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EFFECTS OF CADMIUM AND LEAD CONCENTRATIONS AND ARBUSCULAR MYCORRHIZA ON GROWTH, FLOWERING AND HEAVY METAL ACCUMULATION IN SCARLET SAGE (*SALVIA SPLENDENS* SELLO 'TORREADOR')

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S u m m a r y

The objective of this research was to examine the influ ence of Cd (0, 10, 20, 40 mg Cd·dm ^{3}) and Pb (0, 10, 100, 200 mg Pb·dm ^{3}) in growing substrate and mycorrhizal colonization of root system on growth, flowering, Cd and Pb accumulation in scarlet sage shoots. Both Cd and Pb had a negative effect on my corrhizal colonization of scarlet sage roots. The effect of Cd and Pb on the growth of scarlet sage was negligible. Cd at 40 mg·dm ^{3} lowered the number of inflorescences and caused slight chloro sis of the lowermost leaves. Pb at 200 mg·dm ^{3} caused drying of the lowermost leaves. Both heavy metals accelerated flowering of non mycorrhizal plants, independently of the concentration in growing media. Cd and Pb contents in scarlet sage shoots incre ased with the increasing content of these heavy metals in growing substrate in both non mycorrhizal and mycorrhizal plants. My corrhization decreased the growth of scarlet sage and increased the accumulation of Cd and Pb in shoots of plants grown in media strongly polluted with heavy metals.

Key words: *Salvia splendens*, mycorrhization, heavy metals, growth, flowering

INTRODUCTION

The heavy metals content in urban soils is very often above legally allowable limits (D i a t t a e t al. 2003). Heavy metal pollution of soil enhances plant uptake causing accumulation in plant tissues, growth inhibition and visible phytotoxicity in urban vegetation. The identification and selection of ornamental plant species that possess the ability to accumulate metals and to grow vigorously in contaminated soils can increase the ornamental value of urban vegetation and allow them to be used for phytoremediation.

Cd and Pb are nonessential elements toxic to plants and animals. Significant differences in plant ability to accumulate heavy metals and in plant responses to heavy metal toxicity have been observed (B e g o n i a , 1997; V a s s i l e v et al. 2004). Some plants, so called hyperaccumulators, can absorb relatively high heavy metal concentrations and translocate them to the harvestable shoots where they accumulate. Such plants could be used to remove heavy metals from contaminated soils. W e i s s e n h o r n et. al. (1995) showed that AMF colonization of root system could either reduce or increase the heavy metal content of plants, depending on growth conditions, the fungus and the metal.

The objective of the study was to investigate the influence of Cd and Pb concentrations in growing substrate and AMF colonization of root system on growth, flowering, and Cd and Pb accumulation in scarlet sage which, as a bedding plant, can be used for phytoextraction of heavy metals in urban areas.

MATERIAL AND METHODS

Seeds of scarlet sage (*Salvia splendens* Sello cv. Torreador) were used for the experiments. The seeds were sown in the middle of February and the seedlings were then planted at the beginning of March into Klasmann KTS 3 substrate (pH 6.0, total soluble salts 0.68 g KCl·dm^{-3}). The substrate was inoculated with Endorize-TA AMF inoculum, containing a mixture of different *Glomus* species, mainly *Glomus intraradices* (Biorize Sarl, France), as described earlier (N o w a k , 2004). The substrate was not sterilized. The Klasmann substrate used in this experiment was devoid of AM fungi, as confirmed by the absence of colonization in the non-inoculated treatments. The percentage of root colonization by AM fungi was estimated as described by P h i l i p s and H a y m a n (1970). Pots were arranged on greenhouse benches in a randomized complete bock

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 $Cd \cdot dm^{-3}$ substrate, or 0, 10, 100, and 200 mg $Pb \cdot dm^{-3}$ substrate. Cd was applied as $Cd(NO_3)_2 \cdot 4H_2O$, Pb was applied as $Pb(NO_3)_2$. Different amounts of nitrogen were compensated with respective amounts of $NH_4(NO_3)_2$. The plants were exposed to heavy metals treatment for 8 weeks.

