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Toxicity of Metal Elements on Germination and Seedling Growth of Widely Used Medicinal Plants Belonging to Hyacinthaceae

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Abstract In South Africa, pollution of agricultural soils is on the increase primarily due to excessive application of fertilizers, sewage disposal and mining activities. This study was done to determine the effect of trace elements (Cu, Zn) and heavy metals (Cd, Pb, Hg) on germination and seedling development of *Bowiea volubilis*, *Eucomis autumnalis* and *Merwilla natalensis*. These medicinal plant species are highly recommended for cultivation in South Africa to reduce the pressure on wild populations. Copper and Zn at 1 mg L^{-1} significantly ($p < 0.05$) reduced the percentage germination of *E. autumnalis*. Low concentrations (≥1 mg L^{-1}) of Cu and Zn negatively affected the roots of all three species. Mercury concentrations of 0.5 and 1 mg L^{-1} significantly ($p < 0.05$) decreased the percentage germination of *B. volubilis* and *E. autumnalis* respectively. Cadmium and Hg at 2 mg L^{-1} showed a detrimental effect on the root growth of *B. volubilis*. Concentrations of 0.5 mg L^{-1} of all heavy metals tested significantly ($p < 0.05$) decreased shoot length of *M. natalensis*.

Keywords Metal elements · Medicinal plants · Seed germination · Seedling growth

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Many medicinal plants in South Africa are harvested from the wild, threatening future supplies of the important species. Currently, the top selling bulbs in the country belong to the family Hyacinthaceae, with its species *Bowiea volubilis* Harv. ex Hook.f., *Eucomis autumnalis* (Mill.) Chitt. and *Merwilla natalensis* (Planchon) Speta, trading around 43, 73 and 95 tonnes per annum respectively (Mander 1998). It has been suggested that the most practical solution to ensure their sustainability would be to develop these medicinal plants as small-scale farming crops (Van Staden 1999). Although many studies have recommended cultivation of the above mentioned species (Crouch et al. 2006; Sparg et al. 2005), there is no information on the response of these medicinal plants to trace elements and heavy metals. Copper (Cu) and zinc (Zn) are trace elements essential for normal plant growth, but at higher concentrations they become toxic and can interfere with numerous biological processes (Demirevska-Kepova et al. 2004; Vaillant et al. 2005). Similarly, non-essential heavy metals, commonly found in agricultural soils, such as cadmium (Cd), lead (Pb) and mercury (Hg) are known to cause hazardous effects on plants affecting both growth and development (Patra et al. 2004; Dong et al. 2006).

Heavy metal contamination of agricultural soils is on the increase (Jaja and Odoemena 2004). South Africa has a high concentration of mining activity. The deposition of processed and unprocessed waste materials has led to persistent leaching of dissolved metals into soil and water systems. Hence, heavy metals have been prioritized as leading contaminants due to careless disposal and their carcinogenic nature (Bux et al. 1994). Fuggle and Rabie (1992) estimated that 30,000 ha are watered with polluted water and 150,000 to 250,000 tonnes of dry sewage sludge is disposed annually on South African soils—much of this is on agricultural land.

Germinating seeds and developing seedlings are more sensitive to metal elements than mature plants, as their defence mechanisms are not yet fully developed (Liu et al. 2005). This study was conducted to determine the effects (sensitivity and tolerance) of metal elements on germination and seedling growth of *B. volubilis, E. autumnalis* and *M. natalensis*, which are frequently proposed for cultivation and extensively used in traditional medicine in South Africa.

Materials and Methods

Seeds of *B. volubilis, E. autumnalis* and *M. natalensis* were collected when mature in 2005 from the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg, South Africa. The seeds were stored in brown paper bags at room temperature (25 ± 2°C) for a period of three months before they were used for the experiment.

