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South African Journal of Botany 75 (2009) 611–616
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Short communication

Effect of cadmium uptake and accumulation on growth and antibacterial activity of *Merwilla plumbea* — An extensively used medicinal plant in South Africa

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Received 12 December 2008; received in revised form 25 March 2009; accepted 21 May 2009

Abstract

In South Africa, heavy metal contamination of agricultural soils is on the increase primarily due to excessive application of fertilizers, sewage disposal and mining activities. The aim of this study was to investigate the effect of cadmium (Cd) on plant growth and biological activity. The medicinal plant *Merwilla plumbea* [Syn. *Merwilla natalensis* (Syn. *Scilla natalensis*)] was selected due to the high demand for its bulbs in the traditional medicine markets. Low levels of Cd (2 mg/L) significantly reduced fresh mass of leaves, bulbs and roots in comparison to the control. Although most of the Cd was stored in the roots, the bulbs, which are used medicinally, accumulated 7.1, 5.9 and 11.6 mg/kg when grown in sand watered weekly with 2, 5 and 10 mg Cd/L respectively. The bulbs of *M. plumbea* contained 24-fold more Cd than the World Health Organization guideline of 0.3 mg Cd/kg, when irrigated with 2 mg Cd/L. The bulb extracts showed increased antibacterial activity against the Gram-positive bacterium *Bacillus subtilis* at 2 mg Cd/L. The plants treated with 10 mg Cd/L showed an increased antibacterial activity against *B. subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus* in comparison to non-Cd-treated plants (control). However, there was no change in antibacterial activity of the various extracts against the Gram-negative bacterium *Escherichia coli*. The ability of *M. plumbea* to accumulate Cd not only raises concern for consumer safety, but also the quality of medicinal plants sold may be in question.

Keywords: Antibacterial activity; Cadmium accumulation; Growth; Medicinal plant; *Merwilla plumbea*

1. Introduction

Cadmium (Cd) enters into aquatic bodies through weathering of rocks, industrial effluents and agricultural run offs (Singh et al., 2006). A number of studies regarding metal elements in selected South African rivers and dams have shown a high concentration of Cd, exceeding the South African water quality guidelines (Fatoki and Awofolu, 2003; Okonkwo and Mothiba, 2005). Plant species vary considerably in their tolerance to excess Cd (De La Rosa et al., 2004). Although considered as non-essential (Van der Perk, 2006), Cd is readily taken up by plants (Kabata-Pendias and Pendias, 1984). This poses a potential human health hazard as accumulation in edible plant parts is a major factor with regards to heavy metals entering the food chain (McLaughlin et al., 1999; Clemens, 2006). Human exposure to Cd can lead to many types of pathologies including nephrotoxicity (Nordberg, 1999).

Numerous studies have recommended the cultivation of South African medicinal plants (Sparg et al., 2005; Crouch et al., 2006). This would not only reduce the strain on wild populations, but would also allow for good agricultural and collection practices (GACP), which is the first step in quality assurance upon which the safety and efficacy of plant based medicinal products directly depend (WHO, 2003). According to the World Health Organization (WHO, 1998), heavy metal

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0254-6299/$ - see front matter © 2009 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2009.05.004

contamination of medicinal plants should be monitored to ensure their safety. Thus, a set of quality control methods for medicinal plant materials with regards to the determination of pesticides, micro-organisms and toxic metals have been issued (WHO, 2005). At present there are no regulatory safety standards for South African medicinal plants, thus heavy metal monitoring is uncommon.

Medicinal plants may contain high levels of toxic metals (Chan et al., 1993; Caldas and Machado, 2004). Recent studies have shown the effects of heavy metals on secondary metabolites. Murch et al. (2003) reported that nickel (Ni) in the plant growth media had a negative effect on the secondary metabolite production of *Hypericum perforatum* L. The plants completely lost the ability to produce or accumulate hyperforin and showed a 15- to 20-fold decrease in the concentration of pseudohypericin and hypericin. On the contrary, the presence of Cd caused an increase in phyllanthin and hypophyllanthin in *Phyllanthus amarus* Schum. and Thonn. (Rai et al., 2005). Similarly, increased levels of iron (Fe) caused an increase of bacoside-A in the widely used Indian medicinal plant, *Bacopa monnieri* L. (Sinha and Saxena, 2006). Such changes caused by heavy metals could have serious implications on the quality, safety and efficacy of natural products prepared from medicinal plant species (Murch et al., 2003).