The plants were cultivated under glass to the end of June. The greenhouse was maintained at 16°C during the night and ventilated when the temperature reached 24°C during the day. The plants were fertilized with the 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 a concentration of 1 $g \cdot dm^{-3}$ twice a week.

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

For Cd and Pb determination, the shoots were oven-dried to constant weight at 78°C, milled to homogeneous samples, and then treated with HNO_3 at 180°C, pressure 20 atm. for 40 min. (microwave oven Mars-5, Candela, USA). The concentrations of Cd and Pb were measured spectrophotometrically by using ICP (OPTIMA 2000 DV, Perkin-Elmer, USA).

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

RESULTS AND DISCUSSION

Mycorrhiza was not observed in the root system of the non-inoculated scarlet sage plants. Roots of the inoculated plants were colonized by AM fungi (Tab. 1). The addition of Cd and Pb to the growth substrate decreased root colonization by AMF. Mycorrhizal colonization was earlier shown to be delayed, reduced or even eliminated by high concentrations of heavy metals (Gildon and Tinker, 1983; Koul et al. 2001). No effect of high Cd concentration on AMF colonization of root system was observed by Chen et al. 2003. Differences in tolerance to Cd concentration in soils between different *Glomus* species were also noted (Liao et al. 2003).

Mycorrhizal plants had lower dry weight of shoots than those of the non-inoculated ones (Tab. 2 and 3). Mycorrhization also decreased plant height and inflorescence number. The effect of mycorrhization on shoot number was negligible. Mycorrhization did not affect flowering time of scarlet sage cultivated in the growing medium not contaminated with Cd, and slightly delayed flowering of plants cultivated in Cd and Pb polluted media.

Table 1

The effect of Cd and Pb concentrations in growing medium on percentage of scarlet sage (*Salvia splendens* Sello 'Torreador') root colonization by AMF.

Cd concentration (mg $Cd \cdot dm^{3}$)	Percentage of root coloni zation	Pb concentration (mg $Pb \cdot dm^{3}$)	Percentage of root coloni zation
0	40c	0	40c
10	27b	10	33bc
20	23a	100	30b
40	23a	200	20a

Means within columns followed by the same letter(s) are not significantly different at α 0.05

It is well know 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 mycorrhizal plants have more access to mineral nutrients due to the external hyphae development and they affect positively plant growth. In this experiment, all the plants were fertilized with a complete nutrient solution. In field cultivation, mycorrhizal roots can explore more soil volume than non-mycorrhizal ones, due to their extramatrical hyphae development (Sawaki and Saito, 2001). Results obtained by Dunham et al. (2003) also suggest that under greenhouse conditions AMF act to reduce plant growth despite increased mineral nutrition and photosynthetic activity. The effect of mycorrhization on Cd tolerance could be also less pronounced in pot culture than in field conditions.

Dry weight of shoots was unaffected by Cd concentration in the growing medium in both the non-inoculated and inoculated plants (tab. 2), although the lowermost leaves of the plants grown under the highest Cd concentration were slightly chlorotic. Higher Cd concentration in growing media accelerated flowering of the non-mycorrhizal plants and did not affect flowering time of the AMF inoculated plants. Higher Cd concentration in growing media slightly decreased the number of inflorescences. The inhibition of growth due to Cd pollution was frequently observed in other plants (Rivera – Becerril et al. 2002; Vassilev et al. 2004). Cadmium, if not detoxified rapidly in plant tissue, may induce oxidative stress leading to growth inhibition and finally cell death (Schutzendubel and Polle, 2002).

Pb concentration had no significant effect on growth of scarlet sage but slightly accelerated flowering time in the non-inoculated plants. One month after tre-

Table 2
The effect of cadmium concentration in growing medium and mycorrhizal inoculation on growth and flowering of scarlet sage (*Salvia splendens* Sello 'Torreador').

