the shoots. Importantly in this regard is the finding that the higher critical solution values for toxicity in signal grass appears to result from greater tolerance

of Pb in the plant tissue, rather than from the exclusion of Pb by the roots (see below). Thus, if the grasses were to be consumed by animals, the solution $\{Pb^{2+}\}$ should be ≤ 0.1 μM for signal grass or ≤ 0.2 μM for Rhodes grass for the shoots to have Pb concentrations lower than the indicative critical value of 0.1 mg Pb g^{-1} shoots (National Research Council (U.S.) 2005) (Table 2). For the revegetation of contaminated sites, the presence of AMF may also be of importance (see *Introduction*). Given that AMF have been reported to reduce the toxicity of heavy metals (Audet and Charest 2007), the critical values reported above may possibly be conservative.

Influence of $\{Pb^{2+}\}$ on plant growth

For both species, it is unclear as to whether the primary site of Pb^{2+} toxicity was the root or the shoot. The roots of signal grass did not show any obvious abnormalities (apart from those growing at 9.9 and 10 μM Pb^{2+}), but the shoots displayed an interveinal chlorosis at $\{Pb^{2+}\} \geq 1.5$ μM, possibly consistent with a Pb-induced Mn deficiency (Table 1). The roots of signal grass were, however, more sensitive to Pb^{2+} than were the shoots (Figure 1). A similar disparity exists for Rhodes grass in which visual symptoms indicated that the roots were the primary site of toxicity; at $\{Pb^{2+}\} \geq 0.4$ μM the roots were stunted and had bent tips (Figure 2) whilst the shoots had no symptoms of toxicity over the range of $\{Pb^{2+}\}$ studied. In this species, however, no difference was found between the response of the shoots and the roots to increasing Pb^{2+} (Figure 1).

Root hair growth was prolific in all treatments and did not appear to be inhibited at any $\{Pb^{2+}\}$ (Figure 2), a finding in contrast to those with radish (*Raphanus sativus* L.) (Lane and Martin 1980) and beech (*Fagus sylvatica* L.) (Breckle and Kahle 1992). Lane and Martin (1980) proposed that the reduction in root hair growth resulted from the displacement of Ca from the cell wall resulting in an increase in cell wall rigidity. However, care must be taken when interpreting the results of Lane and Martin (1980), because the high P concentration used (2 mM) has been shown to

influence root hair growth (Foehse and Jungk 1983). The apparent lack of detrimental Pb^{2+} effects on root hair growth is also in contrast to those observed for Al toxicity (Brady et al. 1993; Hecht-Buchholz et al. 1990) and Cu toxicity (Kopittke et al. 2007b; Kopittke and Menzies 2006) in which root hair formation is reduced at lower metal concentrations than those required to reduce root elongation. In the present study, root hairs developed within 1 mm of the root tip (Figure 2i,k) indicating that cells close to the root tip were not killed by Pb^{2+} but that there was a severe disruption of normal root development in which root hairs develop distal to the elongation zone.

Pb tolerance mechanisms

Rengel (1997), in reviewing tolerance and resistance mechanisms to toxicity of Al and heavy metals, made a distinction between external and internal mechanisms. At any given $\{Pb^{2+}\}$ in the current study, there was no significant difference in the total Pb content of the roots between the two species (Figure 3). Similarly, the Pb concentrations of the shoots were similar except at high Pb^{2+} activities where the shoots of signal grass contained significantly more Pb than the shoots of Rhodes grass (Figure 3). Thus, the higher Pb^{2+} tolerance of signal grass, compared to that of Rhodes grass (Figure 1), was not due to external detoxification (and decreased movement of Pb into the root), but rather, to internal detoxification. However, further work is required to provide direct evidence to verify this observation.

Solution composition

Whilst the solution Pb concentration remained relatively constant in the lower Pb treatments, in the four highest signal grass treatments some Pb was observed to precipitate as chloropyromorphite and to accumulate on the roots (Figure 2). Although PhreeqcI modeling predicted that Pb would precipitate at Pb > 0.5 μM (assuming 2 μM P), precipitates were observed only in treatments where the initial Pb concentration was ≥ 7.5 μM (i.e. a $\{Pb^{2+}\} \geq 4.8$ μM). Furthermore, although PhreeqcI

