Toxic Effects of Pb²⁺ on Growth of Cowpea (*Vigna unguiculata*)

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Abstract

A concentration as low as 1 µM lead (Pb) is highly toxic to plants, but previous studies have typically related plant growth to the total amount of Pb added to a solution. In the present experiment, the relative fresh mass of cowpea (*Vigna unguiculata*) was reduced by 10 % at a Pb^{2+} activity of 0.2 µM for the shoots and at a Pb^{2+} activity of 0.06 µM for the roots. The primary site of Pb^{2+} toxicity was the root, causing severe reductions in root growth, loss of apical dominance (shown by an increase in branching per unit root length), the formation of localized swelling behind the root tips (due to the initiation of lateral roots), and the bending of some root tips. In the root, Pb was found to accumulate primarily within the cell walls and intercellular spaces.

Abbreviations: CDTA – cyclohexane-1,2-diaminetetra-acetic acid; DAP – days after planting; DI – deionised; EC – electrical conductivity; I – ionic strength; ICPOES – inductively coupled plasma optical emission spectrometry; ICPMS – inductively coupled plasma mass spectrometry; TEM – transmission electron microscopy

Introduction

Lead is a major pollutant in aquatic and terrestrial ecosystems. Anthropogenic Pb pollution occurs

through a variety of activities, including mining, metal processing, battery manufacture and

disposal, the burning of leaded fuels, the release of Pb from the wearing of tires, and application of

sludge to agricultural land (Kabata-Pendias and Pendias, 2001). Indeed, under some circumstances,

soils subjected to anthropogenic pollution have been reported to contain Pb concentrations of up to

20 mg/g (2 %) (Davies, 1980; Kabata-Pendias and Pendias, 2001). Degryse et al. (2006) reported

that the soil solutions extracted from contaminated soils in Europe contained up to 1.1 µM Pb.

Similarly, Lamersdorf et al. (1991) reported that the leachate from highly acidic (pH_{CaCl2} of 3) soils

in Europe contained up to 0.85 µM Pb, whilst Pb concentrations of up to 2 µM (with 1 µM present

as the Pb^{2+} free-ion) have been reported in a contaminated soil from the USA (Nolan et al., 2003).

Using wheat (*Triticum aestivum* L.), Nolan et al. (2005) showed the phytoavailability of Pb is

related to its soil solution concentration.

Despite the worldwide importance of Pb contamination, it remains unclear as to what concentration

of Pb will cause a reduction in plant growth under conditions similar to those experienced in

'typical' soil solutions. Malkowski et al. (2002) found that Pb at 10, 100 or 1000 µM reduced the

growth of maize (*Zea mays* L.). Similarly, Fodor et al. (1996) reported that 10 µM Pb was toxic to

cucumber (*Cucumis sativus* L.), and Wozny and Jerczynska (1991) found 10 µM Pb to be toxic to

bean (*Phaseolus vulgaris* L.). However, Godbold and Kettner (1991b) reported that as little as 0.1

µM Pb caused a reduction in the root elongation of Norway spruce (*Picea abies* L.) seedlings.

In previous studies utilizing nutrient solutions to investigate the phytotoxicity of Pb (and other

heavy metals), it has been rare to measure the actual concentrations of the heavy metal in the test

solutions during the course of an experiment. Rather, it is almost always assumed, either correctly

or incorrectly, that the heavy metal is present at the concentration at which it was initially added. However, in a study of the tolerance of four native Australian tree species to Zn, Mn, and Cu, Reichman (2001) showed that regular addition (or solution renewal) was required to maintain solution metal concentrations, particularly in longer-term experiments. Furthermore, the authors are unaware of any studies in which plant growth has been related to the activity of the free Pb^{2+} ion calculated from measured Pb concentrations in solution.

The objective of the current work was to determine the critical Pb^{2+} activity associated with a reduction in growth of cowpea. Dilute nutrient solutions were used to replicate conditions found in the soil solution as closely as possible. Growth of both the roots and shoots were related to Pb^{2+} activity as calculated from measured Pb concentrations. Light microscopy and transmission electron microscopy was used to examine the effect of Pb^{2+} on root morphology.

Materials and Methods

Design and establishment of the experiment

The experiment consisted of 12 Pb treatments (0, 0.025, 0.050, 0.10, 0.15, 0.20, 0.30, 0.40, 0.50, 1.0, 1.5, and 2.5 μM) in a randomized block design under semi-controlled glasshouse conditions, replicated twice over time. An additional two pots in both replicates (0 and 2.5 μM Pb) were used to grow plants for microscopic examination (see later). High-pressure sodium lamps were used to supplement natural sunlight, providing 16 h of light daily. Photosynthetically active radiation, measured at plant height, reached a maximum of approximately 1500 μmol/m^2/s during the day. Temperature was maintained at 28 °C during the light period and 25 °C during the dark. A soaker hose was used to keep the floor wet and thus increase humidity, which generally ranged from 40 to 80 % during the day and reached a maximum of 90 to 95 % at night. Average net daily pan evaporation throughout the experimental period was 2.5 mm.