Seeds were surface decontaminated with 0.1% mercuric chloride for 2 min and rinsed thoroughly with distilled water prior to germination testing. *Bowiea volubilis* seeds were scarified with sand paper for optimum germination (Kulkarni et al. 2005). Disposable Petri dishes (9 cm), each containing two Whatman No. 1 filter paper discs were used for germination. Four replications of 20 seeds in each Petri dish were used and 3.5 ml of metallic compound solution was added. The concentrations of Cu ($CuSO_4{\cdot}5H_2O$) and Zn ($ZnSO_4{\cdot}7H_2O$) tested were 1, 2, 5, 10, 20 and 50 mg L^{-1}. Cadmium ($CdCl_2{\cdot}H_2O$), Hg ($HgCl_2$) and Pb ($(CH_3COO)_2Pb{\cdot}3H_2O$) concentrations tested were 0.5, 1 and 2 mg L^{-1}. Distilled water was used for the control treatment. Test solutions were replenished every other day to maintain levels. Petri dishes containing seeds of *B. volubilis* and *M. natalensis* were placed in plant growth chambers set at 25 ± 0.5°C with 16:8 h light and dark conditions. The photosynthetic photon flux density of lamps in the growth chamber was 80.4 ± 3.5 μmol m^{-2} s^{-1}. Germination (2 mm radicle emergence) was recorded every day. The experiment was terminated after 21 days when seedlings were developed. To achieve maximum germination of *E. autumnalis*, the seeds were germinated under constant dark conditions for 45 days at 20 ± 0.5°C (Kulkarni et al. 2006) and germination was recorded every alternate day under a 'safe green light' (0.3 μmol m^{-2} s^{-1}).

Germination and seedling growth data were analyzed using one-way analysis of variance (ANOVA) and Fisher's 95% confidence level ($p < 0.05$) was tested for pair-wise comparison. Percentage germination data were arcsine transformed before analyzing. MINITAB® (Minitab Inc., PA 16801-3008, USA) release 14 statistical software was used.