predicted that in the 20 μM Pb treatment, Pb would precipitate until only 0.003 μM P remained in solution, analysis of the solution 14 DAP revealed that there was 2.2 μM P in solution. Indeed, although nutrient addition was reduced in the high-Pb treatments (to offset the lower rate of nutrient uptake), nutrient addition still exceeded nutrient uptake, with treatments where growth was reduced by > 50 % having an average PO_4^{3-}-P concentration (14 DAP) of 5.1 μM. It was also noted that although no Pb (or P) precipitated from a 20 μM Pb solution aerated for 28 d without any plants (data not presented), if the solution was subsequently gradually acidified with either HCl or HNO_3 (at a rate simulating the acidification caused by plants) and then adjusted back to pH 4.75 using the pH titration unit and peristaltic pump, approximately 25 % of the Pb would precipitate (data not presented). Hence, based upon these observations, it is considered that for the current experimental system: (1) the equilibrium condition predicted by PhreeqcI was not achieved, and (2) in the solutions containing plants, the gradual addition of $Ca(OH)_2$ as a pH titrant and the addition of the high-P delivery solution resulted in the temporary formation of localized regions with conditions favorable for the precipitation of chloropyromorphite.

Conclusions

Signal grass was found to be considerably more tolerant of Pb^{2+} than Rhodes grass, with a 50 % reduction in relative fresh mass occurring at a $\{Pb^{2+}\}$ in solution of 5 μM for the shoots and 3 μM for the roots. This was approximately 10-fold higher than the 0.5 μM Pb that reduced shoot and root growth of Rhodes grass by 50 %. However, the presence of AMF in a Pb-contaminated soil may influence the tolerance of these grasses. The critical Pb concentration (corresponding to a 50 % reduction in relative fresh mass) was calculated to be 0.29 mg g^{-1} shoots and 3.9 mg g^{-1} roots for signal grass, and 0.16 mg g^{-1} shoots and 0.82 mg g^{-1} roots for Rhodes grass. It was concluded that the greater Pb tolerance of signal grass resulted from the internal detoxification of Pb, rather than from the exclusion of Pb from the root. In signal grass, Pb^{2+} caused an interveinal chlorosis of the

shoots (possibly a Pb-induced Mn deficiency), whilst in Rhodes grass, Pb^{2+} caused a bending of the root tips and the formation of a swelling immediately behind some of the root apices. Root hair growth was prolific across all $\{Pb^{2+}\}$ for both species.

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Figure 1. The relative fresh mass of the shoots and roots of signal grass (a) and Rhodes grass (b) after 14 d growth in dilute nutrient solutions containing a range of Pb^{2+} activities.

Figure 2. Micrographs of roots of signal grass ((a) to (e)) and Rhodes grass ((f) to (k)) after 13 d growth in dilute nutrient solutions containing a range in Pb^{2+} activities (as indicated within each photo), stained with crystal violet, except for (e) which was stained with sodium rhodizonate. Without added Pb, the root apex was healthy ((a), (f)) (as was the case with Rhodes grass at 0.4 uM Pb (h)), and there was prolific root hair growth ((b), (g)). The root apex of signal grass showed slight thickening at 10 uM Pb (c) and the root hairs were covered in gelatinous globules (d), red staining of which with sodium rhodizonate indicated the presence of Pb. Root hairs of Rhodes grass developed close to the apex at 0.59 (i) and 3.4 uM Pb (k), and some root apices were deformed ((j), (k)) or swollen (j). All images are of secondary roots other than (d), (e), and (j) which are of primary roots. Bars are equal to approximately 1 mm.

Figure 3. The concentrations of Pb in (a) shoots and (b) roots of signal grass and Rhodes grass after 14 d growth in dilute nutrient solutions containing a range of Pb^{2+} activities. The dotted lines correspond to Pb^{2+} activities which caused a 50 % reduction in relative fresh mass (0.5 μM for Rhodes grass, and 5 μM for signal grass shoots and 3 μM for signal grass roots).

Table 1. The effects of $\{Pb^{2+}\}$ in solution on the nutrient concentrations in shoots of Rhodes grass and signal grass after 14 d of growth in dilute nutrient solutions. Only data for the control (0 μM), the treatment closest to the $\{Pb^{2+}\}$ corresponding to 50 % relative shoot mass, and the highest $\{Pb^{2+}\}$ are presented.

Species		Solution $\{Pb^{2+}\}$ (μM)	K	Ca	S	P	Mg	Pb	Cu	Fe	Zn	Mn
			mg g^{-1}					μg g^{-1}				
Signal grass	Shoots	0	46	5.7	3.6	6.8	3.3		12	190	210	150
		4.8	47	6.6	3.2	4.2	1.6	219	8.2	153	71	46
		10	45	3.5	3.5	2.9	1.2	260	6.4	170	44	20
Rhodes grass	Shoots	0	52	6.7	3.3	7.3	1.7		13	180	140	120
		0.40	45	7.7	2.9	6.0	1.6	150	10	210	100	69
		3.4	29	4.5	1.8	2.8	1.1	180	3.9	180	49	21

Table 2. Critical Pb^{2+} activity in nutrient solution for production of (i) plant shoots safe for animal consumption, (ii) plants with a 10 % reduction in fresh mass, or (iii) plants with a 50 % reduction in fresh mass.