The pots were 22 L polypropylene containers, and were filled with a basal nutrient solution containing (μM) NO_3^--N 680, NH_4^+-N 120, Ca 650, S 502, K 320, Mg 50, P 2, Cl 140, Fe 10, Si 10, B 3, Mn 0.2, Zn 0.1, Cu 0.05, and Mo 0.02. Cyclohexane-1,2-diaminetetra-acetic acid (CDTA) was chosen as the Fe chelating agent because modeling with GEOCHEM v2.0 (Parker et al., 1995) indicated that it would complex only 0.05 to 0.18% of the total Pb under the conditions of the experiment compared with 0.92-2.8 % for EDTA, 12-26 % for NTA (nitrilotriacetate), and 15-38 % for HEDTA. To prepare the 10 mM FeCDTA stock solution, 3.64 g of CDTA (Sigma) was placed in a 1 L volumetric flask and approximately 500 mL of DI water added, plus 7.2 mL of 5 M NaOH used to raise pH. Using approximately 300 mL DI water, 4.04 g $Fe(NO_3)_3.9H_2O$ was dissolved and then slowly added to the CDTA, with the final solution volume raised to 1 L.

Silicon was added to the basal nutrient solution due to its prominence in the soil solution and known beneficial effects on plant growth, including suppression of Mn toxicity (see reviews by Asher (1991) and Epstein (1994)). A 1 L stock Si solution was prepared as a 16.8 mM sodium metasilicate ($Na_2SiO_3.5H_2O$) solution to which 3.0 mL of concentrated HCl had been added to reduce the pH to approximately 8.4.

Twenty-one L of the basal nutrient solution was placed in each pot, and sufficient 0.1 M HCl added to lower the pH to 4.50. An appropriate amount of a 1.05 mM $PbCl_2$ stock solution was then added to each pot to establish the required Pb concentrations (see earlier).

Preliminary work had indicated that the NO_3^-/NH_4^+ ratio (85:15) would cause a slow acidification of the solution over time (see Kopittke et al. (2007) for more details). Hence, after an initial 24 h aeration of the nutrient solutions, each pot was connected to a separate pH titration unit (TPS,

miniCHEM-pH) and peristaltic pump (Masterflex 5 RPM with Masterflex Tygon tubing, L/S 17) using 2 mM $Ca(OH)_2$ to maintain a constant pH 4.50.

Cowpea seeds (*Vigna unguiculata* (L.) Walp. cv Caloona) were imbibed in aerated 200 μM $CaSO_4$ solution for 2 h. Seeds were rolled in paper towel, and germinated for 36 h at 30 ºC with the lower ends of the paper towel immersed in tap water. Four seedlings with radicle lengths of 10 ± 2 mm were transferred to each pot.

Maintenance of the experiment

Nutrient removal by the plants was offset by additions 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, and the probable nutrient removal and quantity of delivery solution required, were calculated as described by Taylor et al. (1998) and Kopittke and Menzies (2006). The delivery solution was added at the calculated rate three times during the first 7 d, then daily afterwards until harvest at 13 DAP. Additional DI water was supplied to the containers throughout the growth period to maintain solution volume.

Electrical conductivity (EC) was measured every 2 d. Nutrient solution samples were taken 0, 2, 6, and 13 DAP, filtered (0.22 μm Millipore GSWP), 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 B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Si, and Zn, and inductively coupled plasma-mass spectrometry (ICPMS) for Pb.

Harvesting, microscopy, and data analysis

Upon completion of the experiment, 13 DAP, roots were sampled from each treatment, stained using 0.5 % crystal violet, and examined using a dissecting microscope. The fresh mass of the remaining roots and shoots was determined, the roots thoroughly rinsed in DI water (5 min), and the concentrations of selected elements in roots and shoots determined using ICPOES after drying for 7 d at 65 ºC and acid digestion as described by Martinie and Schilt (1976). The relative shoot and root fresh masses were calculated from the control (control = 100 %).

For relative shoot and root mass, a grouped regression analysis (fitting logistic curves) was performed using GenStat 7 (GenStat, 2003). Regression analysis was also used to examine the relationship between the mean nutrient solution Pb^{2+} activity (calculated from measured concentrations in the nutrient solution 0, 2, 6 and 13 DAP) and the root and shoot Pb concentrations at harvest (Mitscherlich (exponential) regression model) (GenStat, 2003). All calculations of ionic strength (I) and ion activities were performed using PhreeqcI 2.12.5 with the Minteq database (Parkhurst, 2006). The quantity of Pb chelated by CDTA accounted for < 0.2 % of the total Pb in solution. Of the remaining Pb in solution, Pb^{2+} accounted for approximately 85 % of the Pb, the rest being mostly the uncharged $PbSO_4^0$ ion pair.

