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Effect of cadmium on growth and micronutrient distribution in wild garlic (*Tulbaghia violacea*)

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Abstract

Tulbaghia violacea Harv. (Alliaceae) is one of the few medicinal plants that is also frequently used as a leafy vegetable. Application of cadmium (Cd) at 2 and 5 mg/L to *T. violacea* plants of various sizes (small 8–10 g, medium 16–20 g, large 80–95 g) elicited a difference in growth response, Cd accumulation and micronutrient distribution. Application of Cd up to 5 mg/L had no significant effect on growth parameters of large-sized plants while leaf length and fresh weight of leaves of the medium-sized plants decreased with application of Cd at 2 mg/L, and 5 mg/L. Cadmium significantly decreased the number of leaves in small-sized plants. Small plants accumulated more Cd in the leaves than medium or large-sized plants. Application of Cd at 2 and 5 mg/L lowered the leaf Cu, Fe, Mo and Zn contents in small and medium-sized plants but had no effect on the micronutrients in large-sized plants. This study indicates that *T. violacea* has the ability to accumulate Cd. In addition, plant size plays an important role with regards to Cd accumulation and elemental distribution. The results presented in this study include the first report on the nutritional status of *T. violacea* leaves.

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1. Introduction

In South Africa, cadmium (Cd) is a major environmental contaminant with elevated levels detected in some rivers and dams (Okonkwo and Mothiba, 2005; Coetzee et al., 2006). Cadmium (Cd), although considered as a non-essential element to plants (Van der Perk, 2006), like other elements is readily taken up (Kabata-Pendias and Pendias, 1984). Cadmium may enter the food chain through ingestion of contaminated plants and pose health risks to humans and animals (McLaughlin et al., 1999; Kabata-Pendias, 2001) as Cd is a potent carcinogen (Waisberg et al., 2003). Increasing evidence indicates that Cd has an antagonistic effect on essential elements (Wu and Zhang, 2002; Zornoza et al., 2002; Liu et al., 2003). For example, the addition of Cd to the growth medium of *Triticum aestivum* L. caused higher levels of phosphorus (P), potassium (K) and

manganese (Mn) to accumulate in the roots and this consequently inhibited P, K and Mn translocation to the shoots (Zhang et al., 2002). Similarly, uptake and accumulation of Cd is significantly correlated with copper (Cu), iron (Fe) and zinc (Zn) distribution in rice. Thus interaction between Cd and essential elements in crops is of public concern (Liu et al., 2003).

Tulbaghia violacea Harv. (Alliaceae), commonly known as wild garlic, is a widely used medicinal plant of southern Africa. The bulbs are used for the treatment of fever and colds, asthma, tuberculosis, and gastrointestinal ailments (Kubec et al., 2002). *T. violacea* is one of the few plants where the bulbs are used medicinally and the leaves are used as a vegetable (Watt and Breyer-Brandwyk, 1962). This gives it not only medicinal, but also horticultural potential. Recent studies have demonstrated the ability of commonly used Alliaceae species, including chives (*Allium schoenoprasum* L.) and garlic (*Allium sativum* L.), to accumulate Cd (Jiang et al., 2001; Barazani et al., 2004). The use of indigenous leafy vegetables by local people is still relatively under-developed in South Africa with more research required

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regarding nutrient composition and toxic elements in commonly consumed plant species (Mbhenyane et al., 2005; Odhav et al., 2007). Considering Cd as a major environmental contaminant, the aim of this study was therefore to assess the effect of Cd uptake and accumulation on plant growth and micronutrient distribution in *T. violacea*.

