ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 102, No. 2 (2015), p. 193–200
DOI 10.13080/z-a.2015.102.025

Cadmium effects on mineral accumulation, antioxidant defence system and gas exchange in cucumber

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Abstract

The effects of different concentrations (10, 50, 100 and 500 μM) of cadmium (Cd) on manganese (Mn) and iron (Fe), lipid peroxidation, activities of antioxidant enzymes and photosynthetic function were investigated in hydroponically grown cucumber (*Cucumis sativus* L.). Results indicated that cadmium was accumulated primarily in roots. In the roots and shoots, the cadmium content increased with the increasing cadmium concentrations, and induced decrease of manganese significantly ($P < 0.005$). The levels of iron had an increasing trend with increasing cadmium concentration and duration of treatment. Cadmium-induced oxidation stress and lipid peroxidation in *C. sativus* showed by the increased concentration of MDA (malondialdehyde). Cadmium induced higher SOD (superoxide dismutase) and POD (peroxidase) activities than CAT (catalase) activity, suggesting that SOD and POD provided a better defence mechanism against cadmium-induced oxidative damage in *C. sativus*. The net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration were reduced in the presence of cadmium.

Key words: antioxidant enzymes, cadmium, *Cucumis sativus*, lipid peroxidation, photosynthetic parameters.

Introduction

Cadmium (Cd) has no known function as a nutrient and is toxic to plants. When taken up by plants, cadmium may cause several physiological, biochemical and structural changes (Benavides et al., 2005). Cadmium has been shown to interfere with the uptake, transport and use of several elements (Álvarez-Fernández, 2014; Sperotto et al., 2014). Cadmium has been shown to be one of the most effective inhibitors of photosynthetic activity. It can enter chloroplasts and disturb chloroplast function by inhibiting the enzymatic activities of chlorophyll biosynthesis and chloroplast structure (Ying et al., 2010). Stomatal opening, a transpiration, photosynthesis and antioxidant metabolism have been reported to be affected by cadmium (Feng et al., 2010; Shi et al., 2010). Cadmium can produce disturbances in plant antioxidant defences giving rise to oxidative stress. Cadmium-induced production of the ROS (reactive oxygen species), H_2O_2 and O_2^-, can be attributed to the phytotoxic effect of cadmium, but lower levels of ROS can function as signal molecules in the induction of defence genes against cadmium toxicity (Li, Huang, 2014).

Cucumber (*Cucumis sativus* L.) is an economically important and widely distributed crop. It is reported that fresh fruit of cucumber per 100 g contains 94–97 g water, 1.6–4.1 g carbohydrates, 0.4–1.2 g protein, 4–25 mg vitamin C, and it also contains calcium, iron, phosphorus and other minerals (Lin et al., 2012). *C. sativus* is sensitive to cadmium. Under cadmium stress, its growth was inhibited, reducing the yield and quality of cucumber. *C. sativus* absorbs cadmium from soils and eventually translocates it to edible parts. Therefore, consumption, either directly or indirectly, of edible parts with high levels of cadmium can be a food safety concern. For the present investigation, we examined the cadmium toxic effects on other mineral accumulation, antioxidant enzymes, malondialdehyde content, leaf gas exchange and photosynthetic parameters in *C. sativus* subjected to various levels of cadmium. It is very important to elucidate cadmium toxicity to *C. sativus* and the possible mechanism of action.

Materials and methods

Plant cultivation. The seeds of cucumber (*Cucumis sativus* L., 'Jinyou' No. 35) used in the present investigation were supplied by Tianjin Vegetable Research Institute, China. After soaking for 12 h, they were allowed to germinate and grew in vermiculite in seven containers with 1/2 nutrient solution in a climate chamber with a day/night period of 14/10 h, a day/night temperature and humidity regime of 25/20°C and 55/75% relative humidity, respectively. After the second leaf of *C. sativus*