The roots from the additional two pots (0 and 2.5 μM Pb) plus small samples from a few other pots were removed and fixed in 3 % glutaraldehyde (in 0.1 M sodium cacodylate buffer) and stored at 3.5 °C until further processing. Microwave processing was performed using a Pelco BioWave variable-power microwave oven, equipped with a ColdSpot water recirculating device on the oven floor to prevent specimen heating, and to ensure even distribution of the microwave radiation. The microwave was used as described by Wendt et al. (2004). Following fixation with glutaraldehyde, the roots were rinsed in 0.1 M sodium cacodylate buffer, post-fixed with 1 % osmium tetroxide, subjected to a dehydration series (30 %, 50 %, 60 %, 70 % and 90 % ethanol, and 100 % acetone),

infiltrated with EPON resin, and allowed to polymerize for 48 h at 60 °C. Semi-thin (1 μm) and ultra-thin (approximately 60 to 90 nm) sections were then cut on an ultramicrotome (Leica, EM UC6 Ultramicrotome). The semi-thin sections were examined using light microscopy (Olympus BX61), and the ultra-thin sections using transmission electron microscopy (TEM) (JEOL 1010 at either 80 or 100 kV). Before examination by light microscopy, the semi-thin sections were stained with 1 % toluidine blue in 1 % sodium borate.

For the TEM, it was assumed that most of the Pb was retained within the plant tissues during the fixing and dehydration. Antosiewicz and Wierzbicka (1999) reported that in onion (*Allium cepa* L.), only 4 % of the total Pb was lost during a similar sample preparation. Although not confirmed by energy dispersive X-ray spectroscopy in the current study, all electron-dense (i.e. black or dark grey) deposits were assumed to contain Pb. This assumption was supported by previous work in this laboratory and by other authors demonstrating that, for plants grown in solutions containing Pb, electron-dense deposits in unstained sections represent Pb (or complexed forms of Pb) (Antosiewicz and Wierzbicka, 1999; Lane and Martin, 1982; Qureshi et al., 1986). Furthermore, no electron-dense material was observed in the sections taken from the control (0 μM Pb) plants in the present study.

Results

Plant growth in the control pots was good over the 13 d experimental period, with the fresh mass being approximately 1.25 g/plant for the shoots, and 0.75 g/plant for the roots. However, as the solution Pb increased, both shoot and root growth were observed to decrease (see below).

The solution Pb concentration, over the range studied (0.025 to 2.5 μM), decreased by no more than 29 % during the 13 d experimental period, with the average decrease in solution Pb being 11 %

(data not presented). These decreases in solution Pb concentration corresponded well with the amounts of Pb calculated to be removed by plant uptake, with the greatest proportional decrease in solution Pb being in the lower Pb treatments where plant growth was the highest and the quantity of Pb in solution was the lowest. This relatively stable Pb concentration data allows plant growth to be related to the mean solution Pb^{2+} activity. For the 11 Pb-containing solutions, the mean Pb^{2+} activities were 0.017, 0.031, 0.061, 0.092, 0.13, 0.19, 0.25, 0.33, 0.64, 0.95, 1.6 μM (calculated from the mean Pb concentrations at 0, 2, 6, and 13 DAP).

Increases in Pb^{2+} activity caused strong decreases in the fresh mass of the cowpea shoots and roots ($P < 0.001$) (Figure 1). However, the roots were much more sensitive to increasing Pb^{2+} than were the shoots; with independent equations required to adequately describe the two response curves (Figure 1). A Pb^{2+} activity of 0.06 μM was found to correspond to 90 % relative root mass, whilst an activity of 0.2 μM corresponded to 90 % relative shoot mass.

Nutrient solution EC remained relatively constant at approximately 0.25 dS/m over time (data not presented), being balanced by nutrient uptake, titrant addition (for pH control), and nutrient addition. Indeed, solution EC varied by no more than 6 % in any treatment during the experiment. Concentrations of all measured nutrients also remained approximately constant over the entire experimental period (data not presented).

As expected, root and shoot tissue Pb concentrations increased as the solution Pb^{2+} activity increased (Figure 2). However, root Pb concentrations were 10-50 times greater than the shoot concentrations (note difference in Y-axis scales), with the magnitude of the difference between the root and shoot Pb concentration increasing as the Pb^{2+} activity increased. The critical tissue

concentration (corresponding to 90 % relative shoot or root mass) was 330 µg/g for the roots and 49 µg/g for the shoots (Figure 2).

The nutrient content of the cowpea shoots was dependent upon the solution Pb^{2+} activity. At Pb^{2+} activities lower than that required to cause a 10 % reduction in shoot growth (< 0.2 µM), concentrations of nutrients in the shoots were relatively constant (Table 1) and with the exception of Mg, within ranges considered adequate for healthy growth. However, above this critical Pb^{2+} activity, an increase in Pb^{2+} tended to decrease the shoot tissue concentrations of all nutrient elements measured except P and S, with Mg, K, and Ca falling to levels below the corresponding critical values (Table 1). In the roots there was a marked increase in Fe concentration (> 3 fold) with increase in Pb^{2+} activity, and a much smaller increase in P concentration (Table 1). Root K and Zn concentrations were reduced by about 30% at the highest Pb^{2+} activity (Table 1).

