

Cadmium induces hypodermal periderm formation in the roots of the monocotyledonous medicinal plant *Merwillia plumbea*

Alexander Lux^{1,2}, Marek Vaculík¹, Michal Martinka¹, Desana Lišková², Manoj G. Kulkarni³,
Wendy A. Stirks³ and Johannes Van Staden^{3,*}

¹Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina B-2, SK-842 15 Bratislava, Slovak Republic, ²Department of Glycobiotechnology, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava, Slovak Republic and ³Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

* For correspondence. E-mail rcpgd@ukzn.ac.za

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• **Background and Aims** *Merwillia plumbea* is an important African medicinal plant. As the plants grow in soils contaminated with metals from mining activities, the danger of human intoxication exists. An experiment with plants exposed to cadmium (Cd) was performed to investigate the response of *M. plumbea* to this heavy metal, its uptake and translocation to plant organs and reaction of root tissues.

• **Methods** Plants grown from seeds were cultivated in controlled conditions. Hydroponic cultivation is not suitable for this species as roots do not tolerate aquatic conditions, and additional stress by Cd treatment results in total root growth inhibition and death. After cultivation in perlite the plants exposed to 1 and 5 mg Cd L⁻¹ in half-strength Hoagland's solution were compared with control plants. Growth parameters were evaluated, Cd content was determined by inductively coupled plasma mass spectroscopy (ICP-MS) and root structure was investigated using various staining procedures, including the fluorescent stain Fluorol yellow 088 to detect suberin deposition in cell walls.

• **Key Results** The plants exposed to Cd were significantly reduced in growth. Most of the Cd taken up by plants after 4 weeks cultivation was retained in roots, and only a small amount was translocated to bulbs and leaves. In reaction to higher Cd concentrations, roots developed a hypodermal periderm close to the root tip. Cells produced by cork cambium impregnate their cell walls by suberin.

• **Conclusions** It is suggested that the hypodermal periderm is developed in young root parts in reaction to Cd toxicity to protect the root from radial uptake of Cd ions. Secondary meristems are usually not present in monocotyledonous species. Another interpretation explaining formation of protective suberized layers as a result of periclinal divisions of the hypodermis is discussed. This process may represent an as yet unknown defence reaction of roots when exposed to elemental stress.

Key words: Cadmium, environmental stress, medicinal plants, *Merwillia plumbea*, monocotyledonous plants, periderm, plant anatomy, root structure.

INTRODUCTION

South Africa has a large concentration of metal, mineral and coal-mining industries with high rates of waste disposal (Cooke and Johnson, 2002). The gold and coal-mining industries are responsible for lowering pH levels of South African rivers by the phenomenon known as 'acid mine drainage' (Coetzee, 1995). The waste from mining industries contains heavy metals such as lead, cadmium (Cd), mercury, aluminium, zinc, copper, magnesium and manganese (Department of Water Affairs, 1986; Wren and Stephenson, 1991). Compared with other metals or metalloids, Cd has a higher tendency to accumulate in plant tissues (Kabata-Pendias and Pendias, 1984) and is considered as highly mutagenic and carcinogenic by the International Agency for Research on Cancer (IARC, 1993; Filipic and Hei, 2004). Consumption of edible plant material with high Cd content may cause toxicity in humans (Jackson and Alloway, 1992; FAO/WHO, 1995). Due to its high toxicity,

the maximum permissible limit of Cd in medicinal plants set by the World Health Organization (WHO) is 0.3 p.p.m.

In a recent investigation by Street *et al.* (2008), Cd exceeded the limits recommended by the WHO (1998) in bulb and tuber samples of South African medicinal plants obtained from street markets. In another study, Cd accumulation in bulbs of small and medium-sized plants of *Tulbaghia* species used medicinally increased with increasing Cd concentration (Street *et al.*, 2010). Bulbs of mature *Merwillia plumbea* plants accumulated 24-fold more Cd than the WHO guidelines when irrigated with 2 mg Cd L⁻¹. At the same time, the bulb extracts showed increased antibacterial activity (Street *et al.*, 2009). Cadmium had a detrimental effect on the root growth of *Bowiea volubilis*, which is another important bulbous medicinal plant in South Africa (Street *et al.*, 2007). In most of these studies, it was indicated that bulbs of medicinal plants have a tendency to accumulate Cd. However, the uptake mechanism of Cd is still unclear.