Treatments		DW of shoots (g)	Height of plant (cm)	Number of shoots	Number of days from planting to flowering	Number of infloresc.
Cd concentr. (mg Cd·dm ^{3})	AMF inocul.					
0		7.5c	34.9c	4.9b	31.8b	7.0d
	+	5.5a	29.8ab	4.6b	31.3b	5.4ab
10		6.9bc	34.3c	4.0ab	27.6a	5.7bc
	+	5.5a	28.8a	3.1a	31.3b	3.7a
20		6.7b	32.9c	4.9b	27.3a	6.2cd
	+	5.7a	29.8ab	3.3a	31.4b	4.6ab
40		7.0bc	32.3bc	4.9b	27.7a	5.9bc
	+	5.9a	28.9a	4.2ab	32.6b	4.0a
Significance						
Cd concentration		ns	ns	xx	xxx	xx
Mycorrhization		xxx	xxx	xx	xxx	xxx
Cd x Mycorrhiz.		ns	ns	ns	xx	x

The means in columns followed by the same letter(s) do not differ at α 0.05; ns., x, xx, xxx non significant or significant at α 0.1, 0.05, 0.001, respectively; non mycorrhizal plants, + mycorrhizal plants.

Table 3
The effect of lead concentration in growing medium and mycorrhizal inoculation on growth and flowering of scarlet sage (*Salvia splendens* Sello 'Torreador').

Treatments		DW of shoots (g)	Height of plant (cm)	Number of shoots	Number of days from planting to flowering	Number of infloresc.
Pb concentr. (mg Pb·dm ^{3})	AMF inocul.					
0		7.5c	34.9d	4.9a	31.8b	7.0c
	+	5.5a	29.8a	4.6a	31.3b	5.4b
10		6.8b	32.8bcd	4.8a	28.8a	7.2cd
	+	6.1a	31.0abc	3.9a	31.2b	5.7b
100		7.1bc	34.4d	4.8a	28.2a	6.7c
	+	5.7a	29.7a	4.1a	31.2b	5.4b
200		7.7c	33.7cd	4.9a	27.7a	8.1d
	+	5.6a	30.5ab	4.3a	31.5b	4.6a
Significance						
Pb concentration		n.s.	n.s.	n.s.	xxx	n.s.
Mycorrhization		xxx	xxx	xx	xxx	xxx
Pb x mycorrhiz.		xx	n.s.	n.s.	xxx	x

Explanations as in Table 2.

atment with Pb, chlorotic spots were observed on the lowermost leaves of the non-inoculated and inoculated scarlet sage plants grown at 200 mg Pb·dm^{-3}. Later on, the lowermost leaves became dry. It is well known that phytotoxicity of Pb is relatively low. Pb accumulates in the largest leaves, its transport to younger leaves is inhibited (Cseh et al. 2000). Stunting is a commonly observed growth response in a wide range of plants grown in metal polluted soils (Foy et al. 1978). Stunting, reduced biomass and chlorosis of Pb-treated plants can be due to the inhibition of chlorophyll synthesis and decline in the photosynthetic rate, a specific toxicity of the metal to the plant, antagonism with other nutrients or the inhibition of root penetration in the soil (Sharma and Dubey, 2005). In this experiment, the effect of Pb on growth and flowering of scarlet sage was negligible, probably due to low sensitivity to Pb pollution. The differential growth response of various species to Pb was earlier observed by Begonia (1997). These differences suggest that the phytotoxic mechanism of Pb involve different biochemical pathways in different plant species.

Heavy metals uptake by plants depends on both soil and plant factors (Gleba et al. 1990). The main soil factor affecting heavy metals uptake by plants is

Table 4
The effect of lead concentration in growing medium and AMF inoculation on Pb content in shoots of scarlet sage (*Salvia splendens* Sello 'Torreador').

