

## EFFECTS OF CADMIUM CONCENTRATION AND ARBUSCULAR MYCORRHIZA ON GROWTH, FLOWERING AND CADMIUM ACCUMULATION IN OSTEOSPERMUM (*Osteospermum ecklonis* (DC.) NORL. 'Denebola')<sup>1</sup>

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### Introduction

Cadmium (Cd) is a nonessential heavy metal, which is toxic at low concentration to plants, animals, and people. Increasing amount of Cd in soil comes from some fertilizers, manure, atmospheric deposition, sewage sludge, mining, and smelting of Cd-containing sulphide. Significant differences in plant ability to accumulate Cd and in plant responses to Cd toxicity were observed [VASSILEV et al. 2004]. Some plants, so called hyperaccumulators, can absorb relatively high Cd concentration and translocate them to the harvestable shoots where they accumulate. Hyperaccumulators can be used for cleaning large areas that were contaminated with low to moderate levels of Cd. On highly Cd-polluted soils plant growth is inhibited due to its negative effect on photosynthesis [CHUNG et al. 1999].

Several authors reported that colonization of root system by arbuscular mycorrhizal fungi (AMF) can affect shoot concentration of heavy metals [GILDON, TINKER 1983; RIVERA-BECERRIL et al. 2002]. The effect of AMF colonization on the uptake of heavy metals depends on the concentration of heavy metals in soil. High concentrations of heavy metals in soil have negative effect on AM fungi, whereas at lower levels of heavy metals in soil the metal uptake in mycorrhizal plants increase as compared with non-mycorrhizal ones [WEISSENHORN et al. 1995].

The purpose of the present research was to study the influence of Cd concentration in growing substrate and AMF colonization of root system on the growth, flowering, and Cd accumulation in osteospermum, which, as a bedding plant, can be used for phytoextraction of Cd in urban areas.

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## Materials and methods

Rooted cuttings of osteospermum were used for experiments. The cuttings were planted into Klasmann KTS 3 substrate (pH 6.0, total soluble salts 0.68 g KCl·dm<sup>-3</sup>). The substrate was inoculated by adding 1 dm<sup>3</sup> mixture of osteospermum root pieces and substrate inoculated earlier with Endorize-TA AMF inoculum, containing a mixture of different *Glomus* species, mainly *Glomus intraradices* (Biorize Sarl, France) to 10 dm<sup>3</sup> of inoculated Klasmann substrate. The substrate was not sterilized. The Klasmann substrate used in this experiment was devoid of AM fungi as confirmed by the absence of colonization with the non-inoculated treatments. Mycorrhizal infection was estimated after staining the roots with trypan blue [PHILIPS, HAYMAN 1970]. Pots were arranged on greenhouse benches in a randomized complete block design with four replicates per treatment, using 5 plants as a replicate.

One month after planting, the plants were given 200 ml of solution containing: 0, 10, 20, and 40 mg Cd·dm<sup>-3</sup> substrate. Cd was applied as Cd(NO<sub>3</sub>)<sub>2</sub>·4 HO, different amounts of nitrogen was compensated with respective amounts of NH<sub>4</sub>(NO<sub>3</sub>)<sub>2</sub>. The plants were exposed to Cd treatment for 8 weeks.

The plants were cultivated under glass from April 6th to June 29th. The greenhouse was maintained at 16°C during the night and ventilated when the temperature reached 24°C during the day. Osteospermum was fertilized with commercial fertilizer Symfovita A (12.5 N, 2.1 P, 18.5 K, 2.9 Mg, 0.025 B, 0.025 Zn, 0.0005 Co, 0.1 Mn, 0.02 Cu, 0.003 Mo) at concentration 1 g·dm<sup>-3</sup> twice a week.

All measurements were conducted at the end of experiments. Dry weight of shoots and roots, plant height, leaf number, number of days from planting to flowering, numbers of flower buds and flowers, colonization of root system by AM fungi were determined.

For Cd determination the shoots were oven-dried to constant weight at 78°C, milled to homogeneous samples, and then treated with HNO<sub>3</sub> at 180°C, pressure 20 atm. for 40 min. (microwave oven Mars-5, Candela, USA). The concentrations of Cd were measured spectrophotometrically using ICP (OPTIMA 2000 DV, Perkin-Elmer, USA).

The treatments were statistically analyzed by the analysis of variance and means were compared with Duncan's multiple range test at 95% level of significance.

## Results and discussion

AMF colonization of osteospermum roots at the end of experiment, judged by the percentage of root lengths colonized by hyphae was 30%, 17%, 13%, and 10% for plants grown at 0, 10, 20, and 40 mg Cd·dm<sup>-3</sup>, respectively. The uninoculated plants had no root infection. Negative effect [VIVAS et al. 2003] or no effect [CHEN et al. 2003] of high Cd concentration on AMF colonization of root system was earlier observed. Differences in tolerance to Cd concentration in soil between different *Glomus* species were also noted [LIAO et al. 2003].