Shoots in all Pb treatments were generally healthy in appearance with no obvious symptoms of Pb toxicity even at the highest Pb^{2+} levels, and no sign of the Mn toxicity symptoms which had occurred in a similar experiment conducted on Cu^{2+} toxicity in cowpea (Kopittke and Menzies, 2006). However close inspection of plants grown at > 0.64 µM Pb^{2+} showed the leaves to be marginally paler than the controls. This slight chlorosis may have been due to mild Pb-induced Mg deficiency, a diagnosis consistent with the tissue Mg data (Table 1). However, by contrast, roots grown in solutions containing Pb^{2+} activities ≥ 0.092 µM Pb^{2+} exhibited obvious toxicity symptoms, the severity of these increasing with increasing Pb^{2+} activity (Figure 3). Roots became short and stubby (Figure 3a) and lost apical dominance as demonstrated by an increased number of secondary roots initiated per unit root length (Figure 3e). Almost all roots at toxic Pb activities were swollen behind the root apex (Figure 3e), and some of the root tips were bent (Figure 3) although the bending was generally not as pronounced as that observed for Cu^{2+} toxicity (Kopittke and Menzies,

2006). Examination of longitudinal sections by light microscopy revealed that the swellings immediately behind the roots apices were due to the growth of lateral roots which had initiated but failed to break through the root rhizodermis (Figure 4). Older parts of root systems in the two highest Pb^{2+} treatments were discolored brown (Figure 3a), particularly at the highest Pb^{2+} activity.

Root hair growth was relatively poor in all treatments, including the controls. Several additional pots were included in the second replicate to explore possible reasons for this poor root hair growth. Solution pH (acidity) appeared to be the limiting factor, since an increase in solution pH from 4.50 to 4.75 substantially increased root hair growth. The relatively poor root hair growth observed at pH 4.50 was unexpected since both Kopittke and Menzies (2006) and Kopittke et al. (2007) found prolific root hair growth on cowpea in solutions at pH 4.50 using the same experimental system under similar growing conditions.

Examination of transverse sections 1 mm from the root tip using TEM revealed that most of the Pb had accumulated within the apoplast of the outer cellular layers of the root (i.e. in the rhizodermis and outer cortex) (Figure 5a). Some Pb was also observed to accumulate within the symplast, although these symplastic Pb deposits were typically confined to only a few cells in which comparatively large amounts of Pb had accumulated (Figure 5b). Some of this Pb appeared to be in the form of needle-like crystals, possibly those of Pb oxalate dihydrate (Grases et al., 1993). Such deposits have been observed in fungi exposed to Pb (Fomina et al., 2005) the crystals typically forming 'fans' of needles radiating out from the original Pb deposit (Sayer et al., 1999). In the present study, some TEM sections suggested that these crystalline deposits were located within membrane-bound vesicles. In the cortical apoplast, the Pb appeared to be widely dispersed and not obviously in crystalline forms (Figure 5a). Relatively small amounts of Pb were found in the apoplast of the stele.

At 15mm from the root apex, Pb was again found in the apoplast of the rhizodermis and outer cortex, much of it accumulating in large intercellular spaces (Figure 5c) as well as the cell walls. Only small amounts of Pb were observed in the symplast. Substantial numbers of Pb deposits were also observed in the apoplast of the stele (Figure 5d).

Discussion

Lead was found to be highly toxic to the growth of cowpea, with the roots more sensitive than the shoots; 10 % reductions in relative fresh mass occurring at a Pb^{2+} activity of 0.2 μM for the shoots but at only 0.06 μM for the roots (Figure 1) with visible toxicity symptoms on the roots at activities ≥ 0.09 μM. The absence of clear Pb toxicity symptoms in the shoots together with the much higher Pb concentrations in the roots (10 to 50 times higher) and severe root symptoms in that the higher Pb^{2+} activities suggests that the primary site of Pb^{2+} toxicity is in the root, with the reduced shoot growth a consequence of impaired root growth and function. This finding is in agreement with Godbold and Kettner (1991b) who also concluded that, for Norway spruce, the primary toxic effect of Pb was a reduction in root growth.

It has been reported that Pb reduces root growth by restricting cell division (Eun et al., 2000; Wierzbicka, 1989; Wozny and Jerczynska, 1991) and cell elongation (Malkowski et al., 2002; Obroucheva et al., 1998; Seregin and Ivanov, 1997). Reductions in root growth have been found within 7-12 h of exposure (Wierzbicka, 1994), and possibly result from the displacement of Ca from the cell wall by Pb (Godbold and Kettner, 1991a).

Other than a reduction in growth, the only symptom of Pb^{2+} toxicity in the shoots was a slight chlorosis of the younger leaves with ≥ 0.64 μM Pb^{2+} in solution, possibly caused by Pb-induced Mg

deficiency (see Table 1). However, this chlorosis was not severe and was only evident upon close inspection and comparison with the controls. These observations are similar to those reported by Wozny and Jerczynska (1991) for bean, who noted that whilst Pb reduced root growth, the shoots showed no obvious toxicity symptoms other than a reduction in surface area. Although the shoot Mg concentrations of the low-Pb plants were below that reported to result in deficiency (Table 1), no symptoms of Mg deficiency were observed and solution Mg concentrations were constant over the experimental period (data not presented).