2. Materials and methods

2.1. Experimental design

Stock plants of *T. violacea*, raised in the shade house at the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg Campus (29° 37.55'S; 30° 24.13'E), were used for this study. Plants of various size classes: small (8–10 g), medium (16–20 g) and large (80–95 g) were planted in 30 cm pots containing sterile, acid-washed quartz sand with 16 plants per treatment, and arranged in a randomized block design in the greenhouse. Plants were treated with two different concentrations of Cd (2 and 5 mg/L) in the form of CdCl₂ · H₂O, which was added to 50% Hoagland's nutrient solution (HS) (Hoagland and Snyder, 1933). Test solutions were added weekly (250 mL per pot) until the termination of the experiment (6 weeks). Hoagland's nutrient solution without Cd served as the control.

2.2. Sample preparation and data collection

After harvest, plants were gently washed with distilled water to eradicate any particles of sand that may have adhered to the surface and subsequently various growth parameters were recorded. Thereafter, the plant parts were cut into small pieces and dried at 50 °C for approximately 72 h. Once dry, the individual plant parts were ground into fine powders (<0.5 mm) using an IKA A11 (IKA Works, Inc.) analytical mill, placed into air-tight containers and stored in the dark at room temperature (approximately 23 °C) until analysis.

2.3. Elemental analysis

Borosilicate glass tubes, containing 0.5 g of homogenized plant material and 10 mL HNO₃–HCl–H₂O₂ (8:1:1 v/v/v) were placed on a heating block with the temperature increasing to 120 °C over 3 h. All reagents (55% HNO₃, 32% HCl, 30% H₂O₂), supplied by Merck (Germany), were of analytical grade. Reagent blanks were carried out throughout the process. Elemental analysis was performed using Inductively Coupled Plasma-Optical Emission Spectrophotometry (Varian 720-ES, Varian Inc, Palo Alto, CA, USA). A certified reference material (NCS DC 73349 — bush branches and leaves) was used to validate the analytical procedure (Table 1).

2.4. Statistical analysis

The effects of different treatments were analyzed using one-way analysis of variance (ANOVA) with SPSS 15.0 statistical package and Tukey's test was used to separate means at a 5% level of significance.

Table 1

Results of determination of elements by ICP-OES in certified reference material (mg/kg).

Element	Wavelength (nm)	Certified value	Determined value (mean±S.D.; n=4)
Boron (B)	249.772	34.0±7	34.9±1.14
Cadmium (Cd)	228.802	0.14±0.06	0.14±0.01
Copper (Cu)	327.395	5.2±0.5	4.8±0.14
Iron (Fe)	238.204	1020±67	959±11
Manganese (Mn)	257.610	58.0±6	57.1±4.6
Molybdenum (Mo)	202.032	0.26±0.04	0.26±0.06
Zinc (Zn)	202.548	20.6±2.2	21.1±0.73

3. Results and discussion

3.1. Plant growth and development

Disruption in plant growth homeostasis is a familiar phenomenon caused by Cd toxicity, the most common symptoms being stunting and chlorosis (Das et al., 1997). However, to date, few studies have been conducted to elucidate the effects of heavy metals on plants at various growth stages (Peralta-Videa et al., 2004). The effects of Cd treatments on growth parameters of *T. violacea* are presented in Table 2. In small-sized plants, compared to the control, fresh weight of leaves, bulbs and roots, leaf and root length, and number of roots, were not significantly affected by the Cd treatments (Table 2). However, the addition of Cd at 5 mg/L significantly decreased the number of leaves. Leaf length, fresh weight of leaves and number of roots of the medium-sized plants significantly decreased when supplied with Cd at 2 mg/L. Fresh weight of bulbs decreased when supplied with Cd at 2 and 5 mg/L. However, Cd at 5 mg/L had no significant effect on root growth. Growth parameters of the large-size plants were not significantly affected by increasing concentrations of Cd.

Root length was unaffected in all three plant size classes. However, number of roots in the medium-sized plants was lowered when treated with the lower Cd concentration of 2 mg/L. Conversely, studies have suggested that low concentrations of heavy metals have a stimulatory effect on root growth whilst exhibiting an inhibitory effect at higher concentrations (Nyitrai et al., 2003; Ortiz Castro et al., 2007).