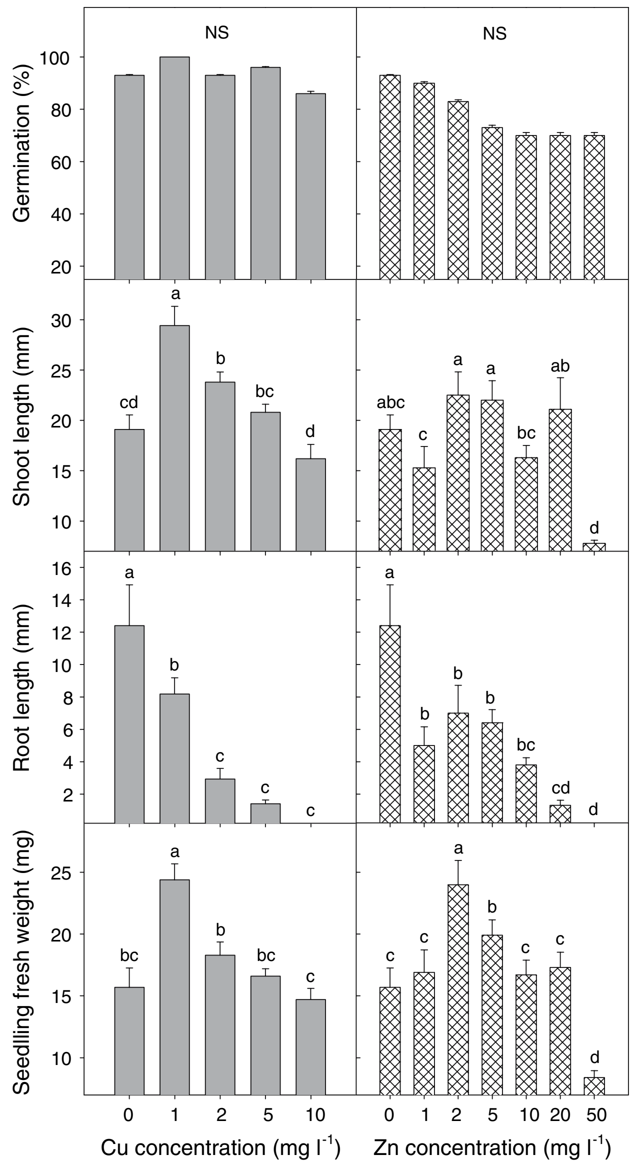

Fig. 1 Effect of trace elements on percentage germination and seedling growth (21-day-old) of *Bowiea volubilis* under 16:8 h light and dark conditions at 25 ± 0.5°C. Control (0) seeds were germinated with distilled water. Standard error bars with different letter(s) are significantly different ($p < 0.05$). Non-significant *NS*

Results and Discussion

Increasing concentrations of Cu had no significant effect on percentage germination of *B. volubilis*. Low concentration of Cu (1 mg L^{-1}) significantly increased shoot length and seedling fresh weight in comparison to the other concentrations (Fig. 1). In the absence of Cu, the seedling significantly achieved the maximum root length. Increasing concentrations of Zn gradually decreased the percentage germination although these results were not significantly different. Seedlings grown in 50 mg L^{-1} of Zn significantly inhibited the shoot/root length. However, significantly longer roots were recorded in the absence of Zn. At 2 mg L^{-1} Zn, seedlings reached significantly greater fresh weight than all other treatments (Fig. 1).

Percentage germination of *E. autumnalis* seeds without Cu (control) was significantly better than seeds germinated in varying Cu concentrations (Fig. 2). Copper at 2 mg L^{-1} significantly decreased shoot length, whilst 1 mg L^{-1} Cu significantly decreased root length. Seedling fresh weight was highest at 1 mg L^{-1} Cu and decreased with increasing concentrations. The effect of different Zn concentrations on germination and seedling growth were similar to the effects of Cu (Fig. 2). These findings clearly suggest that the levels of Cu and Zn should be less than 1 mg L^{-1} for optimum growth of *E. autumnalis*.

Seeds of *M. natalensis* germinated under different concentrations of Cu showed no significant effect on the percentage germination (Fig. 3). Conversely, shoot and root lengths significantly decreased in 2 and 1 mg L^{-1} Cu respectively, and seedling fresh weight at 5 mg L^{-1} Cu (Fig. 3). None of the Zn concentrations tested significantly affected the percentage germination. The results of shoot/root length and seedling fresh weight showed some fluctuations. However, all the values of these parameters significantly decreased at 10 mg L^{-1} (Fig. 3). The fact that Cu