The Pb^{2+} toxicity symptoms observed on roots in the present study were similar to those described for Cu^{2+} toxicity in cowpea by Kopittke and Menzies (2006). They reported that cowpea roots grown at high Cu^{2+} activities had swellings behind the tip and bent root tips. Indeed, the swellings behind the root tip of Pb^{2+}-treated roots (Figure 3) appear to be almost identical to those formed in Cu^{2+}-treated roots (Figure 4 of Kopittke and Menzies (2006)). Microscopic examination in the current study revealed that these localized swellings were due to the growth of lateral roots which had been initiated but had failed to emerge through the rhizodermis (Figure 4). Furthermore, it was noted that toxic levels of Pb and Cu in the root environment caused reductions in the shoot tissue concentrations of most nutrients other than P and S (see Table 1 of current study, and Table 2 of Kopittke and Menzies (2006)). Taken together, these observations may suggest similar mechanisms of toxicity for these two heavy metals. However, it is noteworthy that whilst toxicities of Cu (and Al) have been observed to cause cracking of the rhizodermis and outer cortex (Blamey et al., 2004; Kopittke and Menzies, 2006; Kopittke et al., 2004; Sheldon and Menzies, 2005), no such cracks were observed in the current study.

As noted earlier, Pb concentrations in cowpea roots were 10 to 50 times higher than in shoots in the present study. Research with other species has also revealed similarly large differences, for

example, 15 times higher in maize (Malkowski et al., 2002); 4 to 20 times higher in beans (Hardiman et al., 1984); 9 to 130 times higher in a range of species (Wierzbicka, 1999); and 100 to 300 times higher in Norway spruce (Godbold and Kettner, 1991a). The precise cause of the these large differences in Pb concentration between roots and shoots has not been established, but several workers have suggested that the accumulation of Pb in the roots occurs because the endodermis functions as a barrier to the radial transport of Pb in the root, thereby restricting its movement to the shoots (Hardiman et al., 1984; Jentschke et al., 1991; Seregin and Ivanov, 1997). However, whilst the endodermis does act as a barrier to the apoplastic transport of Pb (and other ions), the endodermis should be just as effective at excluding the apoplastic transport of Cd, Zn and other metals (which are known to enter the stele and accumulate within the shoot) as it is at excluding Pb. Therefore, it is considered that other mechanisms of Pb-exclusion are of more importance in preventing the movement of Pb to the shoots. For example, it is possible that Pb may be immobilized by the negatively charged pectins within the cell wall, precipitated as insoluble Pb-salts in the cell walls or intercellular spaces, or, having crossed the plasma membrane, be sequestered in the vacuoles of rhizodermal and cortical cells.

In cowpea, much of the Pb accumulated within the cell walls and intercellular spaces, with some Pb moving across the plasma membrane into the symplast (Figure 5). This observation is consistent with that reported for several other species including sweet vernal grass (*Anthoxanthum odoratum*) (Qureshi et al., 1986), maize (Tung and Temple, 1996), and onion (Antosiewicz and Wierzbicka, 1999). The distribution of Pb within the cell did not appear to change with increasing distance from the root apex, with apoplastic Pb dominating at both 1 and 15 mm from the apex. Much of the Pb was quite finely divided and not obvious in crystalline form. However within the symplast, clumps of needle-like crystals, possibly of Pb oxalate dihydate (see earlier discussion) were found in some

cells. Similar crystalline deposits have been observed in the roots of (Malone et al., 1974) and sweet vernal grass (Qureshi et al., 1986) which had been exposed to Pb.

Conclusions

Lead was toxic to the growth of cowpea, causing a 10 % reduction in relative shoot fresh mass at a Pb^{2+} activity of 0.2 μM and a 10 % reduction in root mass at an activity of 0.06 μM. The primary site of Pb^{2+} toxicity appeared to be in the root, where it caused a reduction in growth, loss of apical dominance, the formation of a localized swelling behind the root apex (caused by the initiation and arrested development of lateral roots), and sometimes the bending of root tips. Tissue concentrations of Pb were 10 to 50 times greater in the roots than in the shoots, with the critical Pb concentration (corresponding to 90 % relative root and shoot fresh mass) being 330 μg/g for the roots and 49 μg/g for the shoots. An increase in Pb^{2+} was observed to decrease shoot tissue concentrations of several elements and to greatly increase the concentration of Fe in the roots. The Pb was found to accumulate primarily in the cell walls and intercellular spaces of the outer cortex and rhizodermis, although some Pb moved across the plasma membrane into the symplast. It is possible that the immobilization of Pb in the cell wall may have occurred due to the presence of negatively charged pectins, or due to precipitation as insoluble Pb-salts in the cell walls or intercellular spaces.