Criterion	**Critical Pb^{2+} activity in nutrient solution (µM)**		
	Signal grass		**Rhodes grass**
	Shoots	*Roots*	
Animal consumption (≤ 0.1 mg Pb g^{-1} shoots)[1]	0.1	-	0.2
10% fresh mass reduction	0.5	0.3	0.1
50% fresh mass reduction	5	3	0.5

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

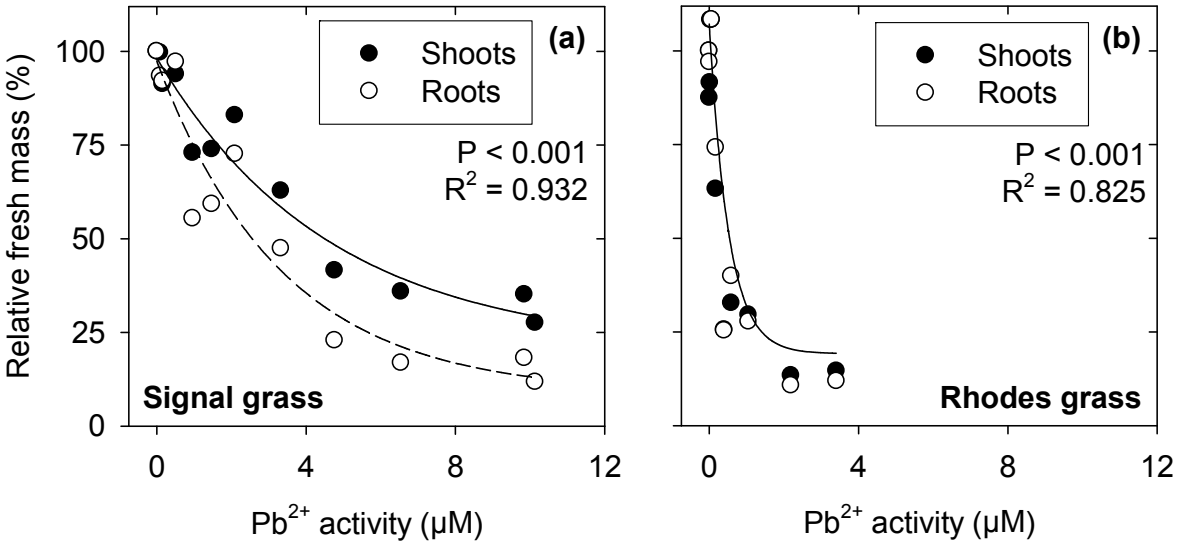

Figure 1. The relative fresh mass of the shoots and roots of signal grass (a) and Rhodes grass (b) after 14 d growth in dilute nutrient solutions containing a range of Pb^{2+} activities.