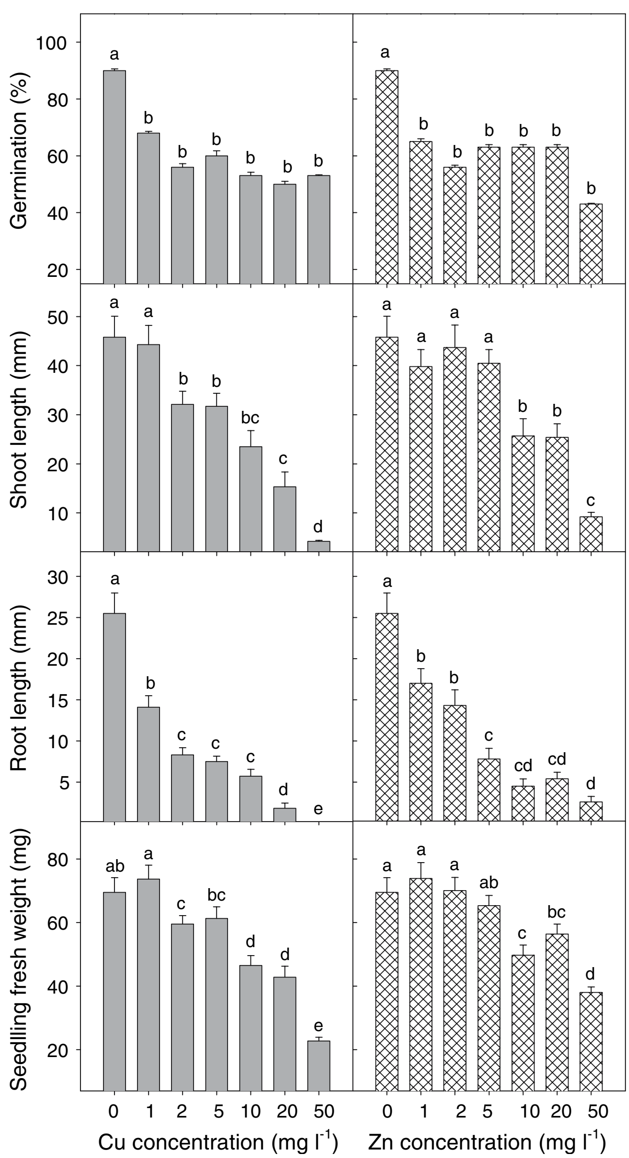

Fig. 2 Effect of trace elements on percentage germination and seedling growth (45-day-old) of *Eucomis autumnalis* under constant dark conditions at 20 ± 0.5°C. Control (0) seeds were germinated with distilled water. Standard error bars with different letter(s) are significantly different ($p < 0.05$)

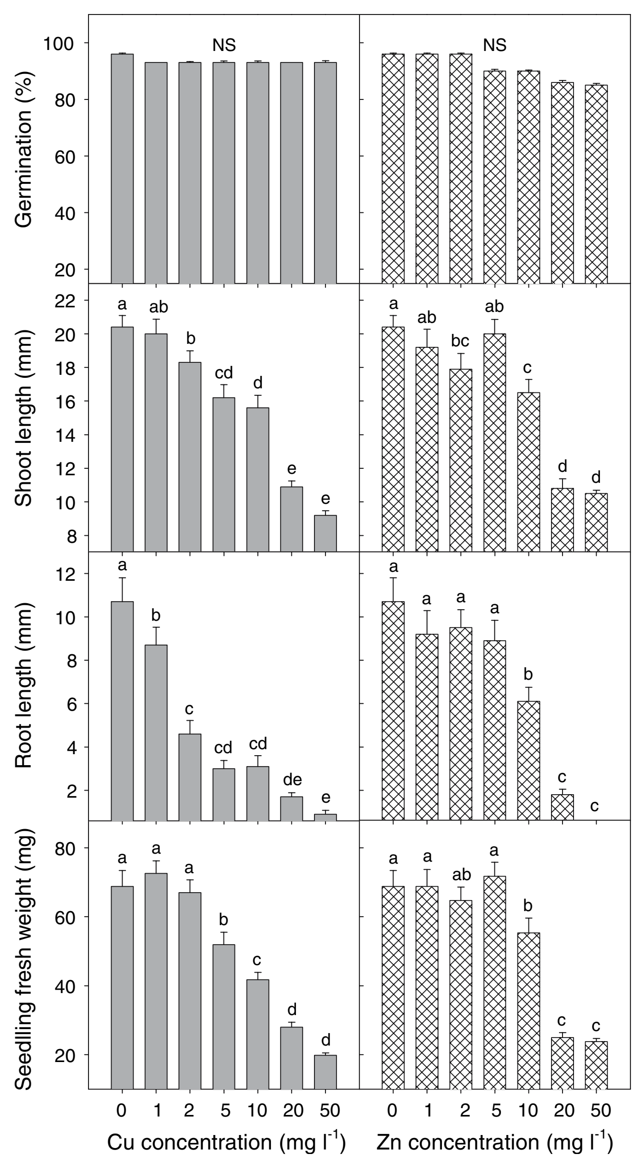

Fig. 3 Effect of trace elements on percentage germination and seedling growth (21-day-old) of *Merwilla natalensis* under 16:8 h light and dark conditions at 25 ± 0.5°C. Control (0) seeds were germinated with distilled water. Standard error bars with different letter(s) are significantly different ($p < 0.05$). Non-significant *NS*

