

CADMIUM-INDUCED CHANGES IN GROWTH AND MICRONUTRIENT COMPOSITION OF TWO PEPPER CULTIVARS

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Abstract. The present research was conducted to investigate the effects of cadmium (Cd) treatments on shoot and root dry weight, SPAD values, shoot and root Cd accumulations and micronutrient compositions of two pepper cultivars (*Capsicum annuum* L. cvs. 'Demre' and 'AT58'). Plants were grown under controlled conditions with 0 and 15 μM Cd supplies. Decreasing root and shoot dry weights were observed with increasing Cd doses ($P < 0.05$). Decrease in shoot and root dry weights with 15 μM Cd supply was higher in Demre cultivar than in AT58 cultivar. Cd-induced decreases in SPAD values were greater in Demre cultivar than in AT58 cultivar. Shoot Cd concentrations were lower and root Cd concentrations were greater in AT58 cultivar than in Demre cultivar. Decreasing shoot zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations and root Zn and Mn concentrations were observed with Cd treatments. Considering the Cd uptake and transport to shoot, it was observed that there were differences in tolerance of cultivars to Cd toxicity, but it was thought that these differences were not necessarily related to microelement uptake and transport of the cultivars.

Keywords: *heavy metal, SPAD value, zinc, iron, manganese, copper*

Introduction

Cadmium (Cd) is a highly toxic heavy metal and poses significant threats to human health and environment. Developed industrial and agricultural practices lead to significant increases in soil cadmium levels (Sarwar et al., 2010). Phosphorus fertilizers, Cd-containing wastewater treatment sludge, livestock manure, wastewater effluents, various metal processing industries, cement facilities and urban traffic are considered to be the greatest sources of Cd for soil (Alloway and Steinnes, 1999; Yang et al., 2004). Although cadmium is not considered as an essential nutrient for plants, soil available Cd is easily up taken through the roots and accumulated in the plant tissues and pose serious health risks on humans through the food chain (Zhou and Qiu, 2005). High cadmium concentrations also damage plant roots, destruct photosynthetic activity, recess plant growth and development and hinder plant nutrient and water uptake from the soil (Jibril et al., 2017). High cadmium levels influence cell membrane permeability (Sengar et al., 2008) and destruct antioxidant defense mechanisms of the plants against oxidative stress conditions through elevated lipid peroxidation (Benavides et al., 2005).

Plant normal cadmium concentrations were reported as between 0.2-0.8 mg kg^{-1} and toxic levels as between 5-30 mg kg^{-1} (Allen, 1989; Kloke et al., 1984). Similar with the other stressors, Cd also hinders plant nutrient uptake, interacts with the soil available nutrients and ultimately results in imbalanced mineral nutrition (Khan et al., 2007). There is a great competition between Cd ions and the other essential plant nutrients including Ca, Mg, K, Cu, Zn, Fe, Ni and Mn required for plant growth and development (Clarkson and Luttge, 1989; Rivetta et al., 1997). In a previous study, either synergistic or antagonistic effects of cadmium were reported on plant nutrients (macro or micro) of different wheat cultivars (Zhang and Huang, 2000). In other studies, either negative

(Bhandal and Kuar, 1992) or positive (Mitchel et al., 2000) correlations of cadmium were reported with nitrogen. Similarly, antagonistic (Li et al., 1990) and synergistic (Nan et al., 2002) interactions of cadmium were reported with zinc. Cadmium treatments increased Cu and Mo levels and decreased K, Ca, Mg and Mn levels of Birch seedlings (Gussarsson, 1994). Increasing Cd levels resulted in greater Cd, Zn, Fe, Mn and Cu accumulations in roots and slight Cd transfer to shoots (Wang et al., 2007).