As roots of seedlings represent the primary contact zone of plants in soils, anatomical alterations occurring during Cd uptake may have an effect on accumulation processes and vegetative growth of the plants when they are exposed to this metal. The root reaction of various plant species was recently reviewed by Lux *et al.* (2011). Inhibition of root growth and branching as a result of the inhibitory effect of Cd on cell division was observed by Fusconi *et al.* (2006) and Ma *et al.* (2010). Species-specific reactions and changes in root tissue organization and development occur after Cd treatment (Lunáčková *et al.*, 2003). Changes in the development of root apoplasmic barriers were shown to be related to Cd uptake and translocation (Lux *et al.*, 2004; Martinka and Lux, 2004; Vaculík *et al.*, 2009). Considering these facts, elevated concentrations of Cd in the rhizosphere may influence root growth and development of *M. plumbea* plants. In the present contribution, two types of cultivation of an extensively used medicinal plant in South Africa, *M. plumbea*, were tested for the evaluation of uptake and translocation of Cd. An anatomical study was performed to investigate the possible effect of Cd treatment on root structure.

MATERIALS AND METHODS

Growth conditions and experimental design

Seeds of *Merwillia plumbea* (Lindl.) Speta, obtained from the Botanical Garden of the University of KwaZulu-Natal in Pietermaritzburg, South Africa, were germinated on wet filter paper in a Petri dish for 10 d. Thereafter, the young seedlings were transferred to perlite and irrigated with tap water for 4 weeks and then by half-strength Hoagland's solution for 8 weeks in the greenhouse at Comenius University in Bratislava, Slovakia. Two different types of cultivation were applied: (1) cultivation of experimental plants directly in hydroponic solution (hydroponics) and (2) cultivation of plants in perlite, which was irrigated with the same solutions as used for hydroponics. For hydroponics, the first set of prepared young plants with already developed bulbs was transferred to 5 L containers filled with half-strength Hoagland's solution. Another set of plants for perlite was transferred to 1 L pots filled with perlite and placed in a growth chamber. The plants in both experiments were grown for 4 weeks at 25 °C, 60 % humidity, 16/8 h light/dark photoperiod, PAR 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

For both experiments, three different treatments were used: (1) control – irrigated with half-strength Hoagland's solution; (2) Cd 1 – irrigated with half-strength Hoagland's solution containing 1 mg Cd L⁻¹; and (3) Cd 5 – irrigated with half-strength Hoagland's solution containing 5 mg Cd L⁻¹.

Cadmium was applied in the form of Cd(NO₃)₂·4H₂O. The hydroponic solution was changed weekly. The pots containing perlite were irrigated with 200 mL of solution on the first day at the start of the experiment and afterwards with an additional 100 mL of solution every seventh day for 4 weeks.

Growth parameter determination

The plants were harvested and fresh weights of roots, bulbs and leaves were determined. The total root length was recorded

and expressed as 'cumulative root length per plant (CRL)'. The length of individual roots was calculated from the CRL divided by the number of roots on each plant. The same measurements and calculations were done with the leaves. Fresh roots, bulbs and leaves were dried at 70 °C for 3 d and after that the dry weight of these organs was determined.

Determination of Cd concentration

Dried samples of roots, bulbs and leaves were analysed for Cd by inductively coupled plasma mass spectroscopy (ICP-MS). The samples were dissolved in HNO₃ and the Cd concentration was determined by an Elan 6000 spectrometer (Pe Sciex, Canada).

Anatomical observations

Series of hand sections of roots were prepared at 1 mm intervals from the root apex to the base. For suberin visualization in fluorescence microscopy, the free hand sections were stained by 0.01 % Fluorol yellow 088 dissolved in lactic acid for 30 min and washed in distilled water according to Lux *et al.* (2005). The samples were placed into a drop of 0.1 % FeCl₃ dissolved in 50 % glycerin prior to observation. The sections for bright-field observations were stained with toluidine blue.

The sections were observed under an Zeiss Axioskop 2 plus epifluorescence microscope (Jena, Germany) and documented by an Olympus DP 72 digital camera.

Statistical analyses

The experimental data shown in the figures are means of 12 replicates \pm s.e. from independent experiments repeated three times. The significance was compared at the 0.05 level using Student's *t*-test (Statgraphics Centurion XV, v. 15.2.05, StatPoint, Inc.).

RESULTS AND DISCUSSION

Growth of the plants

The growth of *M. plumbea* plants in hydroponics was poor, and only in control conditions did some new adventitious roots form. These exhibited limited growth. The roots of plants treated with Cd were stunted and no new root formation occurred. The majority of roots in both Cd treatments gradually died (Fig. 1). The growth parameters of plants from this experiment are shown in Table 1. It was clear that cultivation in hydroponics is not feasible for this species.