It should be mentioned that the used Cd concentrations were not toxic to osteospermum. No leaf chlorosis, necrosis, or differences in flower color due to

anthocyanin production under Cd stress (w) were observed. The lowermost leaves of the other bedding plants, for example *Callistephus chinensis*, *Tagetes erecta*, *Salvia splendens*, *Rudbeckia hirta* were chlorotic and developed marginal necrosis under exposition to the same Cd levels [NOWAK, unpublished data].

The growth and flowering response of *Osteospermum* to different Cd levels and mycorrhization is presented in Table 1. The shoots and roots of non-mycorrhizal plants showed a dry weights decrease in higher Cd treatments (20 and 40 mg Cd·dm<sup>-3</sup>). Inhibition of shoot and root growth due to Cd pollution was frequently observed in other plants [RIVERA-BECERRIL et al. 2002; VASSILEV et al. 2004]. Mycorrhizal plants had lower dry weights of shoots and roots and were lower than non-mycorrhizal ones. It is well known that in the conditions of mineral nutrient availability mycorrhization can depress root and shoot growth primarily by sink competition for photosynthates [DOUDS et al. 1988], while in nutrient-poor growing substrates the mycorrhizal plants have more access to the mineral nutrients due to the external hyphae development. In this experiment all plants were fertilized with complete nutrient solution.

Table 1; Tabela 1

Effect of cadmium and mycorrhizal inoculation on growth and flowering of *Osteospermum ecklonis* (DC.) NORL. 'Denebola')

Wpływ kadmu na wzrost i kwitnienie *Osteospermum ecklonis* (DC.) NORL. 'Denebola')

Treatments Traktowania		DW of shoots Sucha masa pędów (g)	DW of roots Sucha masa korzeni (g)	Height of plant Wysokość rośliny (cm)	Number of flower buds Liczba pąków kwiatowych	Number of flowers Liczba kwiatów
Cd concentr. stężenie Cd (mg Cd·dm <sup>-3</sup> )	AMF inocul. mikoryz.					
0	-	14.0c	7.8d	42.0b	3.8b	6.8bc
	+	11.2ab	4.3ab	36.8a	2.7ab	5.4ab
10	-	13.8c	7.7d	41.9b	2.5ab	5.9abc
	+	10.6a	3.7a	37.8a	1.9a	5.8abc
20	-	12.0b	5.1bc	44.5b	2.8ab	7.7c
	+	10.6a	4.5ab	39.1a	2.9ab	6.1abc
40	-	11.9ab	5.8c	42.3b	2.3ab	7.1c
	+	10.6a	4.8abc	37.0a	3.3ab	6.2bc

means within rows followed by the same letter(s) do not significantly differ at  $\alpha = 0.05$ ; średnie oznaczone tą samą literą nie różnią się istotnie przy poziomie istotności  $\alpha = 0,05$

Cd treatments did not decrease dry weight of shoots and roots, as well as plant height of mycorrhizal plants. It was found earlier that plants in certain mycorrhizal associations are less sensitive to Cd stress [RIVERA-BECERRIL et al. 2002]. The effect of mycorrhization and Cd level on time of flowering of *Osteospermum* was negligible. Cd level and mycorrhization did not affect the number of flower buds and flowers in non-mycorrhizal, as well as mycorrhizal plants.

Cd occurs primarily in exchangeable, readily bioavailable forms in soil [LASAT 2000]. Among the plant factors affecting Cd uptake, plant genotype is con-

sidered the most important. Some plants accumulate small amount of Cd, whereas other can accumulate relatively high Cd concentrations [DAVIS 1984]. Some plants are able to accumulate very high Cd concentrations in roots only, others can accumulate Cd also in shoots [WAGNER, YEARGAN 1986]. Bedding plants accumulating Cd in shoots can be useful in phytoextraction of Cd in urban areas.

In *Osteospermum* untreated with Cd, the Cd concentration in shoot tissue was low and not significantly affected by mycorrhization (Tab. 2). However, in Cd-exposed plants Cd concentration in shoots increased with the increasing Cd level in growing medium. A tendency to increase shoot Cd content in mycorrhizal plants, comparing to non-mycorrhizal ones, was observed, but significant effect of AMF inoculation on Cd accumulation in shoots was noted only in plants exposed to the highest Cd concentration (40 mg Cd·dm<sup>-3</sup>). Root colonization by *Glomus intraradices* significantly increased Cd uptake to shoots of Cd-treated pea genotypes [RIVERA-BECERRIL et al. 2002], whereas Cd accumulation in colonized roots was lower or equal, depending on the genotype. In this experiment Cd content of roots was not determined. The values for Cd in AMF inoculated *Osteospermum* shoots are in the range of those reported in a relatively high-Cd accumulating plants. Shoot Cd levels are usually < 1 mg·kg<sup>-1</sup> DM, Cd hyperaccumulators accumulate and tolerate 100 mg Cd·kg<sup>-1</sup> DM [BAKER et al. 1994].