Different plants have various cadmium accumulation capacities (Yang et al., 1996; Obata and Umebayashi, 1997; Yildiz, 2005). However, variations were also reported among the different cultivars of wheat (Naeem et al., 2016), barley (Tiryakioğlu et al., 2006), maize (Ekmekci et al., 2008), soybeans (Shamsi et al., 2008), tomato (Hussain et al., 2015) and chilli peppers (Xin et al., 2013). Kuboi et al. (1986) classified Cd accumulation capability of the plants into three groups (high, moderate and low accumulators). Pepper is the second crop after tomato produced in greenhouses of Turkey and high-quality yield is the most critical issue in pepper cultivation in greenhouses (Abdel Latef, 2013). Therefore, the present research was implemented to investigate the Cd accumulation in two pepper (*Capsicum annum* L.) cultivars of *Solanaceae* family. The primary target was to identify the effects of cadmium treatments on plant development and micronutrient compositions of pepper cultivars with different Cd accumulation levels.

Materials and methods

Experiment and analyses

Demre and AT58 pepper cultivars were used as the plant material of this study. The plants were grown under controlled climate conditions (26/22 °C day/night temperature, 16/8 h photoperiod, 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity supplied with Osram HQI/2000/D lamps and 65-75% relative humidity). Surface-sterilized seeds with 1% (w/v) calcium hypochlorite for 10 min were rinsed through distilled water. Seeds were then sown in perlite-filled pots and allowed to germinate for 7 days. When the seedlings reached to two true-leaf stage in perlite media (12 days old), they were transferred to 2.7 L plastic pots (four seedlings per pot) filled with continuously aerated and diluted 1:3 nutrient solution for 2 days to ensure time for plant growth. Thinning was performed then as to have two seedlings in each pot. Nutrient solutions were prepared with distilled water and 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 0.88 mM K_2SO_4 , 1 mM MgSO_4 , 0.25 mM KH_2PO_4 , 0.1 mM KCl, 100 μM Fe-EDTA, 10 μM H_3BO_3 , 0.5 μM MnSO_4 , 1 μM ZnSO_4 , 0.2 CuSO_4 and 0.02 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$. Cadmium ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) was supplemented into nutrient solutions at five-to-six true leaf stage (25 days old) of growth for 8 days. All solutions were adjusted as to have a pH of 6.3 ± 0.1 . Nutrient solutions were replaced in every 3-4 days throughout the growing period. Leaf chlorophyll contents were identified with the aid of a chlorophyll meter (SPAD-502, Minolta, Japan) before the harvest. The 32-day old plants were harvested and at harvest, roots were rinsed through 0.5 mM CaSO_4 and de-ionized water for 15 min, and dried at 70 °C to determine the dry weights.

Dried shoots and roots were ground; wet-digested in a microwave with 2 ml 35% H_2O_2 and 5 ml 65% HNO_3 . Digested samples were then subjected to elemental analyses for Cd, Zn, Fe, Mn and Cu by an inductively coupled plasma optical emission spectrometer ICP-OES; (Varian-Vista Pro) device. Measurements were checked with the readings on reference samples of National Institute of Standards and Technology (Gaithersburg, MD, USA).

Statistical analysis

Experimental data were subjected to variance analysis in accordance with randomized plots design with 3 replications. Means were compared by least significant difference (LSD) test at 5% probability level.

Results and discussion

Dry matter yields, SPAD readings and cadmium in shoots and roots

Cadmium treatment (15 μM) reduced shoot dry matter yield by 37% in AT58 cultivar and by 48% in Demre cultivar and reductions in root dry matter yields were respectively observed as 21 and 29% (Table 1). Cd-induced regress in plant growth was also reported for wheat (Naeem, 2016), tomatoes (Kumar et al., 2015), sunflower (Azevedo et al., 2005) and pepper (Abdel Latef, 2013). In this study, more chlorosis and necrotic patches were observed over the oldest leaves of Demre cultivar than the leaves of AT58 at 15 μM Cd dose. Root browning degrees were also greater in Demre cultivar than in AT58 under Cd treatments. Generally, reduced root elongation (Dong et al., 2005) and browning (Liu et al., 1995) are the initial symptoms for cadmium toxicity in roots and chlorosis and rolling are the initial symptoms for cadmium toxicity in shoots (Weigel and Jäger, 1980).

SPAD values significantly decreased with Cd treatment in both cultivars and the decrease rates with 15 μM Cd treatment were found to be 15% in AT58 and 30% in Demre cultivar (Table 1). Sandalio et al. (2001) indicated the reason of Cd-induced reductions in chlorophyll content as chlorophyll degradation or destructions in chlorophyll biosynthesis and membrane integrity.