and Zn did not have a negative influence on percentage germination may be due to sporadic germination of *M. natalensis* seeds. This was not the case for seedling growth. This result shows that even though percentage germination in *M. natalensis* was not affected by the higher concentrations, the lower concentrations of Cu and Zn affected seedling growth. This study indicates that the levels of Cu and Zn should be slightly lower than 1 mg L^{-1} for *M. natalensis*.

With the elevated contamination of agricultural soils due to prolonged use of Cu- and Zn-containing herbicides, pesticides and fertilizers (for example, copper carbonate, copper oxychloride, zinc oxide, zinc sulfate), Cu and Zn tolerance in plants is of great importance. In this study, high concentrations of Cu and Zn did not exhibit detrimental effects on percentage germination of *B. volubilis* and *M. natalensis* seeds, but had a negative effect on seedling growth. Similar results have been reported for *Zea mays* (Mahmood et al. 2005). This minor influence of metal toxicity on germination suggests that the seed utilizes its own reserves, and therefore, there is less chance that metal ions will interference until the process of germination is completed (Stefani et al. 1991). However, this was not the case for *E. autumnalis*, where low concentrations of Cu and Zn had an inhibitory effect on germination. This study showed that although all three species belong to the same family, the response of germination to Cu and Zn was markedly different.

It is clear from our results that seedling development was affected with increasing concentrations of both Cu and Zn. In all three wild-species, root growth was affected more than shoot growth. Our findings are in agreement with similar work verifying that a large copper supply usually inhibits root growth before shoot growth (Chen et al. 2000). This does not necessarily mean that roots are more sensitive to high copper concentrations, but rather that roots are the preferential sites for copper accumulation. In metal-polluted environments, roots are the primary contact zone with soil contaminants. The strong capacity of root tissues to accumulate Cu ions rather than transport them to the shoots has been observed under conditions of both Cu deficiency and excess (Kabata-Pendias 2001; Fuentes et al. 2007). Most plant species and genotypes have great tolerance to excessive amounts of Zn, however, depression in growth is a common symptom of toxicity (Kabata-Pendias and Pendias 1984). A study by El-Ghamery et al. (2003) showed Zn had an inhibitory effect on cell division in root tips causing a reduction in growth. Root growth of *B. volubilis* and *M. natalensis* was strongly inhibited at 50 mg L^{-1} and rooting of *E. autumnalis* was severely affected by Zn. These results indicate that all three species would suffer growth abnormalities in agricultural soils where permissible Cu and Zn concentrations are 6.6 and 46.6 mg L^{-1}, respectively (Water Research Commission 1997).

Mercury significantly reduced the percentage germination of *B. volubilis* seeds at all three concentrations examined (Table 1). In the case of *E. autumnalis*, Cd and Hg significantly lowered the percentage germination at 0.5 and 1 mg L^{-1}, respectively (Table 1). The different concentrations of heavy metals (Cd, Hg and Pb) tested showed no significant effect on the percentage germination of *M. natalensis* seeds. Seedlings of *B. volubilis* had significantly lowest root length at 2 mg L^{-1} Cd. Initially, Pb promoted the root growth at 0.5 mg L^{-1}, but thereafter showed a large variation for 1 and 2 mg L^{-1}. Increasing Hg concentrations decreased the root length of *B. volubilis* seedlings (Table 1). Seedling growth of *E. autumnalis* was not affected significantly by any of the concentrations of heavy metals tested. Cadmium (1 mg L^{-1}) significantly decreased the shoot/root length and seedling weight of *M. natalensis* (Table 1). Whereas Pb and Hg significantly showed a negative effect on shoot length at 0.5 mg L^{-1}.