seedlings just came out, seedlings were uniformly selected and treated with different concentrations of cadmium – 0, 10, 50, 100 and 500 μM for 5, 10, 15 and 20 days. The solution consisted of 0.75 mmol L^{-1} K_2SO_4, 0.65 mmol L^{-1} $MgSO_4 \times 7H_2O$, 0.01 mmol L^{-1} KCl, 0.25 mmol L^{-1} KH_2PO_4, 2 mmol L^{-1} $Ca(NO_3)_2 \times 4H_2O$, 100 μmol L^{-1} FeEDTA, 10 μmol L^{-1} H_3BO_3, 1 μmol L^{-1} $MnSO_4 \times H_2O$, 0.1 μmol L^{-1} $CuSO_4 \times 5H_2O$, 0.05 μmol L^{-1} $(NH_4)_6Mo_7O_4 \times 4H_2O$ and 1 μmol L^{-1} $ZnSO_4$ at a pH of 5.5 (Zhang et al., 2009). The nutrient solutions were continuously aerated and changed regularly every 5 days until the seedlings were harvested. Cadmium (Cd) was provided as cadmium chloride ($CdCl_2 \times 2.5H_2O$). Cadmium stock solution was prepared in deionized water. The test solutions were changed every 5 days. Meanwhile the 1/2 strength Hoagland solution without cadmium was used for the control plants. Macroscopic observations and all determinations were conducted at the end of each time interval (5 days). The seedlings were used to determine contents of Cd and other minerals, antioxidant enzyme activities, concentration of MDA (malondialdehyde) and photosynthetic parameters in the present investigation.

Determination of cadmium and other minerals. Twenty plantlets from each treatment were harvested based on uniformity of size and colour (removing the greatest and the smallest plantlets and then selected randomly) after 20 days of incubation. The seedlings were removed from solution and washed thoroughly with running tap water for 30 min and then with deionized water to remove traces of nutrients and cadmium ions from seedling surfaces. Concentrations of cadmium (Cd), manganese (Mn) and iron (Fe) were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Leeman Labs Inc., USA).

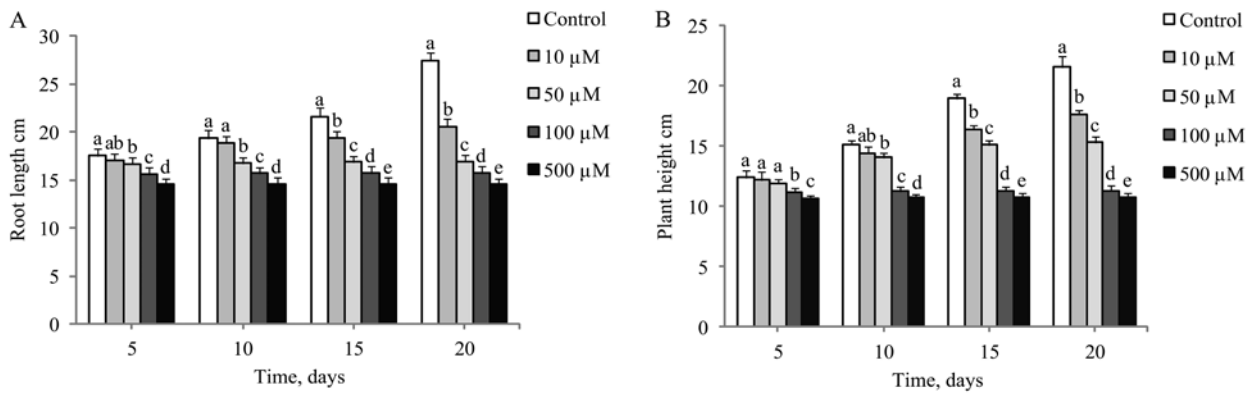
Determination of antioxidant enzyme activities and MDA concentration. The fresh roots or leaves from each treatment were homogenized in a pestle and mortar with 0.05 M sodium phosphate buffer (pH 7.8) at the end of each time interval (5 days). The homogenate was centrifuged at 10 000× g for 20 min, and the supernatant was used for analyzing superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). The above steps were carried out at 4°C. The methods are described in more detail (Li et al., 2013). The fresh leaf (1 g) in each treatment was homogenized in 10 mL of 10% TCA (trichloroacetic acid) with a pestle and mortar. The homogenates were centrifuged at 4000× g for 20 min. To 2 mL aliquot of the supernatant, 2 mL of 0.6% thiobarbituric acid in 10% TCA was added. The mixture was heated at 100°C for 15 min and then quickly cooled in an ice bath. After centrifugation at 10 000× g for 10 min, the absorbance of the supernatant was recorded at 532 and 450 nm. Lipid peroxidation was expressed as MDA content in l M kg^{-1} fresh weight.