Table 1. Effects of cadmium (-Cd = 0 and +Cd = 15 μM) treatments on shoot-root dry weights and SPAD values of AT 58 and Demre pepper cultivars

Cultivars	Dry matter yields (mg plant ⁻¹)*				SPAD values	
	Shoot		Root		- Cd	+ Cd
	- Cd	+ Cd	- Cd	+ Cd		
AT58	1139 aA	723 bA	154 aB	122 bB	53 aA	45 bA
Demre	1240 aA	643 bA	234 aA	167 bA	42 aB	30 bB

*Means indicated with different small letters (between Cd treatment, in each cultivar) and by the same capital letters (between cultivars, in each Cd treatment) are significantly different at $p < 0.05$

Cadmium treatment (15 μM) significantly increased shoot Cd concentrations and contents of both cultivars (Table 2). Demre had higher shoot Cd concentration and content than AT58. While shoot Cd concentration and content of Demre cultivar at 15 mM Cd treatment were 148 mg kg⁻¹ DW and 95.1 $\mu\text{g plant}^{-1}$, the values in AT58 cultivar were 90.5 mg kg⁻¹ DW and 65.1 $\mu\text{g plant}^{-1}$, respectively. Root Cd concentrations and contents of AT58 were greater than Demre (Table 2). Root Cd concentration and content of AT 58 were respectively measured as 1529.7 mg kg⁻¹ DW and 186.8 $\mu\text{g plant}^{-1}$. For both cultivars, roots had greater Cd concentrations and contents than the shoots (Table 2). Cataldo et al. (1983) pointed out that large portion of cadmium retained in plant roots and a small portion was transferred to shoots. Blum (1997) reported the greatest Cd content for roots and the least Cd content for seeds;

stem, leaves and fruits were placed in between them. Regardless of the concentrations, both cultivars differ in their root and shoot Cd accumulation capacity. Although root Cd contents were not influenced significantly by cadmium treatments, high root Cd content of AT58 than Demre was found to be compatible with root Cd concentrations of these cultivars (Table 2). Previous researches tried to explain the differences in Cd-tolerance of plants with the differences in their Cd-uptake and accumulation levels (Hall, 2002; Kochian et al., 2002). Complying with these hypotheses, AT58 cultivar of the present study had greater root Cd concentration and smaller shoot Cd concentration than Demre cultivar. Such findings indicated that AT58 cultivar retained greater Cd quantities in roots.

Table 2. Effects of cadmium (-Cd = 0 and +Cd = 15 µM) treatments on shoot-root Cd concentration and content of AT58 and Demre pepper cultivars

Cultivars	Cd concentrations (mg kg ⁻¹ DW)*				Cd contents (µg plant ⁻¹)			
	Shoot		Root		Shoot		Root	
	- Cd	+ Cd	- Cd	+ Cd	- Cd	+ Cd	- Cd	+ Cd
AT58	0.3 bA	90.5 aB	2.9 bA	1529.7 aA	0.4 bA	65.1 aB	0.5 bA	186.8 aA
Demre	0.4 bA	148.0 aA	3.7 bA	1036.1 aB	0.5 bA	95.1 aA	0.9 bA	173.0 aA

*Means indicated with different small letters (between Cd treatment, in each cultivar) and by the same capital letters (between cultivars, in each Cd treatment) are significantly different at $p < 0.05$

Shoot and root micronutrients

Cadmium treatment (15 µM) significantly decreased ($p < 0.05$) shoot Zn, Mn, Cu and Fe concentrations and contents of both cultivars (Table 3). Shoot Zn, Mn, Cu and Fe concentrations of AT58 cultivar decreased with Cd treatment at slightly greater rates than at Demre cultivar. The average Cd-induced decrease was 55% for Zn and Mn and 69% for Fe and Cu (Table 3). Cd-treatment also reduced root Zn and Mn levels significantly. Cd treatments reduced Zn and Mn levels of wheat root and shoots, but did not influence shoot and root Fe and Cu levels (Jalil et al., 1994). Cadmium toxicity mostly comes from the interactions of Cd with the other essential nutrients, especially with the same valence (Skrebsky et al., 2008). In a hydroponic experiment with barley, Cd treatments significantly decreased root and shoot Zn, Cu and Mn concentrations and shoot Fe concentrations (Wu et al., 2003).