3.2. Cd content in bulbs

When supplied with 2 mg/L Cd, the small, medium and large-sized bulbs accumulated 3.4, 6.2 and 8.7 mg Cd/kg respectively (Fig. 1A). Cadmium accumulation in bulbs of small and medium-sized plants increased with increasing Cd concentration (Fig. 1B). However, the Cd concentration in the bulbs of the large-sized plants remained the same (8.7 mg/kg) when supplied with Cd at 2 or 5 mg/L.

Freshly harvested *T. violacea* bulbs are commonly boiled in water and the decoction is either taken orally or as an enema for a variety of ailments (Van Wyk et al., 1997). In this study, when supplied with 2 mg/L Cd, the small, medium and large-sized bulbs accumulated 11, 21 and 29 times more Cd than the WHO

Table 2
Effect of Cd application on growth parameters of *Tulbaghia violacea* of varying size classes after 6 weeks. Mean values in a column (\pm S.E) with dissimilar letter(s) are significantly different ($p < 0.05$).

Size class	Treatment (Cd mg/L)	Leaf length (cm)	No. of leaves	Leaf fresh weight (g)	Bulb fresh weight (g)	Root length (cm)	No. of roots	Root fresh weight (g)
Small	0	28.3 \pm 0.94 a	4.5 \pm 0.23 a	8.66 \pm 1.00 a	7.57 \pm 0.85 a	14.5 \pm 1.20 a	14.5 \pm 1.11 a	20.6 \pm 2.14 a
	2	26.3 \pm 1.25 a	4.4 \pm 0.28 a	7.89 \pm 0.84 a	10.0 \pm 1.01 a	14.9 \pm 1.25 a	11.5 \pm 0.94 a	20.2 \pm 2.27 a
	5	27.4 \pm 0.81 a	3.3 \pm 0.18 b	6.16 \pm 0.49 a	7.72 \pm 0.48 a	15.9 \pm 1.22 a	12.3 \pm 0.97 a	16.2 \pm 1.89 a
Medium	0	38.9 \pm 1.46 a	9.08 \pm 0.41 a	33.7 \pm 2.61 a	22.8 \pm 1.98 a	18.1 \pm 1.03 a	12.4 \pm 0.78 ab	33.3 \pm 3.67 a
	2	33.3 \pm 1.38 b	7.50 \pm 0.39 a	19.8 \pm 1.52 b	17.02 \pm 0.94 b	17.4 \pm 0.93 a	9.00 \pm 0.70 b	27.1 \pm 1.78 a
	5	36.2 \pm 1.75 ab	8.50 \pm 0.62 a	24.7 \pm 3.52 ab	16.4 \pm 1.22 b	18.5 \pm 1.26 a	13.6 \pm 1.72 a	29.5 \pm 4.76 a
Large	0	43.1 \pm 1.70 a	11.5 \pm 0.43 a	57.9 \pm 2.84 a	39.6 \pm 3.35 a	19.7 \pm 0.97 a	15.6 \pm 1.14 a	73.8 \pm 6.93 a
	2	46.8 \pm 1.82 a	10.0 \pm 0.51 a	56.1 \pm 8.29 a	34.9 \pm 2.76 a	22.4 \pm 1.28 a	14.0 \pm 1.67 a	79.1 \pm 12.3 a
	5	48.0 \pm 2.02 a	10.3 \pm 0.48 a	62.3 \pm 8.92 a	35.1 \pm 2.73 a	23.0 \pm 1.64 a	16.3 \pm 1.58 a	82.2 \pm 15.0 a

(1998) medicinal plant guideline of 0.3 mg Cd/kg, respectively. As Cd is a potent human carcinogen and human exposure has been associated with a range of cancers (Waisberg et al., 2003), the accumulation of Cd to these high concentrations raises safety issues and indicates that precautions should be taken.