Leaf gas exchange and photosynthetic parameter measurements. Leaf gas exchange and photosynthetic parameters: net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i), were measured in the first fully expanded leaf from the top shoot at the end of each time interval (5 days), using handheld photosynthesis system CI-340 (CID Inc., USA). The measurements were performed between 10:00 and 11:00 a.m. under the greenhouse conditions described above. Data were given and recorded by the machine.

Statistical analysis. In treatment groups, seedlings were examined for macroscopic observations and all determinations at the end of each time interval (5days). Each treatment was run in triplicate for statistical validity. Data from this investigation were analyzed with standard statistical software *Sigma Plot 8.0* using means ± standard error. For equality of averages the *t*-test was applied. Results were considered statistically significant at $p < 0.05$.

Results

Effects of cadmium on seedlings. The seedling growth of *C. sativus* varied with the cadmium concentration and duration of treatment (Fig. 1). During the 10 days, 10 μM Cd exposure had no toxic effect on root length. After 15 days, the root growth was inhibited significantly ($P < 0.05$). In the other three treatment groups the root length was inhibited significantly ($P < 0.05$) during the duration of the experiment when compared to control except for the group treated with 50 μM Cd for 5 days (Fig. 1 A). At lower concentrations of Cd (10 and 50 μM), the roots became yellow, and at 500 μM Cd, most seedling roots appeared black. The seedlings treated with 10 and 50 μM Cd for 5 days showed the same growth trend as the control. At the 50 to 500 μM Cd dose, the seedling growth was inhibited significantly ($P < 0.05$) during the entire experiment except for the group exposed to 50 μM Cd for 5 days. At 100 and 500 μM Cd concentrations, the seedling growth almost completely stopped during the whole course of treatment (Fig. 1 B).



Note. Vertical bars denote standard error, n = 10; values with different letters differ significantly from each other ($p < 0.05$, *t*-test).

Figure 1. Effects of different concentrations of cadmium (Cd) on *Cucumis sativus* seedlings stressed for 5, 10, 15 and 20 days

Leaves of control plants showed green in colour during the whole experiment. In contrast to control leaves, the serious chlorotic leaves with slight green around veins were exhibited after exposure to cadmium. In the group of plants treated with 500 μM Cd, the leaves showed chlorotic symptoms like the plants with iron-deficiency (Fig. 2).

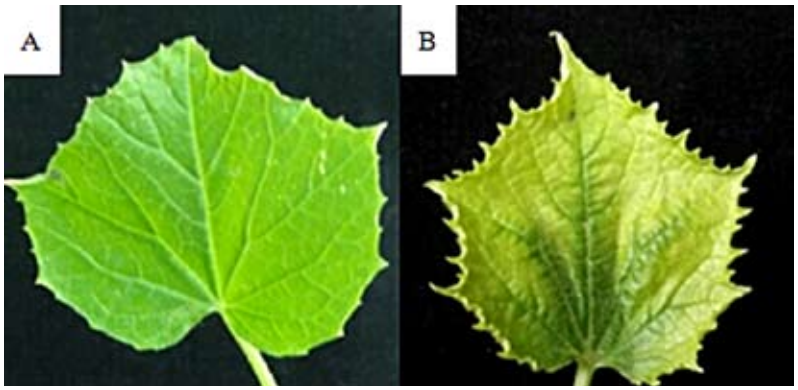


Figure 2. Visible symptoms in leaves of *Cucumis sativus* exposed to cadmium (Cd) for 20 days (A – control, B – 500 μM Cd)

by roots, stems and shoots increased with increasing cadmium concentration and prolonged treatment time. The accumulation of cadmium ions primarily was in roots, and small amounts of cadmium were transported to stems and leaves. Levels of cadmium in *C. sativus* treated with cadmium were in the order: roots > stems > leaves. In the presence of cadmium, the contents of manganese in roots, stems and leaves of *C.sativus* decreased with increasing cadmium concentration. Manganese accumulation was inhibited significantly ($P < 0.005$). The levels of iron in roots, stems and leaves of *C. sativus* had an increasing trend with increasing cadmium concentration and duration of treatment.