The growth of plants cultivated in perlite under control conditions was adequate and plants exhibited increases in both root and shoot growth (Figs 2 and 3). Use of solid substrate is necessary for this species. Street *et al.* (2009) used sterilized acid-washed quartz sand wetted with Hoagland's solution for cultivation of this species. In our experiments the growth of *M. plumbea* plants was retarded after Cd application; the root system was reduced in comparison with the control and leaves were shorter and narrower, and yellowish compared with those of control plants (Fig. 1). Shorter leaves of *M. plumbea* were observed after CdCl₂ treatment, but

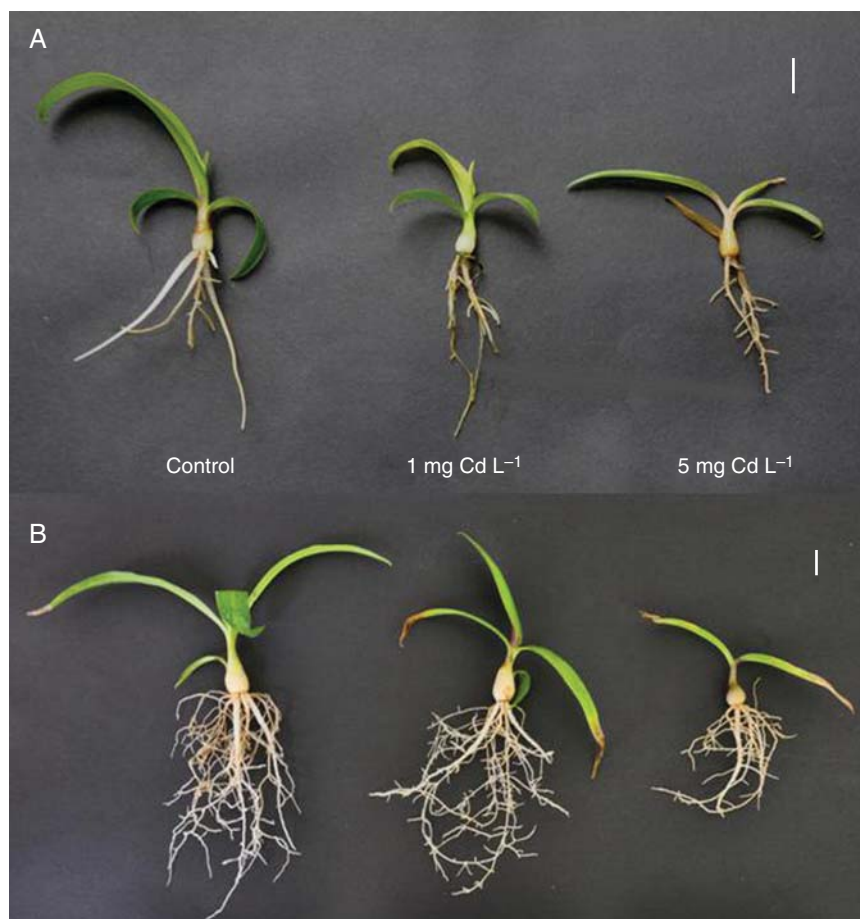


FIG. 1. Effect of cadmium on 17-week-old *Merwillia plumbea* plants grown for 4 weeks in (A) hydroponics and (B) perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Scale bars = 1 cm.

TABLE 1. Growth parameters of *Merwillia plumbea* plants grown for 4 weeks in hydroponics

Growth parameter	Treatment		
	Control	Cd1	Cd5
Root f. wt (g)	0.0904 ± 0.017 ^a	0.0534 ± 0.016 ^b	0.0483 ± 0.019 ^b
Root d. wt (g)	0.0051 ± 0.0009 ^a	0.0033 ± 0.0012 ^b	0.0026 ± 0.0008 ^b
Leaf f. wt (g)	0.49 ± 0.083 ^a	0.131 ± 0.032 ^b	0.1 ± 0.018 ^b
Leaf d. wt (g)	0.032 ± 0.0045 ^a	0.0127 ± 0.0009 ^b	0.0123 ± 0.0026 ^b
Bulb f. wt (g)	0.134 ± 0.048 ^a	0.095 ± 0.029 ^a	0.011 ± 0.054 ^a
Bulb d. wt (g)	0.013 ± 0.0075 ^a	0.017 ± 0.0057 ^a	0.013 ± 0.0024 ^a
CRL (cm)	18.22 ± 3.45 ^a	10.62 ± 2.21 ^b	9.38 ± 0.81 ^b
ARL (cm)	4.742 ± 1.46 ^a	2.948 ± 0.46 ^b	2.818 ± 0.48 ^b

Three different treatments were used: control, half-strength Hoagland's solution; Cd1, half-strength Hoagland's solution containing 1 mg kg⁻¹ Cd; and Cd5, half-strength Hoagland's solution containing 5 mg kg⁻¹ Cd.