3.3. Cd content in leaves

Accepting that bulb diameter is positively correlated with plant age and fitness (Williams et al., 2007), younger or small-sized plants accumulated more Cd in the leaves than the older plants (medium and large-sized plants). Leaf Cd content was the

same for medium and large-sized plants (1.4 mg/kg) when supplied with Cd at 2 mg/L compared with 2.1 mg/kg in the leaves of the small plants. With an increase of Cd to 5 mg/L, the small plants accumulated 5.5 mg/kg in the leaves, once again higher than medium and large-sized plants which accumulated 3.1 and 2.9 mg/kg respectively.

Urban agriculture has gained increasing recognition as a survival strategy for poor urban dwellers in developing countries (Nabulo et al., 2006). Mapanda et al. (2007) evaluated Cd concentrations in *Brassica juncea* (L.) Czern. and *Brassica napus* L. leaves from gardens irrigated with wastewater from the Mukuvisi River and partially treated sewage effluent at Pension farm in Harare, Zimbabwe. Cadmium leaf concentrations ranged from 0.7 to 2.4 mg/kg, and emphasis was placed on potential public health hazards. Although growing locally available crops can create socio-economic benefits, consumer safety must be safeguarded.

3.4. Micronutrient distribution

Boron (B) content in both leaves and bulbs of small-sized *T. violacea* plants significantly decreased when supplied with Cd at 5 mg/L (Table 3). However, in the bulbs of medium and large-sized plants, B content was not significantly affected by Cd at either 2 or 5 mg/L. Copper content in the bulbs of the small and large-sized plants significantly decreased when supplied with Cd at 2 mg/L, whilst Cu content in bulbs of medium-sized plants was significantly lowered by Cd at 5 mg/L.

Iron content in bulbs of small-sized plants was not affected when the plants were supplied with Cd at 2 mg/L, but Fe levels decreased significantly when the plants were supplied with Cd at 5 mg/L (Table 3). In contrast to our findings, Zhou and Qiu (2005) reported that Cd significantly increased Fe content in *Sedum alfredii* Hance (Crassulaceae). Leaf Fe content in the small-sized *T. violacea* plants was not significantly affected by Cd treatments. At 2 mg/L Cd, Fe levels in the bulbs of medium-sized plants were significantly lowered compared to non-Cd-treated bulbs (Table 3), a similar trend was observed for the bulbs of large-sized plants (Table 3). Conversely, the leaf Fe levels in the large-sized *T. violacea* plants significantly increased when supplied with Cd at 2 mg/L, when compared to the control (Table 3). This may be due to the age and coping ability of the plant. Medium-sized plants showed a similar trend