Cd concentration (mg Cd·dm ^{3})	AMF inocul.	Cd content of shoots (mg·kg ^{1} DW)	Pb concentration (mg Pb·dm ^{3})	AMF inocul.	Pb content of shoots (mg·kg ^{1} DW)
0		0.86a	0		34.7a
	+	0.75a		+	31.2a
10		8.81b	10		22.7a
	+	12.55b		+	20.0a
20		20.4c	100		189.0b
	+	24.5c		+	282.0c
40		38.65d	200		147.5b
	+	50.10e		+	330.0c
Significance			Significance		
Pb concentration		xxx	Pb concentration		xxx
Mycorrhization		xx	Mycorrhization		xx
Pb x mycorrhiz.		xx	Pb x mycorrhiz.		xxx

Explanations as in Table 2.

soil heavy metal content. Among the plant factors affecting heavy metal uptake, plant genotype is considered the most important. Some plants have an exceptional capacity to accumulate Cd in high Cd exposure without any toxicity symptoms, great differences in Cd accumulation due to species and cultivars were also suggested (Tsadilas, 2000; Angelova et al. 2004). Cadmium and lead concentrations in scarlet sage shoots are shown in Table 4. Cd accumulation in scarlet sage shoots was dependent on Cd concentration in growing media. With the increased Cd doses, Cd content of the shoots increased as well, in both the non-mycorrhizal and mycorrhizal plants, although in the highest Cd exposure the mycorrhizal plants took up more Cd than the non-mycorrhizal ones. The ability of AM fungus hyphae to acquire and translocate Cd was earlier confirmed using separated zones for hyphae and roots (Guo et. al. 1996).

Low levels of Pb accumulated in shoots of the plants growing in the absence of added Pb (Tab. 4). Pb accumulation in shoots of scarlet sage increased greatly with the increasing Pb concentration in the growing medium. Earlier results showed that different plant species exhibit differential ability to take up Pb and to transport and accumulate it in shoots (Begonia, 1997). Mycorrhization increased Pb accumulation in shoots of scarlet sage grown at 100 and 200 mg Pb·dm^{-3}. Increased accumulation of Pb in shoots of AMF inoculated red kidney and wheat plants was noted by Rabie (2005). The higher metal concentration in the AMF inoculated plants could be explained by the fact that inoculation enlarges the absorbing area and efficient hyphal translocation.

CONCLUSIONS

1. The effect of Cd and Pb on growth and flowering of scarlet sage was negligible, but heavy metal pollution lowered the decorative value of plants due to chlorosis and necrosis of leaves.

2. Accumulation of Cd and Pb in scarlet sage shoots is dependent on metals content in the growing medium.

3. Mycorrhization increased Cd and Pb accumulation in shoots of scarlet sage grown at high concentrations of these metals in the growing medium.

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Wpływ stężeń kadmu i ołowiu oraz mikoryzacji na wzrost, kwitnienie i akumulację metali ciężkich w szałwii lśniącej (*Salvia splendens* Sello 'Torreador')

Streszczenie

Badania miały na celu określenie wpływu zawartości kadmu (0, 10, 20, 40 mg $Cd \cdot dm^{-3}$) i ołowiu (0, 10, 100, 200 mg $Pb \cdot dm^{-3}$) w podłożu oraz mikoryzy arbuskularnej na wzrost, kwitnienie i akumulację Cd i Pb w pędach szałwii lśniącej. Zarówno Cd jak i Pb wpływały ujemnie na kolonizację korzeni przez grzyby tworzące mikoryzę arbuskularną. Wpływ obu metali ciężkich na wzrost szałwii był niewielki. Cd w stężeniu 40 $mg \cdot dm^{-3}$ obniżał liczbę kwiatostanów i powodował lekką chlorozę liści dolnych, a Pb w stężeniu 200 $mg \cdot dm^{-3}$ zasychanie liści dolnych. Oba metale ciężkie przyspieszały kwitnienie roślin nie poddanych mikoryzacji, niezależnie od stężenia w podłożu. Zawartości Cd i Pb w pędach szałwii lśniącej wzrastały wraz ze wzrostem zawartości tych pierwiastków w podłożu, zarówno u roślin nie zmikoryzowanych jak i zmikoryzowanych. Mikoryzacja wpływała ujemnie na wzrost szałwii oraz zwiększała akumulację Cd i Pb w pędach roślin rosnących w podłożach silnie zanieczyszczonych tymi pierwiastkami.