Cadmium is not an essential element to plants but the contamination therewith could lead to anatomical and physiological changes (Chaoui and Ferjani 2005; Liu et al. 2005). A number of studies regarding metal elements in selected rivers and dams in South Africa showed a high concentration of Cd, with most of them exceeding the South African water quality guidelines (Fatoki and Awofolu 2003; Okonkwo and Mothiba 2005). Consequently, irrigating these medicinal plants with high levels of Cd-polluted water may have an adverse growth effect. Currently, the maximum permissible limit for Cd in agricultural soils is 2 mg L^{-1}. However, at this concentration all three species studied were negatively affected in either shoot/root growth (*B. volubilis* and *M. natalensis*) or germination (*E. autumnalis*).

Lead has received much attention as a major chemical pollutant of the environment (Nriagu et al. 1996a, b; Kabata-Pendias 2001). There are many reports regarding stimulatory (Öncel et al. 2000; Nyitrai et al. 2003) and inhibitory (Jaja and Odoemena 2004) effects of low concentrations of Pb on plant growth. This study similarly showed that 0.5 mg L^{-1} Pb promoted and inhibited seedling growth of *B. volubilis* and *M. natalensis* respectively (Table 1).

Mercury is considered one of the most readily accumulated toxic metal elements. It accumulates in living organisms causing harmful damage (Su et al. 2005). At low concentrations of Hg, seedling growth of rice and cucumber was inhibited (Du et al. 2005; Cargnelutti et al. 2006). Our study showed that the seedling growth (shoot + root length) of all Hyacinthaceae species examined was reduced at 0.5 mg L^{-1} indicating the threshold levels for Hg.

The negative impact of trace elements and heavy metals was pronounced at the post-germination stage. All three

Table 1 Effects of heavy metals on germination and seedling growth of medicinal plant species of Hyacinthaceae