Table 2; Tabela 2

The effect of cadmium content in growing medium and AMF inoculation on Cd content in *Osteospermum* shoots  
(*Osteospermum ecklonis* (DC.) NORL. 'Denebola')

Wpływ stężenia Cd w glebie i mikoryzacji na zawartość Cd w pędach *Osteospermum* (*Osteospermum ecklonis* (DC.) NORL. 'Denebola')

Cd concentr. Stężenie Cd (mg Cd·dm <sup>-3</sup> )	AMF inoculation Mikoryzacja	Cd content of shoots Zawartość Cd w pędach (mg·kg <sup>-1</sup> DM)
0	-	0.67a
	+	2.23a
10	-	22.8b
	+	27.2bc
20	-	31.0c
	+	38.2cd
40	-	47.1d
	+	88.1e

means within rows followed by the same letter(s) do not significantly differ at  $\alpha = 0.05$ ; średnie oznaczone tą samą literą nie różnią się istotnie przy poziomie istotności  $\alpha = 0,05$

## Conclusions

1. *Osteospermum* 'Denebola' is very tolerant to high concentration of Cd in growing substrate.

2. AMF inoculation increased the accumulation of Cd in shoots of *Osteospermum* subjected to high Cd concentration in growing substrate.

### Literature

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**Key words:** osteospermum, growth, flowering, Cd, arbuscular mycorrhiza, phytoextraction

### Summary

The influence of Cd concentration in growing substrate (0, 10, 20, 40 mg·dm<sup>-3</sup>) and AMF colonization of root system on growth, flowering, and Cd accumulation in osteospermum shoots were examined. Cd in the applied concentrations did not affect decorative value of osteospermum, no chlorosis or necrosis were observed on leaf margins frequently visible on leaves of other bedding plants. Cd at higher concentrations (20 and 40 mg·dm<sup>-3</sup>) lowered dry weights of shoots and roots in non-mycorrhizal plants. Mycorrhizal plants were lower and had lower dry weights of shoots and roots. Cd did not affect dry weights of shoots and roots in mycorrhizal plants. The effects of Cd and mycorrhization on flowering of osteospermum were negligible. Cd content of osteospermum shoots increased with the increasing content of Cd in growing substrate. Mycorrhizal plants grown in substrate containing 40 mg Cd·dm<sup>-3</sup> accumulated more Cd in shoots than non-mycorrhizal plants. Obtained results showed that osteospermum is very tolerant to Cd toxicity and can accumulate great amount of Cd in shoots.

### WPEŁYW STĘŻENIA KADMU I MIKORYZY ARBUSKULARNEJ NA WZROST, KWITNIENIE I AKUMULACJĘ KADMU W OSTEOSPERMUM (*Osteospermum ecklonis* (DC.) NORL. 'Denebola')

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**Słowa kluczowe:** osteospermum, wzrost, kwitnienie, Cd, mikoryza arbuskularna, fitoekstrakcja

### Streszczenie

Badania miały na celu określenie wpływu zawartości kadmu w podłożu (0, 10, 20, 40 mg·dm<sup>-3</sup>) i mikoryzy arbuskularnej na wzrost, kwitnienie i akumulację Cd w pędach osteospermum. Cd w zastosowanych stężeniach nie obniżał wartości dekoracyjnej osteospermum, nie powodował chlorozy i nekroz na brzegach liści, wyraźnie widocznych u innych roślin rabatowych. Cd w wyższych stężeniach (20 i 40 mg·dm<sup>-3</sup>) powodował spadek suchej masy części nadziemnych i korzeni roślin niezmikoryzowanych. Rośliny zmikoryzowane były niższe i miały mniejszą suchą masę niż niezmikoryzowane. Cd nie obniżał suchej masy pędów i korzeni roślin zmikoryzowanych. Wpływ Cd i mikoryzacji na kwitnienie osteospermum był nieznaczny. Zawartości Cd w pędach osteospermum wzrastały wraz ze wzrostem zawartości tego pierwiastka w podłożu. Rośliny zmikoryzowane rosnące w podłożu zawierającym 40 mg Cd·dm<sup>-3</sup> akumulowały znacznie więcej Cd w pędach niż

rośliny niezmikoryzowane. Otrzymane wyniki wskazują, że osteospermum można zaliczyć do roślin bardzo tolerancyjnych w stosunku do kadmu i zdolnych do akumulacji znacznych ilości tego pierwiastka w pędach.

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