Despite the importance of monitoring heavy metal accumulation in medicinal plants coupled with increasing emphasis on cultivation, it is interesting to note that to date no work has been done on heavy metal accumulation in South African medicinal plants. *Merwilla plumbea* (Lindl.) Speta [Syn. *Merwilla natalensis* (Planchon) Speta (Syn. *Scilla natalensis* Planchon)] is ranked as one of the most popular medicinal plants sold in South Africa with a trade of 95 tonnes annually (Mander, 1997). In 2006, 2.1 million bulbs were estimated to be sold in the Durban and Johannesburg medicinal plant markets (Williams et al., 2007). Bulbs of *M. plumbea* are widely used for the treatment of numerous ailments, including stomach aches, constipation, intestinal worms, diarrhea, nausea and indigestion (Hutchings, 1989; Hutchings et al., 1996). Considering Cd as one of the most toxic pollutants and its high solubility in water, this study was conducted to assess the effect of Cd uptake and accumulation on plant growth and antibacterial activity of *M. plumbea*.

2. Materials and methods

2.1. Plant growth and treatment

Stock plants of *M. plumbea* were obtained from the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg. Plants with a bulb diameter of 1.2±0.3 cm and a total fresh weight of 8–11 g were transferred to pots (13.5×10 cm) containing sterilized, acid-washed quartz sand and wetted with 100 mL of 50% Hoagland's nutrient solution (HS) (Hoagland and Snyder, 1933). Plants were treated with three different concentrations of Cd (2, 5 and 10 mg/L) in the form of $CdCl_2 \cdot H_2O$, which was added to the HS. The treatments were added weekly (100 mL per pot) until the termination of the experiment (6 weeks). Hoagland's nutrient solution without Cd served as the control. Each treatment consisted of 10 replicates. Plants were placed in growth chambers under 16:8-h light:dark conditions with a photosynthetic photon flux density of 80.4± 3.5 μmol m^{-2} s^{-1} at 25 °C. All the plants were watered (100 mL) twice weekly. Reagents used in this experiment were of analytical grade.

2.2. Sample preparation and data collection

After harvesting, the plants were lightly washed to remove any particles of sand that may have adhered to the surface, and the growth parameters recorded. Thereafter, the plant parts (leaf, bulb and root) were cut into small pieces and dried at 50 °C for 72 h. Dried individual plant parts were ground into fine powders (<0.5 mm) using an analytical mill (IKA A11, Works Inc., USA), placed into air-tight containers and stored in the dark at 25±2 °C until analysis.

2.3. Cadmium analysis in plant material

Borosilicate glass digestion tubes, containing 0.5 g of homogenized plant material and 10 mL HNO_3–HCl–H_2O_2 (8:1:1 v/v/v) were placed on a heating block with increasing temperature up to 120 °C over 3 h. All reagents (55% HNO_3, 32% HCl, 30% H_2O_2), supplied by Merck (Germany), were of analytical grade. Reagent blanks were carried through the process. Elemental analysis was performed using Inductively Coupled Plasma-Optical Emission Spectrophotometry (Varian 720-ES, Varian, Palo Alto, CA, USA). The operating conditions are presented in Table 1. A certified reference material (NCS DC 73349 — bush branches and leaves) was used to check the analytical procedure (certified value 0.14±0.06 mg Cd/kg; determined value 0.14±0.01 mg Cd/kg).

2.4. Extraction of plant material (bulbs)

Previous work on *M. plumbea* concluded that the ethanolic extract gave the highest inhibitory activity against bacterial strains (Sparg et al., 2002). Therefore, in the present investigation ethanol extracts were made where 500 mg of powered bulbs in 5 mL ethanol were sonicated (Julabo Labortechnik, Seelbach, Germany) for 30 min. The bulb extract was then filtered under vacuum through Whatman No. 1 filter paper using a Büchner funnel, dried under vacuum using a rotary

Table 1
Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES) operating conditions for determination of Cd.

Power [kW]	1.00
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Replicate read time (s)	1
Instrument stabilizer delay (s)	15
Sample uptake delay (s)	50
Pump rate (rpm)	15
Wavelength (nm)	228.802

evaporator and stored at 5 °C. Bulb extract residues were resuspended in ethanol to a concentration of 50 mg/mL.

2.5. Bacterial strains screened

Each bulb extract was bioassayed against two Gram-positive bacteria, *Bacillus subtilis* (ATCC No. 6051) and *Staphylococcus aureus* (ATCC No. 12600) and two Gram-negative bacteria, *Escherichia coli* (ATCC No. 11775) and *Klebsiella pneumoniae* (ATCC No. 13883). The bacterial strains were obtained from the American Type Culture Collection (ATCC). The bacterial strains were maintained on Mueller–Hinton nutrient agar (Biolab) at 4 °C.