Treatment ($mg\ L^{-1}$)		Shoot length (mm)	Root length (mm)	Seedling weight (mg)	Germination (%)
B. volubilis[a]					
Cadmium	0	19.1 ± 1.4 a	12.4 ± 2.5 a	15.7 ± 1.5 a	93 ± 0.4 a
	0.5	17.2 ± 1.5 a	7.6 ± 1.0 b	17.1 ± 1.2 a	90 ± 0.4 a
	1	19.5 ± 1.6 a	7.4 ± 1.6 b	15.4 ± 1.2 a	90 ± 0.6 a
	2	17.2 ± 1.4 a	5.1 ± 0.7 b	17.0 ± 1.0 a	90 ± 0.6 a
Lead	0	19.1 ± 1.4 a	12.4 ± 2.5 a	15.7 ± 1.5 b	93 ± 0.4 a
	0.5	23.3 ± 2.5 a	13.8 ± 2.7 a	21.5 ± 2.0 a	98 ± 0.4 a
	1	21.0 ± 1.7 a	7.1 ± 1.5 b	18.8 ± 1.7 ab	93 ± 0.7 a
	2	22.9 ± 1.6 a	11.8 ± 2.4 ab	22.1 ± 1.9 a	93 ± 0.4 a
Mercury	0	19.1 ± 1.4 a	12.4 ± 2.5 a	15.7 ± 1.5 a	93 ± 0.4 a
	0.5	15.0 ± 0.8 a	12.2 ± 0.3 ab	13.8 ± 0.3 a	80 ± 1.5 b
	1	20.1 ± 1.9 a	9.6 ± 1.9 ab	15.1 ± 1.8 a	73 ± 1.2 b
	2	17.2 ± 1.6 a	7.6 ± 1.2 b	14.8 ± 1.2 a	80 ± 0.7 b
E. autumnalis[b]					
Cadmium	0	45.8 ± 4.2 a	25.5 ± 2.4 a	69.5 ± 4.6 a	90 ± 0.5 a
	0.5	35.4 ± 4.2 a	21.0 ± 1.3 a	60.3 ± 3.2 a	63 ± 0.9 b
	1	41.1 ± 3.4 a	25.2 ± 1.9 a	69.5 ± 3.5 a	56 ± 0.7 b
	2	35.1 ± 3.5 a	24.0 ± 1.5 a	64.3 ± 4.4 a	63 ± 1.2 b
Lead	0	45.8 ± 4.2 a	25.5 ± 2.4 a	69.5 ± 4.6 a	90 ± 0.5 a
	0.5	44.3 ± 3.6 a	22.7 ± 2.0 a	79.9 ± 5.9 a	85 ± 0.9 a
	1	44.2 ± 3.6 a	24.5 ± 1.4 a	73.1 ± 3.8 a	86 ± 0.9 a
	2	39.1 ± 3.7 a	21.0 ± 1.1 a	67.0 ± 4.2 a	86 ± 0.9 a
Mercury	0	45.8 ± 4.2 a	25.5 ± 2.4 a	69.5 ± 4.6 a	90 ± 0.5 a
	0.5	44.1 ± 3.1 a	21.7 ± 1.5 a	68.0 ± 3.6 a	86 ± 0.9 ab
	1	46.8 ± 3.9 a	19.8 ± 2.1 a	64.7 ± 3.9 a	83 ± 0.4 b
	2	46.2 ± 3.0 a	21.0 ± 1.8 a	66.9 ± 3.7 a	83 ± 0.4 b
M. natalensis[a]					
Cadmium	0	20.4 ± 0.6 a	10.7 ± 1.1 a	68.8 ± 4.6 a	96 ± 0.4 a
	0.5	12.5 ± 0.4 b	8.3 ± 1.0 ab	50.0 ± 4.0 b	94 ± 1.1 a
	1	12.7 ± 0.5 b	4.3 ± 0.5 b	42.3 ± 3.6 b	94 ± 0.7 a
	2	15.3 ± 0.5 b	5.8 ± 0.5 b	57.3 ± 3.1 ab	93 ± 0.7 a
Lead	0	20.4 ± 0.6 a	10.7 ± 1.1 a	68.8 ± 4.6 a	96 ± 0.4 a
	0.5	16.0 ± 0.6 bc	7.6 ± 0.6 a	52.6 ± 2.1 b	100 ± 0 a
	1	13.4 ± 0.5 c	8.8 ± 0.6 a	58.0 ± 2.1 ab	95 ± 0.7 a
	2	18.0 ± 0.4 ab	10.6 ± 0.4 a	72.0 ± 2.0 a	100 ± 0 a
Mercury	0	20.4 ± 0.6 a	10.7 ± 1.1 a	68.8 ± 4.6 a	96 ± 0.4 a
	0.5	15.5 ± 0.1 b	10.0 ± 0.4 a	62.8 ± 2.5 a	95 ± 0.7 a
	1	15.6 ± 0.4 b	8.1 ± 0.7 a	57.7 ± 2.1 a	100 ± 0 a
	2	16.2 ± 0.7 b	8.2 ± 0.8 a	55.0 ± 3.8 a	95 ± 0.7 a

Mean values (± SE) of each species and heavy metal within the column with different letter(s) are significantly different at 5% level of significance

[a] 21-day-old seedling

[b] 45-day-old seedling

medicinal plant species showed a similar trend of sensitivity with increasing concentrations of Cu and Zn. However, these species responded differently to heavy metals. In most cases seedling growth was below that of the control. This study clearly indicates that the maximum permissible concentrations of Cd ($2\ mg\ L^{-1}$), Cu ($6.6\ mg\ L^{-1}$), Hg ($0.5\ mg\ L^{-1}$), Pb ($6.6\ mg\ L^{-1}$) and Zn ($46.5\ mg\ L^{-1}$) in South African agricultural soils set by the Water Research Commission (1997) are too high for growing these wild medicinal plants. This study therefore recommends separate threshold limits of metal elements for important traditional medicinal plants.

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