Acknowledgments

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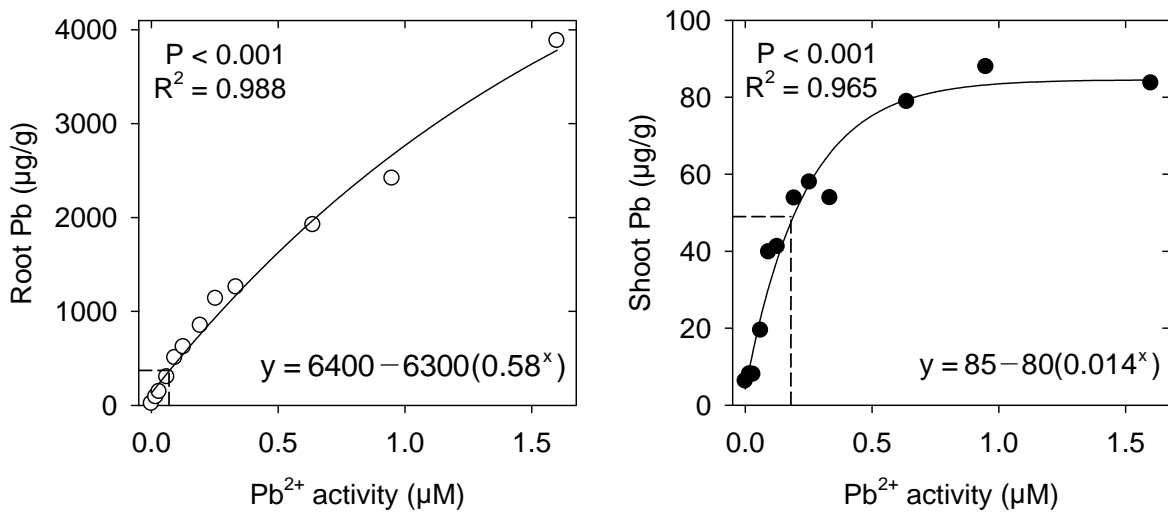
Table 1. The effects of Pb^{2+} activity on the nutrient concentrations of cowpea roots and shoots after 13 d of growth in dilute nutrient solutions. Only selected treatments are presented: (1) the treatment with the lowest Pb^{2+} activity (other than the control) (0.017 μM), (2) the treatment with a Pb^{2+} activity closest to (but less than) the critical solution Pb^{2+} activity for a 10 % reduction in either the growth of roots and shoots, and (3) the 0.33 μM Pb^{2+} treatment.

|	Solution Pb^{2+} activity	K	Ca	S	P	Mg	Pb	Cu	Fe	Zn	Mn
	(μM)	----------------------- mg/g -----------------------					----------------------- μg/g -----------------------				
Roots	0.017	57	2.2	7.8	3.3	1.6	90	14	300	310	14
	0.061	45	2.4	7.4	3.2	1.6	300	18	370	390	15
	0.33	45	2.5	7.8	3.6	2.5	1300	16	630	410	18
Shoots	0.017	38	19	5.1	3.7	2.1	8.1	7.9	120	110	94
	0.13	34	17	4.6	3.2	2.0	41	7.6	100	91	120
	0.33	26	13	4.4	3.1	1.5	54	5.4	91	60	74
Shoots	Critical concentration[†]	25-35	15-20	2.5-3.5	1.5-3.0	3.0-3.5			70-75	20-35	< 70

[†]Approximate critical concentration for deficiency of various nutrients for shoots of cowpea (Reuter and Edwards, 1997; Smith et al., 1984)
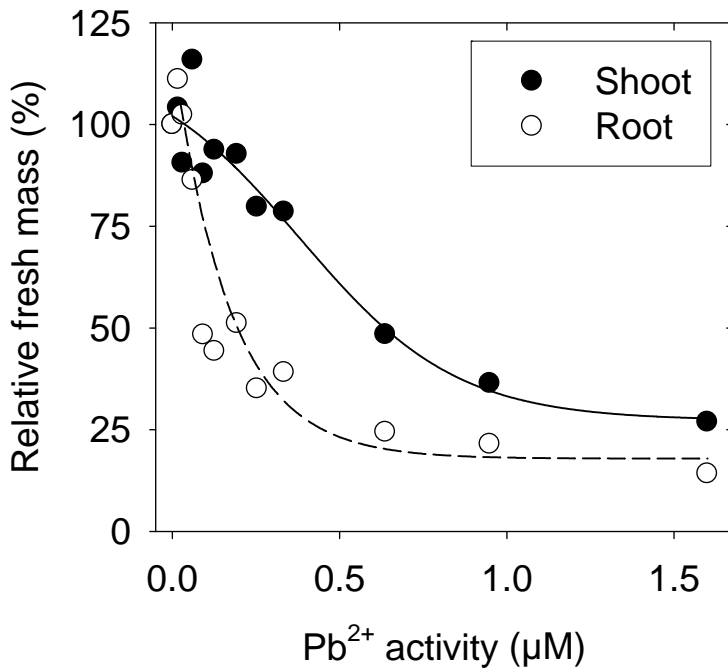
Figure 1. The relative fresh masses of the shoots and roots of cowpea grown for 13 d in nutrient solutions containing various Pb^{2+} activities. Curves were fitted using a grouped regression (logistic curves) ($P < 0.001$, $R^2 = 0.932$) for the shoots: $y = 27+90/(1+\exp(4.3(x-0.38)))$, and for the roots: $y = 18+6500/(1+\exp(5.9(x+0.70)))$.