Cadmium accumulation and its effect on other minerals. Statistical analysis showed the presence of significant correlations between the concentration of cadmium and microelements (manganese and iron). The accumulation of cadmium in *C. sativus* roots, stems and shoots varied with cadmium concentration and treatment time. As shown in Table 1, levels of cadmium accumulation

Effects of cadmium on the activities of SOD, CAT and POD. Effects of cadmium on SOD activities in roots and leaves of *C. sativus* varied with the different concentrations of cadmium and the duration of treatment (Fig. 3 A and B). Figure 3 A showed that SOD activities in roots exposed to cadmium during the whole experiment time were observed to be significant ($P < 0.05$) in comparison with control. The activity of SOD in leaves exposed to cadmium increased significantly ($P < 0.05$) during the whole treatment when compared with control (Fig. 3 B). Both controls and cadmium treatments showed higher SOD activity in leaves than in roots. The activities of POD in *C. sativus* are presented

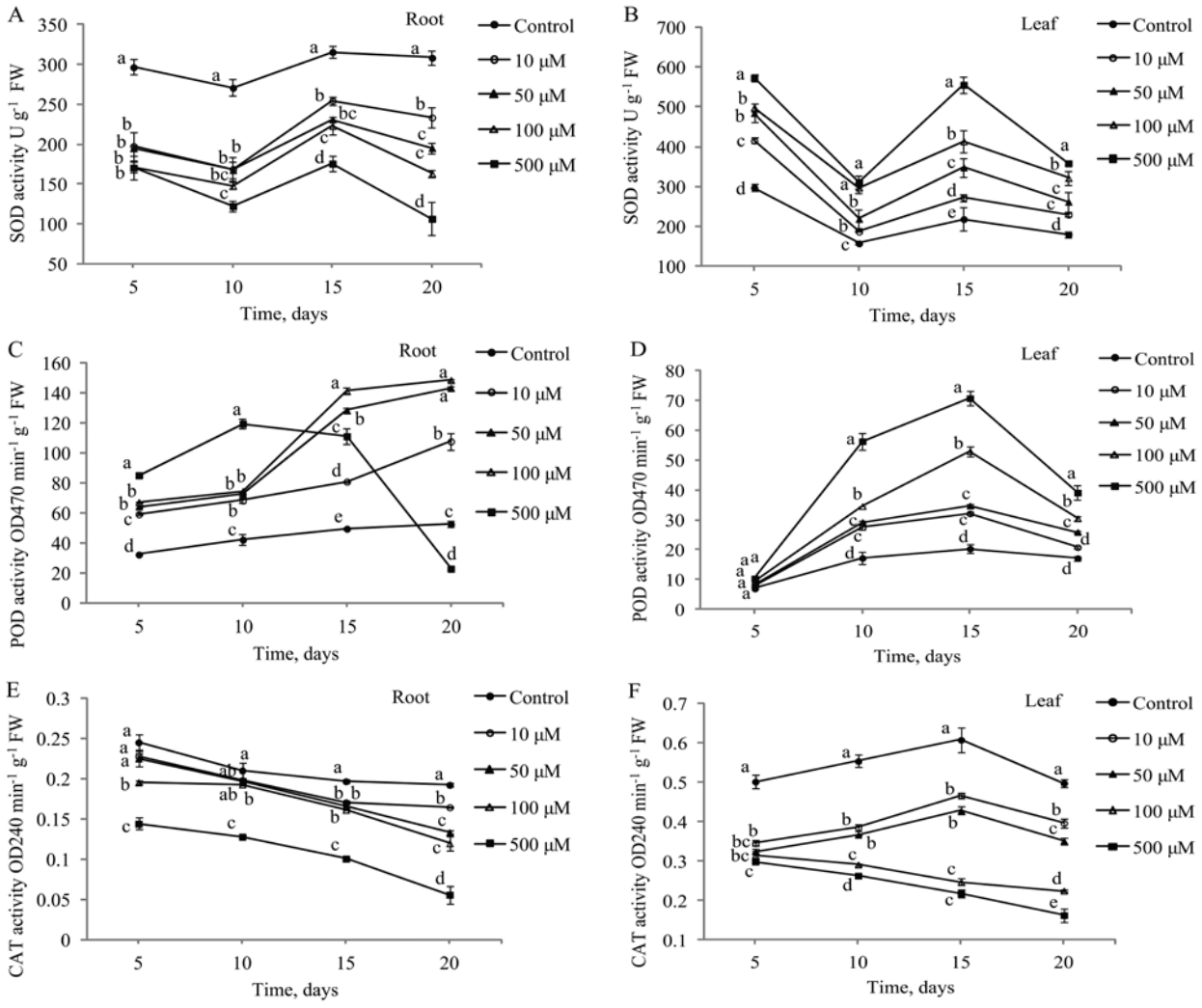
Table 1. Cadmium (Cd), manganese (Mn) and iron (Fe) accumulation by *Cucumis sativus* root, stem and leaf