In the present study, while root Cu content of AT58 decreased significantly with Cd treatment, root Cu content of Demre and root Cu concentrations of both cultivars did not change (Table 3). Iron concentration and accumulation in roots of spinach was not affected by Cd treatments (Abul Kashem and Kawai, 2007). Similar with those findings, present root Fe concentrations and contents of both pepper cultivars remained unchanged with Cd supply (Table 3). Kabata-Pendias and Pendias (2001) indicated strong bonds of Cu and Fe in root cells.

Present differences in shoot Zn and Cu levels were mostly attributed to Cd-induced regress in plant growth and development. Thusly, shoot Zn and Cu concentrations were not significantly different (Table 3). Similarly, shoot Fe and Mn concentrations-contents were not also significantly different at 15 µM Cd treatment (Table 3). Under Cd supply, there seems to be antagonistic relationships between root Cd concentrations-contents and root Zn concentrations-contents (Table 3). Under controlled conditions, Demre

cultivar generally had greater quantities of Zn, Fe, Mn and Cu than AT58 cultivar (Table 3). Such a case may result in significantly different Zn and Cu accumulation levels under Cd supply. Differences in cultivars may be related to present Cd dose, exposure duration to this dose and micronutrient levels of the cultivars under controlled conditions.

Table 3. Effects of cadmium (-Cd = 0 and +Cd = 15 μ M) treatments on shoot-root Zn, Fe, Mn and Cu concentration and content of AT 58 and Demre pepper cultivars

Cultivars	Zn		Fe		Mn		Cu	
	- Cd	+ Cd	- Cd	+ Cd	- Cd	+ Cd	- Cd	+ Cd
<i>Shoot concentrations (mg kg⁻¹ DW)*</i>								
AT58	36.2 aB	15.2 bB	175.0 aA	51.1 bA	26.6 aB	11.1 bA	8.3 aA	2.4 bB
Demre	41.1 aA	19.6 bA	215.1 aA	66.0 bA	31.5 aA	14.4 bA	9.1 aA	3.2 bA
<i>Root concentrations (mg kg⁻¹ DW)</i>								
AT58	101.3 aA	54.7 bB	976.0 aA	1032.1aA	112.1aA	11.0 bA	36.0 aA	35.8 aA
Demre	100.8 aA	66.7 bA	708.0 aA	734.1 aB	49.8 aB	7.0 bB	31.0 aA	33.6 aA
<i>Shoot content (μg plant⁻¹)</i>								
AT58	41.0 aA	11.0 bA	199.3 aA	37.0 bA	30.2 aB	8.0 bA	9.5 aA	1.7 bA
Demre	51.2 aA	12.6 bA	266.2 aA	42.4 bA	39.2 aA	9.2 bA	11.3 aA	2.1 bA
<i>Root content (μg plant⁻¹)</i>								
AT58	15.5 aB	6.7 bB	150.6 aA	126.5 aA	17.2 aA	1.4 bA	5.5 aA	4.4 bB
Demre	23.3 aA	11.1 bA	169.7 aA	122.6 aA	12.0 aA	1.2 bA	7.3 aA	5.6 aA

*Means, indicated with different small letters (between Cd treatment, in each cultivar) and by the same capital letters (between cultivars, in each Cd treatment) are significantly different at $p < 0.05$

Conclusion

Cadmium treatment generated significant decreases in both root and shoot dry matter yields. Such decreases were greater in Demre cultivar than in AT58 cultivar. Similarly, greater decreases were observed in SPAD values of Demre cultivar than of AT58 cultivar with Cd treatment. Regardless of shoot Cd uptake and accumulation, it was observed that there were differences in Cd toxicity tolerance of the cultivars, but these differences were not attributed to differences in microelement uptake and transport of the cultivars. For Cd-polluted sites, AT58 can be recommended to reduce yield losses. Further studies are recommended to compare antioxidative defense mechanisms of AT58 and Demre pepper cultivars with different root and shoot Cd accumulation levels.

Conflict of interests. The author has not declared any conflict of interests.

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