Figure 2. Effects of increasing Pb^{2+} activity on the Pb concentration in the roots (left) and shoots (right) of cowpea grown for 13 d in dilute nutrient solutions. The data plotted are the arithmetic means of two replicates. The dashed lines correspond to the Pb^{2+} activity found to cause a 10 % reduction in the fresh mass of the roots (0.06 μM Pb^{2+}) and shoots (0.2 μM Pb^{2+}).
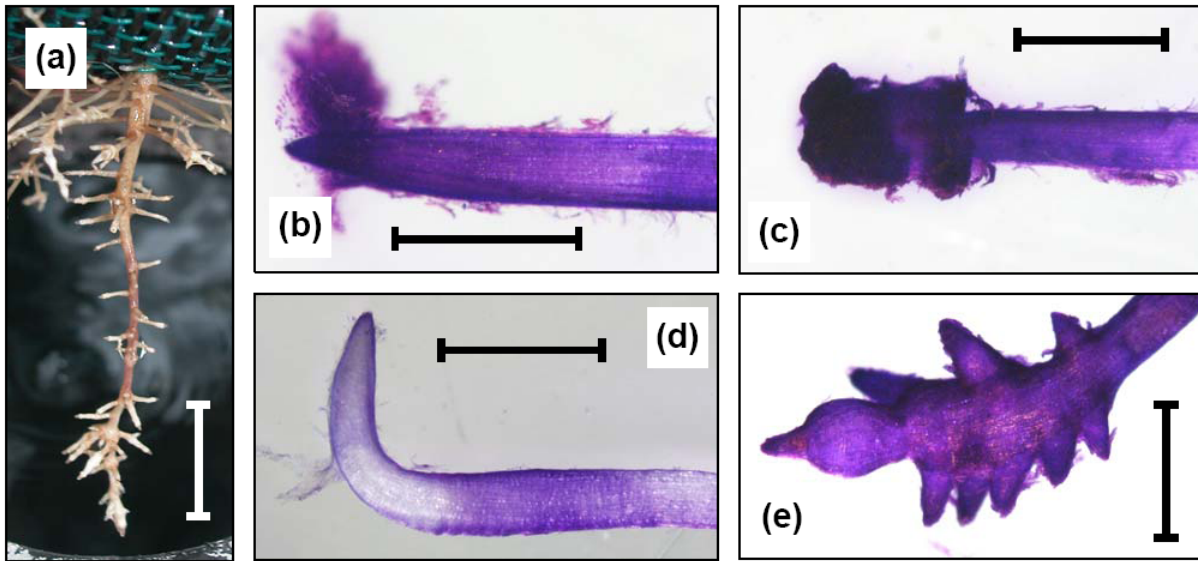
Figure 3. Roots of cowpea stained with 0.5 % crystal violet after growth for 13 d at various Pb^{2+} activities. (a) Photograph of root system at 0.95 µM Pb^{2+}, and optical micrographs of (b) primary root tip in the control (0 µM), (c) primary root tip at 0.092 µM Pb^{2+} (root apex obscured by mucigel and sloughed-off root cap cells), (d) a lateral root at 0.33 µM Pb^{2+} showing bending of the root tip, and (e) a lateral root tip at 0.95 µM Pb^{2+} showing loss of apical dominance and swelling behind the root apex. (Bar is approximately equal to 10 mm in (a), and 2 mm in (b) to (e)).