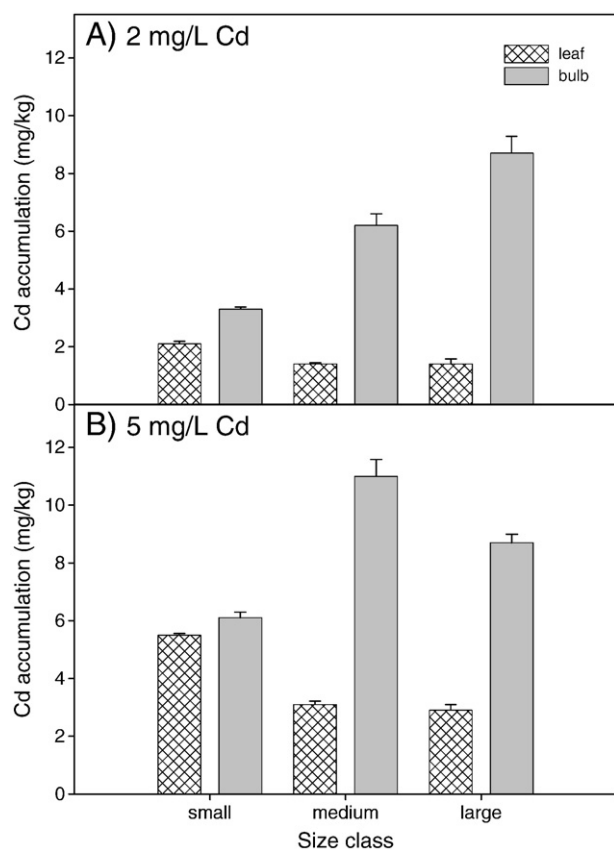


Fig. 1. Cadmium accumulation (mg/kg) in leaves and bulbs of *Tulbaghia violacea* after 6 weeks of exposure to either 2 mg/kg (A) or 5 mg/kg cadmium (B). Error bars indicate S.E. Cadmium was not detected in the control plants.

Table 3

Effect of various Cd treatments on microelement distribution (mg/kg) in *Tulbaghia violacea*. Mean values (\pm S.E.) in a column with dissimilar letter(s) are significantly different ($p < 0.05$).

Size class	Treatment (Cd mg Γ^{-1})	Leaf	Bulb	Leaf	Bulb
		B (mg/kg)		Cu (mg/kg)	
Small	0	40.5 \pm 2.23 a	12.2 \pm 0.68 a	2.75 \pm 0.03 b	7.73 \pm 0.26 a
	2	37.1 \pm 1.51 a	11.1 \pm 0.35 ab	5.70 \pm 0.06 a	3.06 \pm 0.36 b
	5	30.0 \pm 1.72 b	9.93 \pm 0.29 b	0.85 \pm 0.01 c	3.15 \pm 0.28 b
Medium	0	43.4 \pm 1.21 b	15.3 \pm 0.43 a	4.43 \pm 0.28 a	10.5 \pm 0.20 a
	2	38.7 \pm 1.39 b	13.8 \pm 0.48 a	5.41 \pm 0.04 a	11.5 \pm 0.59 a
	5	52.9 \pm 2.48 a	12.8 \pm 0.75 a	3.20 \pm 0.37 b	3.6 \pm 0.23 b
Large	0	36.3 \pm 0.44 b	15.8 \pm 0.87 a	3.82 \pm 0.30 b	14.2 \pm 0.21 a
	2	48.4 \pm 0.45 a	14.6 \pm 0.32 a	3.22 \pm 0.11 b	6.23 \pm 0.68 b
	5	29.1 \pm 0.71 c	15.3 \pm 0.81 a	4.83 \pm 0.22 a	4.15 \pm 0.11 c
		Fe (mg/kg)		Mn (mg/kg)	
Small	0	81.8 \pm 3.71 a	53.4 \pm 3.91 a	30.0 \pm 0.49 ab	10.8 \pm 0.21 a
	2	84.1 \pm 2.59 a	48.7 \pm 0.92 a	32.1 \pm 1.12 a	11.9 \pm 0.56 a
	5	77.1 \pm 5.42 a	23.7 \pm 1.54 b	28.7 \pm 0.59 b	10.8 \pm 0.33 a
Medium	0	81.3 \pm 3.26 a	144 \pm 3.95 a	20.93 \pm 0.15 a	21.8 \pm 0.63 a
	2	67.2 \pm 1.71 b	38.3 \pm 1.26 b	22.3 \pm 0.37 b	20.1 \pm 0.44 a
	5	72.4 \pm 3.26 ab	34.3 \pm 0.83 b	21.7 \pm 0.04 ab	15.7 \pm 0.45 b
Large	0	60.2 \pm 1.66 b	124 \pm 2.1 a	21.7 \pm 0.18 a	18.9 \pm 1.06 a
	2	119 \pm 2.26 a	59.5 \pm 4.1 b	19.8 \pm 0.26 b	12.9 \pm 0.64 b
	5	55.7 \pm 2.58 b	62.0 \pm 3.0 b	17.9 \pm 0.13 c	15.8 \pm 0.51 ab
		Mo (mg/kg)		Zn (mg/kg)	
Small	0	1.31 \pm 0.01 b	2.15 \pm 0.06 a	16.8 \pm 0.25 b	14.6 \pm 0.14 a
	2	1.51 \pm 0.02 a	2.19 \pm 0.02 a	48.5 \pm 3.41 a	15.2 \pm 0.52 a
	5	1.26 \pm 0.02 b	2.24 \pm 0.18 a	12.8 \pm 1.22 b	12.6 \pm 0.46 b
Medium	0	1.35 \pm 0.00 a	3.34 \pm 0.13 a	24.3 \pm 1.01 a	30.9 \pm 1.91 a
	2	1.31 \pm 0.02 a	3.03 \pm 0.08 a	21.6 \pm 0.80 a	29.5 \pm 0.55 a
	5	1.38 \pm 0.02 a	2.51 \pm 0.02 b	23.0 \pm 0.07 a	26.5 \pm 0.81 a
Large	0	0.69 \pm 0.01 b	2.08 \pm 0.23 ab	25.2 \pm 0.71 a	35.3 \pm 0.47 a
	2	0.79 \pm 0.05 ab	2.79 \pm 0.11 a	25.2 \pm 3.31 a	31.1 \pm 0.41 b
	5	0.86 \pm 0.02 a	1.69 \pm 0.12 b	27.6 \pm 2.22 a	28.6 \pm 0.59 c