ARL, average root length; CRL, cumulative root length (means ± s.e., different letters represent significant differences at $P < 0.05$).

without statistically significant differences between control and treated plants (Street *et al.*, 2009). However, Cd at 2 mg L⁻¹ resulted in a significant reduction of fresh weight of leaves, bulbs and roots, when compared with the control (Street *et al.*, 2009). Similarly in our experiment in the plants treated with Cd(NO₃)₂·4H₂O, the fresh and dry weight of roots were significantly lower, >50 %, after cultivation in

hydroponics with 1 mg Cd L⁻¹ when compared with the control plants. This difference was even higher when the higher Cd concentration (5 mg Cd L⁻¹) was used. However, the difference between 1 and 5 mg Cd L⁻¹ was not significant. The same trend was observed for the leaves. The main differences were observed with bulbs; the fresh and dry weights of bulbs of plants treated with 1 mg Cd L⁻¹ were not different

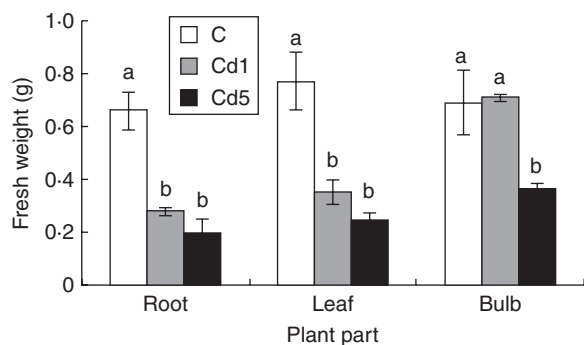


FIG. 2. Effect of cadmium on fresh weight of 17-week-old *Merwillia plumbea* plants grown for 4 weeks in perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Means (\pm s.e.) with different letters are significantly different ($P < 0.05$).

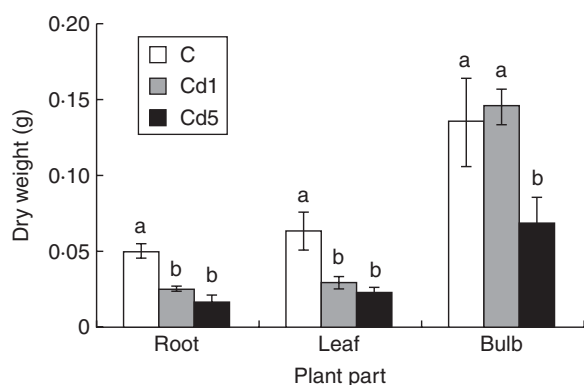


FIG. 3. Effect of cadmium on dry weight of 17-week-old *Merwillia plumbea* plants grown for 4 weeks in perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Means (\pm s.e.) with different letters are significantly different ($P < 0.05$).

from those of the controls. After higher Cd application (5 mg Cd L⁻¹), the fresh and dry weights of bulbs were lower, about 50%, when compared with the control and 1 mg Cd L⁻¹-treated plants (Figs 2 and 3).

The number of adventitious roots was lower in plants cultivated with Cd; the mean numbers for control, 1 mg Cd L⁻¹ and 5 mg Cd L⁻¹ were 6.5, 6.0 and 4.9, respectively. CRL as well as the average length of roots (ARL) was not different between control and 1 mg Cd L⁻¹-treated plants. However, both CRL and ARL were significantly different between the control and 5 mg Cd L⁻¹ treatments and also between both Cd treatments (Figs 4 and 5). The inhibition of root growth was probably caused by Cd-induced depolymerization of microtubules of the cell cytoskeleton and chromosome aberrations, which resulted in lower mitotic activity of meristematic cells (Fusconi *et al.*, 2006; Seth *et al.*, 2008), and thus inhibition of root elongation. Cumulative leaf length per plant and also the average length of leaves were significantly different between the control and both treatments. The growth of roots of carrot and radish seedlings was inhibited at 20 mg Cd L⁻¹ and this inhibitory effect increased with increasing concentrations of Cd in both liquid culture and pot experiments (Chen *et al.*, 2003). In the present study, the inhibitory effect was more pronounced at 5 mg Cd L⁻¹, suggesting that

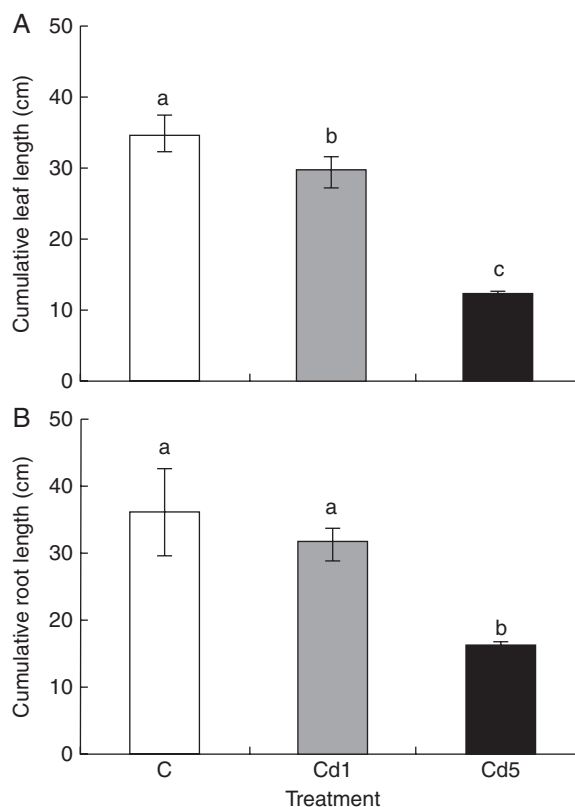


FIG. 4. Effect of cadmium on cumulative leaf and root length of 17-week-old *Merwillia plumbea* plant grown for 4 weeks in perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Means (\pm s.e.) with different letters are significantly different ($P < 0.05$).