2.6. Minimum inhibitory concentration (MIC) bioassay

Extracts were screened for antibacterial activity using the microdilution method for MIC determination as described by Eloff (1998). Suspension cultures were inoculated in Mueller–Hinton (MH) broth (Oxoid) from bacterial stock cultures and incubated overnight at 37 °C in a water bath on an orbital shaker. Bulb extract (100 µL of 50 mg/mL) were serially diluted two-fold with 100 µL of sterile distilled water in a sterile 96-well microtitre plate (Greiner Labortechnik). A similar two-fold serial dilution of neomycin (Sigma) (100 µg/mL) was used as a positive control, and extraction solvent, extracts and bacteria-free controls were included as negative controls. The bacterial-saturated suspension cultures were diluted 1:100v/v with sterile MH broth, with 100 µL being added to each of the wells containing the test and control solutions. The plates were covered with parafilm and incubated overnight at 37 °C. Bacterial growth was indicated by adding 50 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (Sigma) to each of the wells. The plates were incubated at 37 °C for a further 30 min. The MIC was taken as the lowest concentrations of plant extract to elicit an inhibitory effect on the growth (last well not to exhibit a colour change) of test bacterium. The experiment was conducted in duplicate.

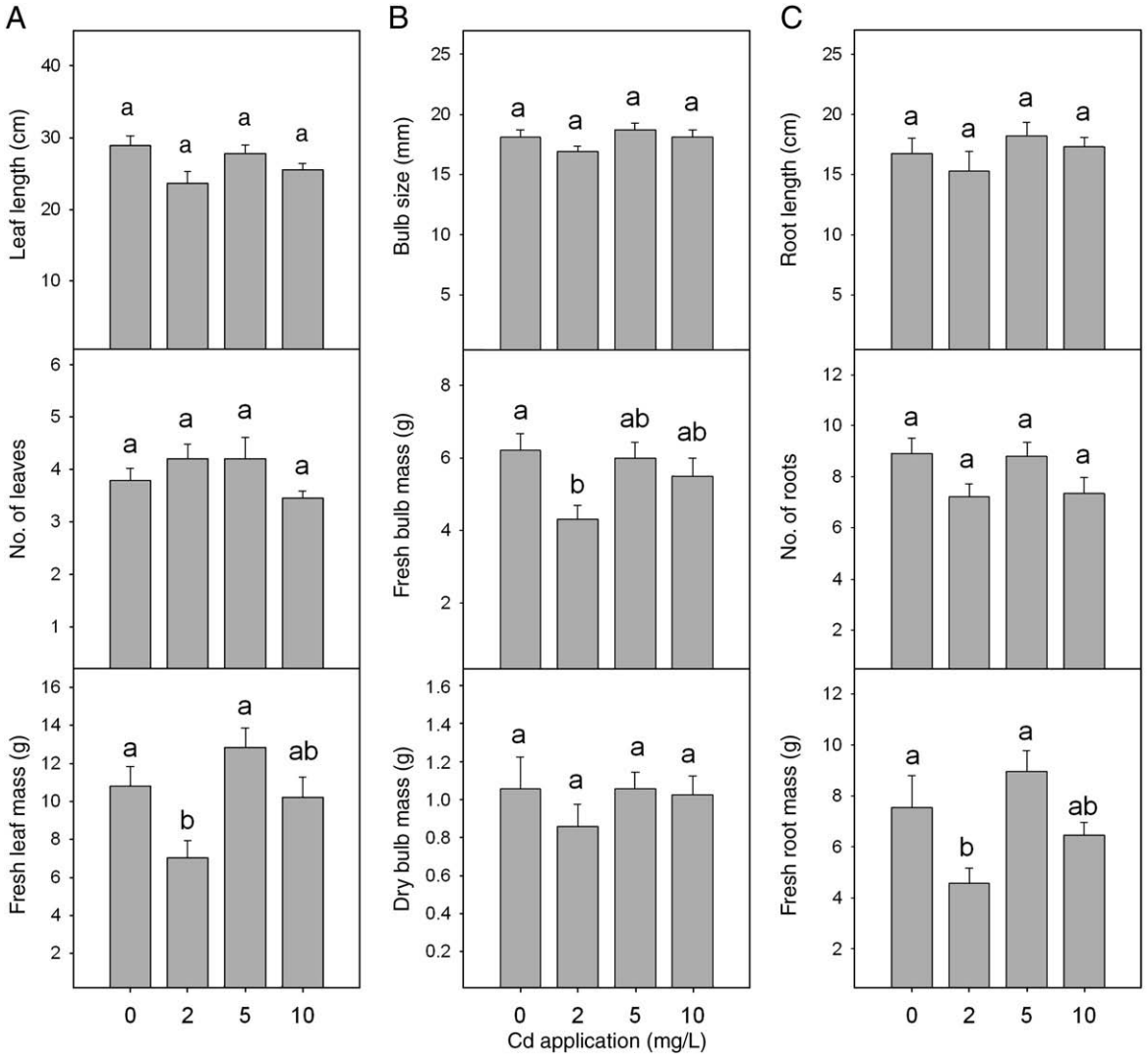

Fig. 1. Effect of Cd on growth parameters of *Merwilla plumbea*. Standard error bars with similar letter(s) are not significantly different ($P<0.05$).

2.7. Analysis of results

Growth data from different treatments were analyzed using one-way analysis of variance (ANOVA) with SPSS 15.0 statistical package (SPSS Inc., Chicago, USA). Tukey's test was used at a 5% level for significant differences.

3. Results and discussion