Time, days	Treatment μM	Root			Stem			Leaf		
		μg g^{-1} dry weight ± standard error								
		Cd	Mn	Fe	Cd	Mn	Fe	Cd	Mn	Fe
1	2	3	4	5	6	7	8	9	10	11
5	Control	0.00 ± 0.00 a	83.16 ± 0.28 a	620 ± 0.36 a	0.00 ± 0.00 a	11.26 ± 0.08 a	5.20 ± 0.06 a	0.00 ± 0.00 a	146 ± 0.26 a	49.53 ± 0.16 a
	10	3178 ± 16.64 b	63.18 ± 0.48 b	1133 ± 3.93 b	285 ± 1.71 b	9.69 ± 0.09 b	9.90 ± 0.38 b	82.45 ± 2.06 b	78.51 ± 0.23 b	58.27 ± 0.36 b
	50	5270 ± 13.87 c	56.84 ± 0.49 c	1264 ± 10.19 c	721 ± 1.24 c	7.78 ± 0.03 c	17.69 ± 0.09 c	151 ± 0.75 c	73.47 ± 0.26 c	92.51 ± 1.40 c
	100	8592 ± 5.26 d	37.57 ± 0.23 d	1624 ± 3.51 d	1185 ± 2.10 d	7.35 ± 0.05 d	23.31 ± 0.58 d	161 ± 0.79 d	70.81 ± 0.12 d	118 ± 0.37 d
	500	12861 ± 112.05 e	31.71 ± 0.21 e	1745 ± 5.31 e	1833 ± 1.49 e	6.38 ± 0.10 e	31.08 ± 0.38 e	307 ± 0.94 e	67.23 ± 0.38 e	141 ± 0.04 e
10	Control	0.00 ± 0.00 a	85.36 ± 0.29 a	1412 ± 8.41 a	0.00 ± 0.00 a	26.42 ± 0.24 a	12.41 ± 0.65 a	0.00 ± 0.00 a	154 ± 0.03 a	98.47 ± 0.04 a
	10	4445 ± 9.25 b	71.87 ± 0.15 b	1619 ± 3.85 b	363 ± 3.02 b	21.52 ± 0.04 b	17.62 ± 0.75 b	163 ± 0.46 b	76.31 ± 0.25 b	101 ± 0.14 b
	50	6295 ± 14.24 c	61.94 ± 0.23 c	1842 ± 12.09 c	919 ± 1.83 c	19.12 ± 0.15 c	21.57 ± 0.78 c	243 ± 0.63 c	70.62 ± 0.33 c	105 ± 0.22 c
	100	10075 ± 15.47 d	48.69 ± 0.09 d	2014 ± 7.81 d	1224 ± 0.18 d	16.47 ± 0.17 d	24.97 ± 0.57 d	262 ± 1.49 d	63.97 ± 0.10 d	122 ± 0.29 d
	500	13936 ± 22.66 e	35.03 ± 0.14 e	2329 ± 3.98 e	2218 ± 1.91 e	14.40 ± 0.10 e	32.75 ± 0.48 e	362 ± 0.81 e	48.58 ± 0.04 e	154 ± 0.54 e