M. plumbea seedlings are more sensitive to Cd than the seedlings of carrot and radish. Recently, Street *et al.* (2009) showed that 2 mg Cd L⁻¹ negatively affected the fresh weight of leaves, bulbs and roots of mature plants of *M. plumbea* exhibiting a high accumulation of Cd in the roots. This suggests that inhibition of root growth at high Cd concentration may affect nutrient and water uptake, resulting in inhibition of shoot elongation. However, there is not enough information on the mechanism of Cd toxicity in the root system, which is the main organ for uptake in plants (Chen *et al.*, 2003).

Cadmium concentration

Large differences were found in accumulation of Cd by different plant organs of *M. plumbea* (Fig. 6). Roots accumulated considerably higher amounts of Cd when compared with the bulbs and leaves. This phenomenon is well known for many plant species. It is assumed that this is one of the main mechanisms to protect the above-ground parts of plants against toxic Cd effects (Lux *et al.*, 2011). The concentration of Cd significantly increased in roots treated with 1 mg Cd L⁻¹ and even more Cd was found in roots when grown at higher Cd stress (5 mg Cd L⁻¹). The same tendency was observed in bulbous species cultivated at 50 and 250 μ M Cd (Soudek *et al.*, 2009). Similarly to the roots, the Cd concentrations in the other plant organs were elevated correspondingly with increasing concentration of Cd in the media. However, the

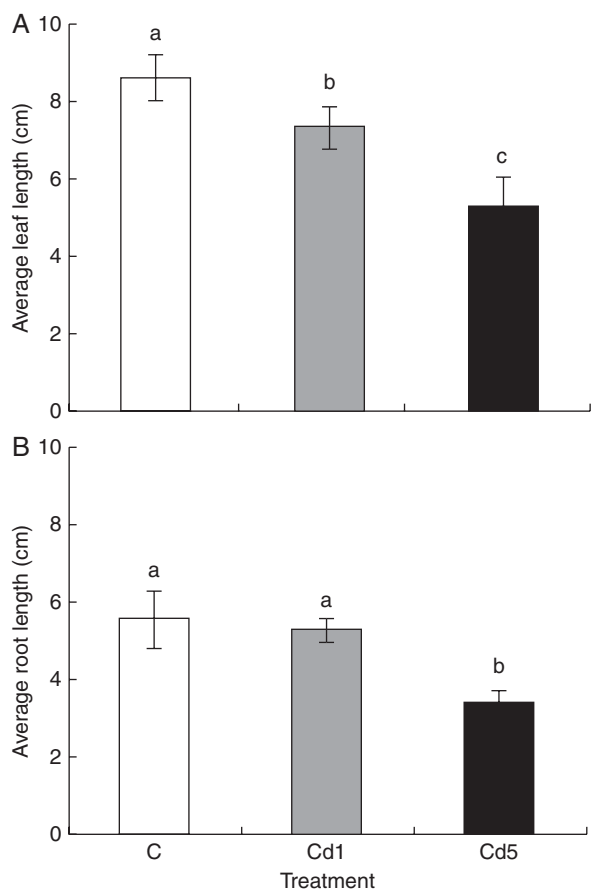


FIG. 5. Effect of cadmium on average leaf and root length of 17-week-old *Merwillia plumbea* plants grown for 4 weeks in perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Means (\pm s.e.) with different letters are significantly different ($P < 0.05$).

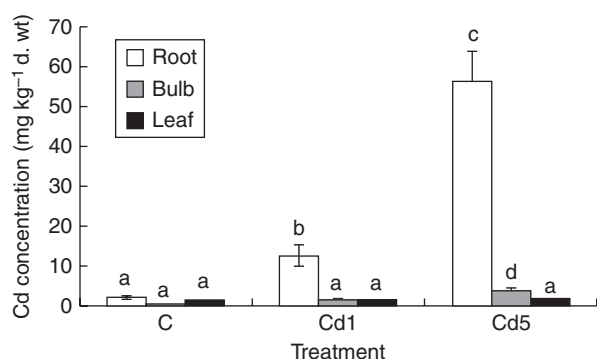


FIG. 6. Effect of cadmium application on cadmium concentration in roots, bulbs and leaves of 17-week-old *Merwillia plumbea* plants grown for 4 weeks in perlite. Three different treatments were used: control (C); 1 mg Cd L⁻¹ (Cd1); and 5 mg Cd L⁻¹ (Cd5). Means (\pm s.e.) with different letters are significantly different ($P < 0.05$).