with regards to B and Fe translocation. When supplied with Cd at 2 mg/L, leaf B and Fe content decreased. However, when supplied with Cd at 5 mg/L, leaf B and Fe content increased (Table 3).

Manganese and Mo content in bulbs of small-sized plants was not significantly affected by the presence of Cd. However, in medium-sized bulbs, the content of these microelements was significantly decreased by Cd at 5 mg/L. In the case of bulbs of large-sized plants, Mn content was significantly decreased when supplied with Cd at 2 mg/L with Mn leaf content significantly decreasing with an increase in Cd (Table 3). Similar results have been reported where Cd toxicity caused a reduction in leaf Mn content (Chizzola, 2001; Gardea-Torresdey et al., 2004).

There are many reports of Cd/Zn antagonistic and synergistic effects (Chakravarty and Srivastava, 1997; Chaoui et al., 1997; Aravind and Prasad, 2005). It is suggested that the toxic effects of Cd may be preventable or treatable by Zn application (Wajda et al., 1989; Das et al., 1997). In the case of *T. violacea*, the only Cd/Zn interaction observed was in the small-sized plants whereby plants supplied with Cd at 2 mg/L accumulated Zn to 48.5 mg/kg in the leaves compared to 16.8 mg/kg in the control. However, when supplied with Cd at 5 mg/L, only

12.8 mg Zn/kg accumulated in the leaves suggesting Cd/Zn antagonism at 2 mg Cd/L (Table 3).

The results presented in this study include the first report on the nutritional status of *T. violacea* bulbs and leaves. The occurrence of essential elements in medicinal plants may be correlated with therapeutic properties against a range of health disorders. Our findings indicate that the leaves of *T. violacea* may be an important supplementation vegetable with the content of certain micronutrients comparable to similar South African traditional leafy vegetables (Odhav et al., 2007). Investigating the relationship between Cd and other elements is essential to supplement the current knowledge on Cd accumulation in plants that enter the food chain (Wu and Zhang, 2002). It is clear that Cd had an effect on elemental distribution which could result in compromising the nutritional value of the bulbs and leaves.

4. Conclusion

The ability of *T. violacea* to accumulate Cd illustrates a potential Cd exposure route to consumers. As Cd exposure can produce a variety of adverse effects on human health, monitoring Cd levels in soils is strongly advocated for the

cultivation of *T. violacea*. In addition, age of the plant plays an important role with regards to Cd accumulation and elemental distribution. Evaluating and monitoring Cd contamination is an essential step in improving the overall safety and quality of widely used *T. violacea* which will in turn result in safeguarding the consumer.

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