Table 2 continued

1	2	3	4	5	6	7	8	9	10	11
15	Control	0.00 ± 0.00 a	125± 0.21 a	1821 ± 10.07 a	0.00 ± 0.00 a	30.56 ± 0.12 a	15.99 ± 0.33 a	0.00 ± 0.00 a	163 ± 2.29 a	100 ± 0.38 a
	10	5147 ± 4.65 b	98.59 ± 0.35 b	2151 ± 13.57 b	501 ± 4.34 b	25.48 ± 0.10 b	18.17 ± 0.419 b	217 ± 1.19 b	70.34 ± 0.02 b	105 ± 1.41 b
	50	7772 ± 23.01 c	64.60 ± 0.32 c	2200 ± 10.25 c	1191 ± 6.24 c	21.44 ± 0.04 c	23.51 ± 0.16 c	293 ± 0.44 c	60.07 ± 0.02 c	112 ± 0.25 c
	100	11332 ± 18.93 d	53.02 ± 0.20 d	2336 ± 10.37 d	1377 ± 11.81 d	19.20 ± 0.06 d	26.86 ± 0.16 d	333 ± 1.30 d	57.99 ± 0.16 c	140 ± 1.53 d
	500	18829 ± 33.27 e	37.44 ± 0.35 e	3080 ± 7.06 e	2697 ± 20.57 e	18.66 ± 0.05 e	40.15 ± 0.27 e	385 ± 0.94 e	45.16 ± 0.24 d	159 ± 1.54 e
20	Control	0.00 ± 0.00 a	208 ± 1.19 a	2190 ± 1.57 a	0.00 ± 0.00 a	33.17 ± 0.07 a	19.34 ± 0.19 a	0.00 ± 0.00 a	169 ± 0.45 a	122 ± 1.23 a
	10	7049 ± 10.50 b	104 ± 0.39 b	2581 ± 21.88 b	588 ± 7.46 b	25.74 ± 0.10 b	22.38 ± 0.23 b	241 ± 1.33 b	54.59 ± 0.07 b	128 ± 0.48 b
	50	9596 ± 16.02 c	70.72 ± 0.12 c	2817 ± 4.60 c	1348 ± 5.69 c	22.20 ± 0.20 c	24.59 ± 0.23 c	329 ± 0.46 c	43.96 ± 0.23 c	137 ± 0.39 c
	100	12471 ± 41.52 d	65.78 ± 0.42 d	3537 ± 42.98 d	1651 ± 2.32 d	19.36 ± 0.11 d	37.73 ± 0.76 d	387 ± 1.15 d	21.54 ± 0.20 d	142 ± 0.56 d
	500	25741 ± 83.77 e	39.57 ± 0.27 e	5050 ± 12.29 e	3603 ± 10.75 e	18.98 ± 0.07 d	54.34 ± 0.17 e	409 ± 1.32 e	19.25 ± 0.07 e	170 ± 0.75 e

Note. Values followed by same letters are not significantly different ($P < 0.005$); means ± standard error, n = 10.



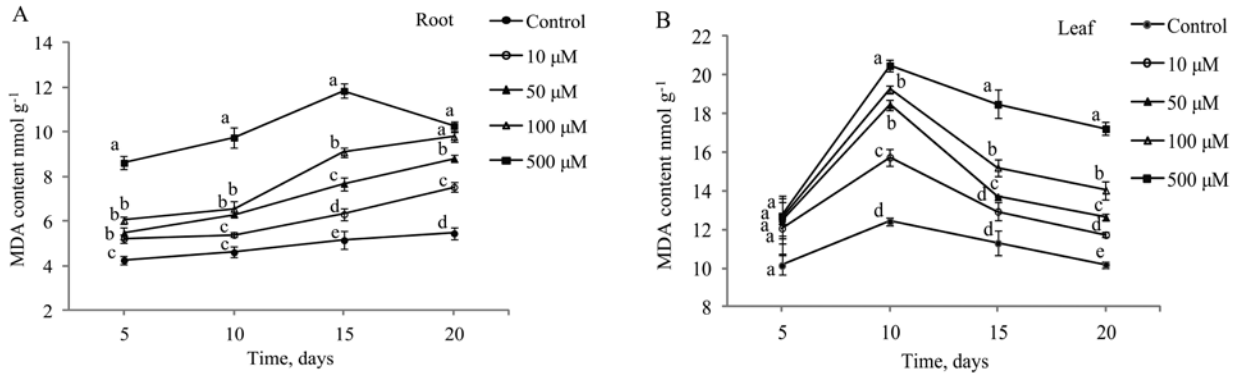
Explanations under Figure 1

Figure 3. Effects of different cadmium (Cd) concentrations on the activities of SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) in roots and leaves of *Cucumis sativus*