The effects of Cd treatments on the growth parameters of *M. plumbea* are presented in Fig. 1. Cadmium in the growth media did not show significant effects on leaf length or number of leaves of *M. plumbea*. However, Cd at 2 mg/L resulted in a significant reduction of fresh leaf mass in comparison to the control (Fig. 1A). Similarly, this concentration of Cd brought about a significant lower fresh mass of the bulbs and roots (Fig. 1B, C respectively). In addition, a reduction in lateral root formation was observed. However, Cd concentrations of up to 10 mg/L had no significant effect on bulb size, bulb dry mass, root length or number of roots (Fig. 1B, C).

The results clearly show that *M. plumbea* is more sensitive to a low level of Cd. This disruption in homeostasis is a common phenomenon caused by Cd toxicity. Contrary to our findings, similar studies have reported that low concentrations of heavy metals stimulate and high concentrations inhibit root growth (Uveges et al., 2002; Nyitrai et al., 2003). Despite high stomatal resistance, Cd-treated plants maintain less water than untreated plants, resulting in reduced water intake and flow (Öncel et al., 2000). This may, to a certain extent, be associated with root damage (Öncel et al., 2000). Polec-Pawlak et al. (2005) showed that Cd inhibited the growth of root hairs, lateral root formation (as seen in *M. plumbea*) and lowered biomass in *Arabidopsis thaliana* (L.). Wang et al. (2007) reported similar findings whereby the roots of maize (*Zea mays* L.) appeared thinner and more sparsely branched due to Cd toxicity. However, in accordance with the findings of Arduini et al. (2004), these changes in root morphology did not affect Cd translocation to the above ground parts of the plant.

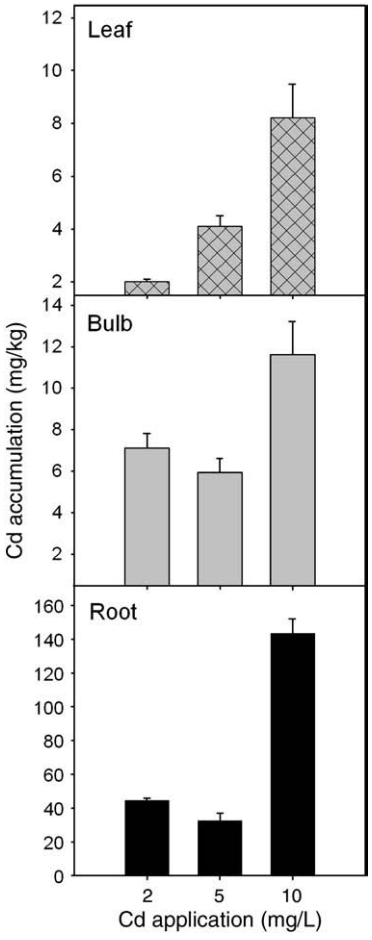

Fig. 2. Cadmium accumulation in different parts of *Merwilla plumbea* plants after 6 weeks of growth with different concentrations of Cd.

Table 2
Antibacterial activity (MIC mg/mL) of *Merwilla plumbea* bulb extracts obtained from plants grown at different Cd concentrations.