concentration of Cd was more than eight times higher in the roots than in the leaves and bulbs when plants were grown in 1 mg Cd L⁻¹. The differences between plant parts were considerably bigger when the higher Cd concentration (5 mg Cd L⁻¹) was used. Comparable results were obtained with

CdCl₂ treatment of the same species by Street *et al.* (2009). The concentration of Cd in the roots was approx. 15 times higher compared with the bulbs and about 30 times higher when compared with the leaves. The concentration of Cd was the same in the leaves and the bulbs when a lower Cd concentration (1 mg Cd L⁻¹) was used, and about twice as high in the bulbs than in the leaves at higher Cd treatment (5 mg Cd L⁻¹) (Fig. 6). Soudek *et al.* (2009) have observed that experimentally tested bulbous plants retained on average 75 % of accumulated Cd in the roots, and only 18 % and 7 % in the bulbs and leaves, respectively. Despite the translocation factor being quite low in *M. plumbea*, the Cd accumulated in the bulbs exceeded, at all experimental variants, the limit for maximum Cd content (0.3 p.p.m.) set by the WHO for medicinal plants. Another medicinal plant species, *Tulbaghia violacea*, accumulated Cd in its bulbs in the range of 3.4–8.7 $\mu\text{g g}^{-1}$ d. wt depending on the size of test plants after 6 weeks treatment with 2 mg L⁻¹ Cd. *Tulbaghia violacea* accumulated Cd in the leaves at the level of 2.1–1.2 and 5.5–2.9 $\mu\text{g g}^{-1}$ at 2 and 5 mg L⁻¹, respectively, depending on plant age – the older the plant, the less accumulation (Street *et al.*, 2010). Several plant species, such as *Thlaspi caerulescens*, *Arabidopsis halleri* and *Sedum alfredii*, can accumulate Cd in concentrations exceeding even the level set for hyperaccumulators (which is 100 $\mu\text{g Cd g}^{-1}$ d. wt) (Salt *et al.*, 1995; Zhao *et al.*, 2006; Deng *et al.*, 2007; Banasova *et al.*, 2008). Usually important crop plants, such as maize, rice, wheat and sunflower, do not reach this hyperaccumulation limit when treated with Cd concentrations $< 10 \text{ mg kg}^{-1}$ of soil (Greger and Landberg, 2008; Faessler *et al.*, 2010; Liu *et al.*, 2010). Monocotyledonous bulbous plant species, such as *Allium cepa*, *Allium sativum*, *Allium schoenoprasum* and *Allium porrum*, behave similarly, but in some cases they can exceed the hyperaccumulation limits when grown under specific conditions, mostly in hydroponics and at high Cd concentrations in the media (Soudek *et al.*, 2009). *Allium schoenoprasum* was able to accumulate this metal to >1700 and 200 $\mu\text{g g}^{-1}$ in roots and leaves, respectively, during 28 d of 50 μM Cd treatment (Barazani *et al.*, 2004).

Root anatomy

The adventitious roots of *M. plumbea* seedlings show the typical structure of a monocotyledonous root (Fig. 7A). One layer of a rhizodermis is formed by small cells as the outermost tissue. The cortex is relatively broad, with a one-layered hypodermis. Hypodermal cells are radially extended and develop Casparian bands and suberin lamellae 10–15 mm from the root apex (Fig. 7B, C); this layer can be classified as exodermis (Peterson and Perumala, 1990). Cortical parenchyma is multilayered and cells are alternatively arranged with small intercellular spaces. The single-layered endodermis develops Casparian bands close to the root apex, at a distance of approx. 3 mm. A secondary state of endodermal development (suberin lamellae development) occurs approx. 30 mm from the root apex. The central cylinder is narrow; it consists of a one-layered pericycle, 6–12 radially arranged poles of xylem and phloem elements, and a central parenchymatous region.