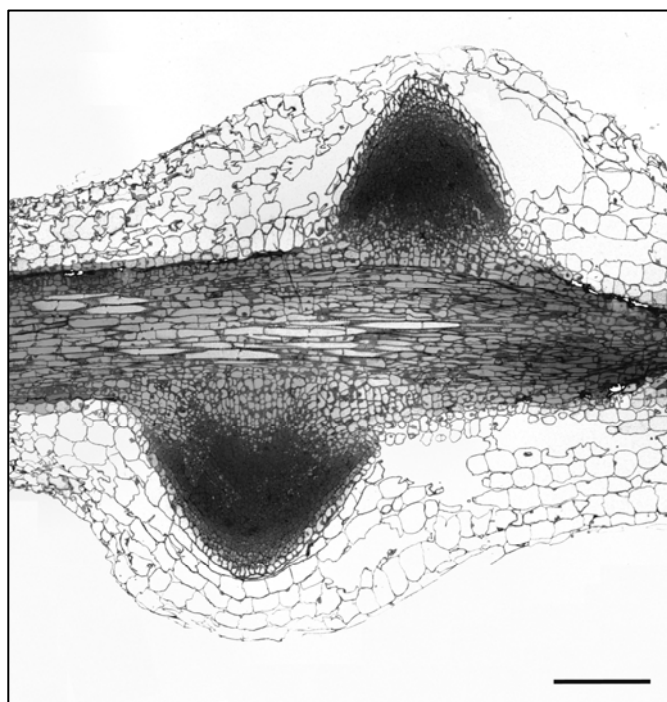
Figure 4. A longitudinal section (1 μm thick, examined using light microscopy) of the swelling behind the apex of a primary root (see Figure 3e) of cowpea grown for 13 d at 1.6 μM Pb^{2+}, and stained using 1 % toluidine blue in 1 % sodium borate (bar is equal to 0.2 mm).
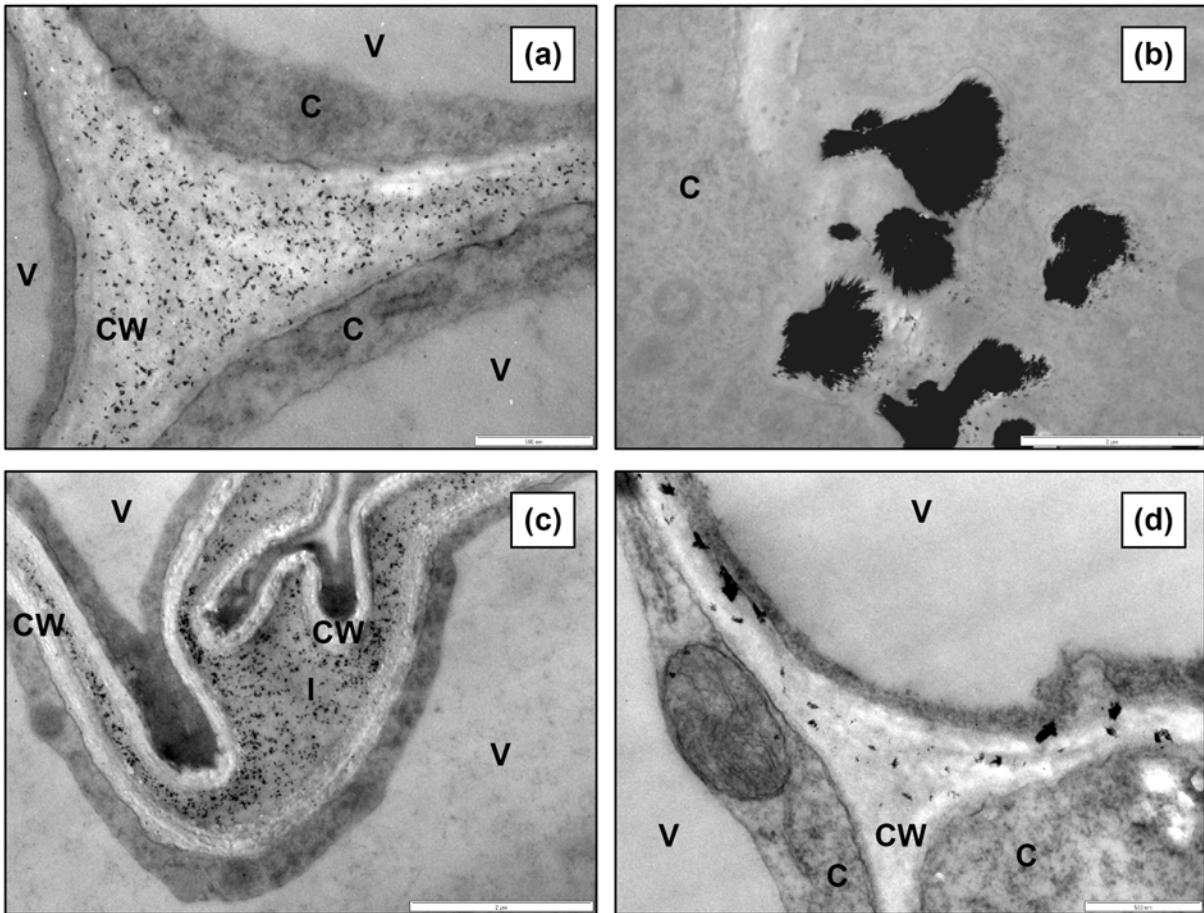
Figure 5. Transmission electron micrographs of roots of cowpea grown for 13 d at 1.6 μM Pb^{2+}; (a) Pb (black specks) in the apoplast of the outer cortex (1 mm from root apex, bar = 0.5 μm), (b) masses of needle-like Pb-containing crystals in the symplast of the inner cortex (1 mm from root apex, bar = 2 μm), (c) Pb in large intercellular spaces of the outer cortex (15 mm from root apex, bar = 2 μm), and (d) Pb in the apoplast of the stele (15 mm from root apex, bar = 0.5 μm). C = cytoplasm, CW = cell wall, I = intercellular space, V = vacuole.