Treatment (mg Cd/L)	Bacterium [a]			
	B.s.	S.a.	E.c.	K.p.
0	6.25	6.25	6.25	6.25
2	3.13	6.25	6.25	6.25
5	6.25	3.13	6.25	6.25
10	3.13	3.13	6.25	3.13
Neomycin	3.13×10^{-2}	3.13×10^{-2}	1.56×10^{-2}	1.56×10^{-2}

[a] Abbreviations: B.s. — *Bacillus subtilis*; E.c. — *Escherichia coli*; K.p. — *Klebsiella pneumoniae*; S.a. — *Staphylococcus aureus*.

The distribution of Cd in leaves, bulbs and roots of *M. plumbea* differed with increasing Cd concentrations. Cadmium accumulation in the leaves increased with increasing Cd in the media (Fig. 2). The medicinally used bulbs accumulated 7.1, 5.9 and 11.6 mg/kg when grown in 2, 5 and 10 mg Cd/L respectively (Fig. 2).

The results of this study show that bulbs of *M. plumbea* contained about 24-fold more Cd than the WHO guideline of 0.3 mg/kg, when irrigated weekly with Cd at 2 mg/L. *M. plumbea* is medicinally used to treat both adults and children. According to Trzcinka-Ochocka et al. (2004), childhood Cd exposure may have a more significant impact on renal function, predominantly tubular reabsorption, than adult exposure. This study indicates that when administering medicinal plants, especially to children, care must be taken when using Cd susceptible species.

Cadmium had an effect on the antibacterial activity of *M. plumbea* bulbs against certain bacterial strains (Table 2). When the plants were treated with Cd at 2 mg/L, the bulb extract was more active against the Gram-positive bacterium *B. subtilis* than the control (Table 2). When treated with Cd at 5 mg/L, the activity was comparable to the control, but when treated with Cd at 10 mg/L there was an increase in activity against *B. subtilis*. The activity may be correlated to the Cd accumulation in the bulb, which was the lowest (5.9 mg/kg) when plants were treated with Cd at 5 mg/L (Fig. 2). Cadmium at 5 and 10 mg/L increased antibacterial activity against *S. aureus*. The activity against *K. pneumoniae* was greater than the control when plants were grown with 10 mg Cd/L (Table 2). This may be correlated with the high Cd accumulation (Fig. 2). There was no change in

the activity of *E. coli* with different concentrations of Cd tested. These results indicate that the presence of Cd in the bulb extract may have an effect on the antibacterial activity of the plant. The results can be interpreted in two ways. Firstly, the presence of Cd in the plant extract may affect the antibacterial activity in the bioassay. To verify this, one would need to test the activity of the Cd. However, testing pure Cd would not necessarily be valid due to the presence of other extracted substances which may interact with the Cd and modify its form. Secondly, the presence of Cd as an environmental stress may increase or decrease secondary metabolite production. To confirm this, one would need to quantify the effect of Cd on the biosynthesis of the active constituent. However, in the case of *M. plumbea* the active compound is not yet known, despite its wide use in South African traditional medicine. To date, very few South African medicinal plants have been studied in detail (Drewes et al., 2006).

Nonetheless, this study shows that Cd in the plant growth media and subsequently Cd uptake by plants has an effect on the antibacterial activity of *M. plumbea*. Similar results were reported following the isolation of the active compound in *Cyrtanthus suaveolens* Schönland, which revealed that the activity was due to the commercial pesticide Captan, with known mutagenic, genotoxic and teratogenic activity (Elgorashi et al., 2004). Thus, reporting of biological activity of plant crude extracts without the isolation and identification of an active compound raises concern, as the activity may be due to the presence of toxic substances.

4. Conclusions

This is the first report regarding Cd accumulation in *M. plumbea*. As this widely used medicinal plant enters the food chain, precaution should be taken because of its Cd accumulatory traits. More importantly, this study suggests the need for upgrading the safety regulations of South African medicinal plants for heavy metal contaminants. The findings reported in this study lay emphasis on Cd contamination as an important factor in the optimization of quality control of plants used in traditional medicine. In addition, researchers should be aware of the impact of environmental contaminants when reporting on biological activity of crude plant extracts — especially when the plant material is from an untraceable unknown source. Consequently, the results of the reported biological activity may be skewed due to the presence of heavy metals in the medicinal plants tested.

Acknowledgements

We thank the National Research Foundation (NRF), Pretoria and the University of KwaZulu-Natal for financial support.

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Edited by JN Eloff