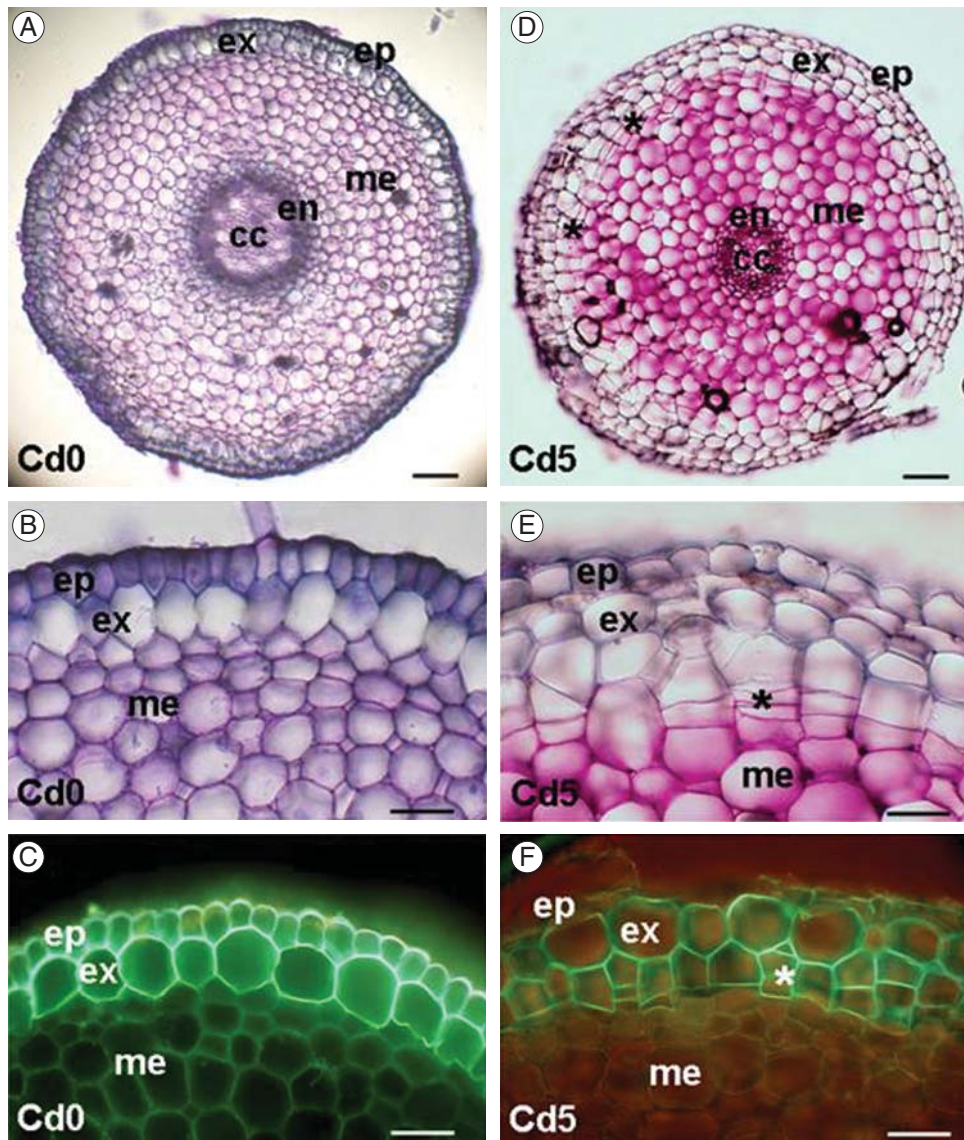


FIG. 7. Cross-sections of adventitious roots of *Merwillia plumbea*. Plants were grown in control conditions in perlite (A–C) and treated with 5 mg kg^{-1} Cd (D–F). Roots in control conditions exhibit the typical structure of monocotyledonous roots formed by single-layered epidermis and single-layered exodermis (A, B); exodermal cells develop suberin lamellae close to the root apex, as shown in C after Fluorol yellow 088 staining in fluorescence microscopy. After cadmium treatment the hypodermal periderm is formed in the peripheral cortical zone close to the root apex (D–F). Cork cambium produces cells by periclinal division (E); these become impregnated by suberin, as shown in F after Fluorol yellow 088 staining in fluorescence microscopy. The distances of sections from the root apex are 5 mm (A, B, D, E) and 30 mm (C, F). Abbreviations: ep, epidermis; ex, exodermis; me, mesodermis (mid-cortical layers); en, endodermis; cc, central cylinder; asterisks indicate periclinal divisions in cork cambium. Scale bars: (A, D) = $100 \mu\text{m}$. (B, C, E, F) = $50 \mu\text{m}$.

The oldest part of *M. plumbea* root, close to the bulb, is characterized by the presence of a contractile zone. Contractile roots pull bulbs down into the soil. The association between contractile roots, water uptake and habitat aridity was investigated for agaves, yuccas and aloes by North *et al.* (2008).

Cadmium application in our experiments at a higher concentration (5 mg Cd L^{-1}) caused changes in the root tissue organization and development. Exposure to Cd induced the formation of cork cambium in cortical tissues internally, adjacent to the exodermis (Fig. 7D). Cells divided periclinally and the derivatives impregnated their cell walls with suberin

(Fig. 7E, F). Hypodermal periderm formation occurred in the young, sub-apical part of the root. The first periclinal divisions were present approx. 5 mm from the root apex.