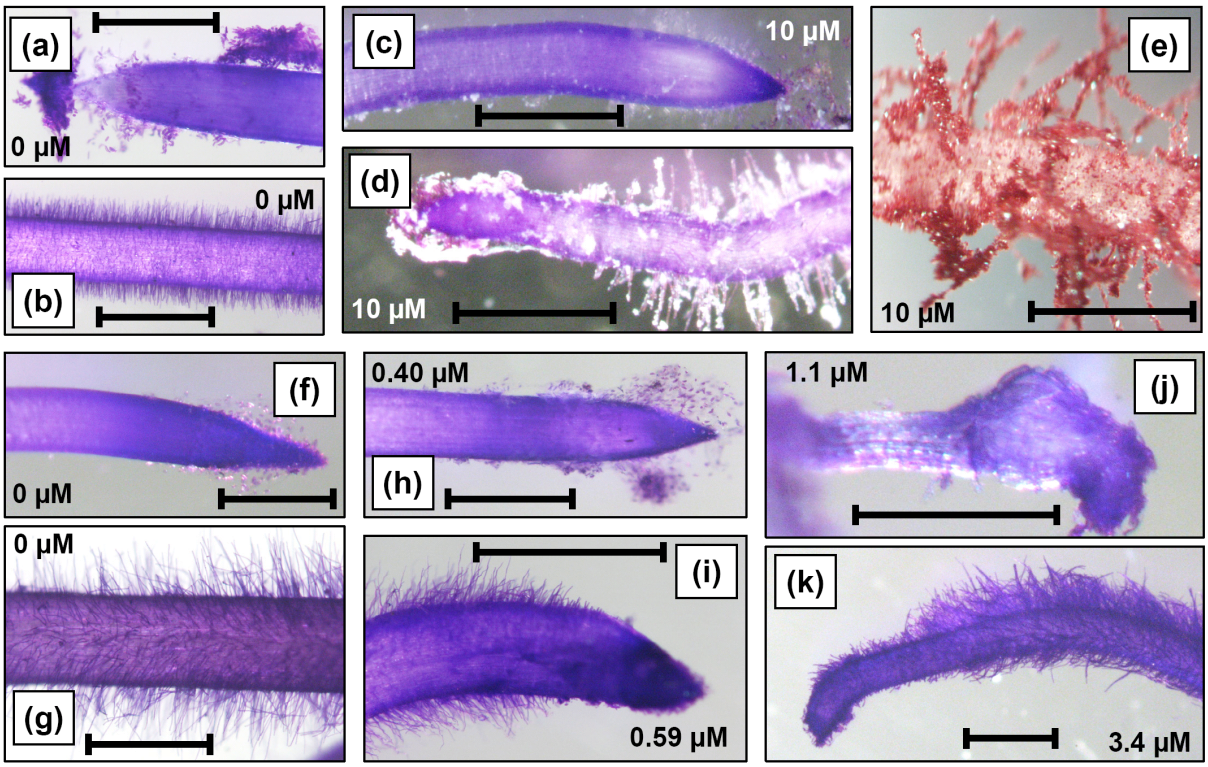

Figure 2. Micrographs of roots of signal grass ((a) to (e)) and Rhodes grass ((f) to (k)) after 13 d growth in dilute nutrient solutions containing a range in Pb^{2+} activities (as indicated within each photo), stained with crystal violet, except for (e) which was stained with sodium rhodizonate. Without added Pb, the root apex was healthy ((a), (f)) (as was the case with Rhodes grass at 0.4 uM Pb (h)), and there was prolific root hair growth ((b), (g)). The root apex of signal grass showed slight thickening at 10 uM Pb (c) and the root hairs were covered in gelatinous globules (d), red staining of which with sodium rhodizonate indicated the presence of Pb. Root hairs of Rhodes grass developed close to the apex at 0.59 (i) and 3.4 uM Pb (k), and some root apices were deformed ((j), (k)) or swollen (j). All images are of secondary roots other than (d), (e), and (j) which are of primary roots. Bars are equal to approximately 1 mm.

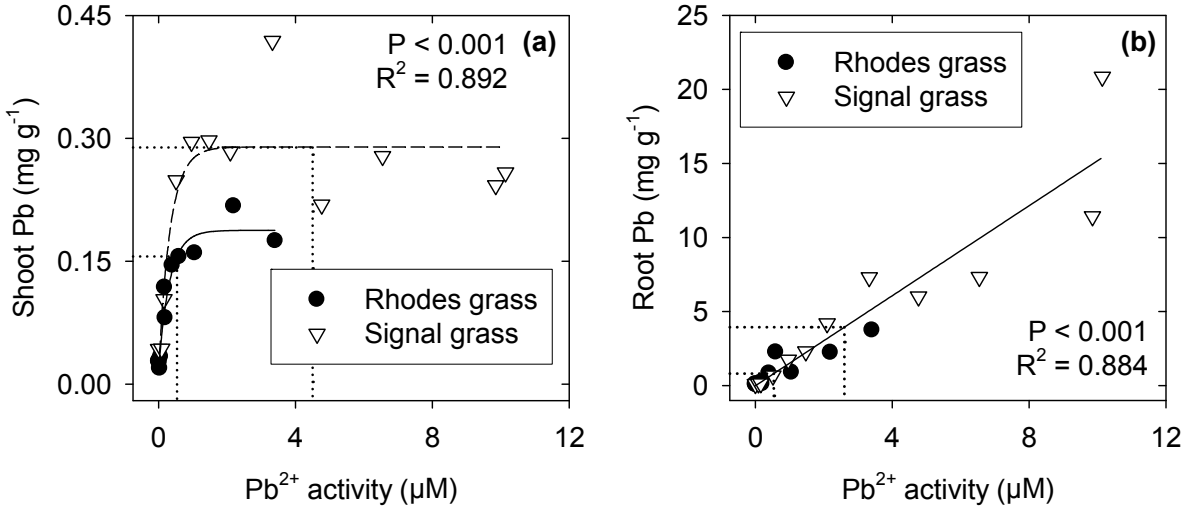

Figure 3. The concentrations of Pb in (a) shoots and (b) roots of signal grass and Rhodes grass after 14 d growth in dilute nutrient solutions containing a range of Pb^{2+} activities. The dotted lines correspond to Pb^{2+} activities which caused a 50 % reduction in relative fresh mass (0.5 µM for Rhodes grass, and 5 µM for signal grass shoots and 3 µM for signal grass roots).