The root of most monocotyledons is covered by the original primary epidermis during their whole life; under some conditions it becomes lignified or even suberized. These cell wall modifications may reduce the growth of plant organs and can affect the transport of nutrients and water. Only a few studies of periderm formation in monocotyledons have been completed, mostly focused on arborescent monocotyledons (Dickinson, 2000). In bulbous plants, wound periderm formation was observed in roots after infection caused by

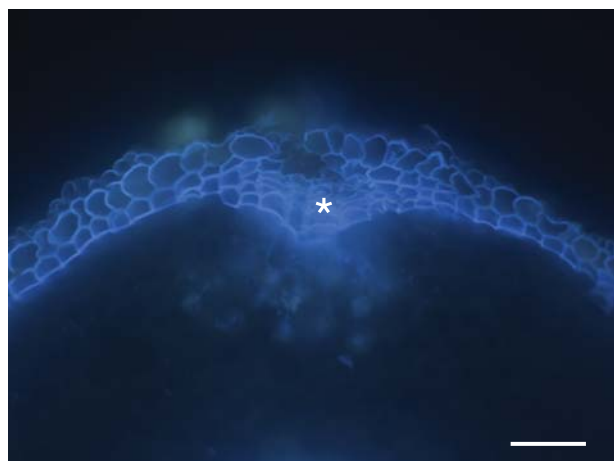


FIG. 8. Cross-section of adventitious roots of an adult *Merwillia* plant growing in soil (Botanical Garden of University of KwaZulu-Natal, Pietermaritzburg, South Africa) stained with Fluorol yellow 088 in UV light. The formation of multiseriate cell layers with suberized cell walls (*) resembling typical periderm occurs after injury of the root surface. Scale bar = 100 μm .

various species of *Penicillium* (Saaltink, 1971). Exceptional periderm is sometimes present below the exodermis in some species of Asparagales (Kauff *et al.*, 2000). We suggest that formation of hypodermal periderm in young sub-apical parts of *Merwillia* roots represents a defence reaction of roots exposed to toxic metal treatment. Periderm, with impermeable cell walls, impregnated by suberin may reduce radial uptake of Cd ions by roots.

The formation of protective sub-epidermal layers in response to Cd treatment is interpreted here as hypodermal periderm or wound periderm formation. It can probably also be interpreted as multiseriate hypodermis. This interpretation is based on multiseriate hypodermis shown in roots of some monocotyledonous species by Seago and Marsch (1989), Seago *et al.* (2000) and Heimsch and Seago (2008), and in *Iris* by Meyer *et al.* (2009). These studies showed developmental schemes of multiseriate exodermis and hypodermis which can be formed from the root apical meristem (RAM), in some cases many millimetres behind the tip. Exodermis absent in control conditions and induced by salt stress was found in roots of dicotyledonous plant species (cotton) by Reinhardt and Rost (1995). These data suggest the possibility of formation of sub-epidermal layers observed by us as a result of meristematic activity derived from the RAM. We can support our opinion about the formation of secondary meristem in sub-epidermal root layers of *Merwillia* in response to stress by additional observations. Adventitious roots of adult plants growing in soil (Botanical Garden of University of KwaZulu-Natal, Pietermaritzburg, South Africa) were investigated. In wounded or damaged areas of older root parts, formation of multiseriate cell layers with suberized cell walls resembling typical periderm (wound periderm) of dicotyledonous roots was observed (Fig. 8). Depending on interpretation, we can call these structures in monocotyledonous plant species hypodermal periderm, or regard their formation as a result of additional divisions of cortical cells. Otherwise we can accept the possibility of cork cambium formation resulting in periderm layers in monocotyledons.

Conclusions

Accumulation of toxic amounts of heavy metals in plants represents a health threat for humans. Medicinal plants used in traditional medicine are little studied with respect to Cd intake. The capacity of *M. plumbea*, an intensively traded medicinal plant of South Africa, was studied here. The data show that this plant can accumulate considerable amounts of Cd. Plants limit shoot Cd accumulation by restricting Cd movement to the xylem through both the symplasmic and the apoplasmic pathways. Restriction of apoplasmic movement of Cd was found in various plant species by root cell wall modifications and impregnations, accelerated maturation of apoplasmic barriers – exodermis and endodermis – and lignification of peripheral root tissues. In roots of *M. plumbea*, early formation of hypodermal periderm close to the root apex occurs after Cd treatment. Periderm is only rarely developed in roots of monocotyledonous plants; therefore, another interpretation may be additional periclinal division of hypodermal layers. We hypothesize that this process and production of secondary tissues impregnated by suberin in *M. plumbea* treated by Cd have functional significance in reducing the radial transport of toxic Cd ions to the xylem and subsequently to the shoot. This may be a novel defence reaction of plants exposed to Cd.

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