in Figure 3 C and D when the seedlings were exposed to different concentrations of cadmium. In roots, cadmium induced significantly high POD activity during the whole experiment time except for the group exposed to 500 μM Cd at 20th day (Fig. 3 C). In leaves, the presence of cadmium in the solution increased markedly POD activity ($P < 0.05$) at the 10th, 15th and 20th days in comparison with control (Fig. 3 D). Information on CAT activity was given in Figure 3 E and F. In roots, the CAT activities decreased significantly ($P < 0.05$) with increasing cadmium concentration and treatment time, except for the groups treated with10 and 50 μM Cd during 10 days treatment when compared to control (Fig. 3 E). At high concentration of Cd (500 μM), the CAT activity was severely inhibited during the whole experiment in comparison with control and the other treatment groups. The CAT activity was found to be inhibited significantly ($P < 0.05$) during 20 days treatment and to be the lowest in the leaves at 500 μM Cd when compared with control and other treatment groups (Fig. 3 F).

Effects of cadmium on lipid peroxidation. The effects of cadmium on MDA concentration are presented in Figure 4 A and B. The MDA contents in roots exposed to cadmium were high significantly ($P < 0.05$) when compared with control during the whole treatment time, and the ones in control were the lowest except for the group treated with 10 μM Cd at 10th days. Compared to control, MDA concentration increased significantly ($P < 0.05$) in leaves treated with cadmium used in the present investigation during the whole experiment.

Effects of cadmium on photosynthetic gas exchange. Effects of cadmium on the photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration in leaves of *C. sativus* varied with the different concentrations of cadmium and the duration of treatment (Table 2). When compared to the controls, the photosynthetic rate in the treated plants was inhibited during the whole experiment. The photosynthetic rate in the group exposed to 500 μM Cd was the lowest. The transpiration rate in the treatment groups exposed to cadmium was inhibited with increasing cadmium concentration and prolonged duration of treatment in comparison with control. At 10, 50, 100 and 500 μM Cd treatments for 20 days the transpiration rate was reduced by 32.61, 61.73, 74.39 and 88.14 %, respectively. Cadmium could induce the decrease in intercellular CO_2



Explanations under Figure 1

Figure 4. Effects of different cadmium (Cd) concentrations on the contents of MDA (malondialdehyde) in roots and leaves of *Cucumis sativus*

Table 2. Effects of different cadmium concentrations on net photosynthetic rate (P_n), transpiration rate (T_r), intercellular CO_2 concentration (C_i) and stomatal conductance (G_s) in *Cucumis sativus*

Time, days	Treatment μM	Photosynthetic gas exchange			
		P_n, μmol m^{-2} s^{-1}	T_r, mmol m^{-2} s^{-1}	C_i, μmol mol^{-1}	G_s, μmol m^{-2} s^{-1}
5	control	7.05 ± 0. 26 a	3.45 ± 0.25 a	506 ± 6.85 a	114 ± 5.38 a
	10 μM	6.29 ± 0.10 ab	2.77 ± 0.75 b	483 ± 3.99 ab	97.57 ± 3.79 a
	50 μM	5.49 ± 0.25 b	1.94 ± 0.06 b	445 ± 6.59 b	73.02 ± 3.91 b
	100 μM	3.20 ± 0.13 c	1.44 ± 0.07 c	408 ± 2.21 c	58.30 ± 2.81 b
	500 μM	2.41 ± 0.09 d	0.98 ± 0.05 d	387 ± 7.47 c	31.60 ± 1.49 c
10	control	9.47 ± 0.25 a	3.51 ± 0.12 a	535 ± 3.46 a	138 ± 6.71 a
	10 μM	7.34 ± 0.24 b	2.69 ± 0.11 b	496 ± 4.16 b	105 ± 2.36 b
	50 μM	6.57 ± 0.28 b	1.81 ± 0.07 c	458 ± 3.13 b	83.33 ± 3.30 c
	100 μM	3.32 ± 0.12 c	1.16 ± 0.06 d	414 ± 5.36 c	66.78 ± 2.47 c
	500 μM	1.86 ± 0.07 d	0.86 ± 0.04 e	383 ± 2.15 d	29.26 ± 1.42 d
15	control	11.12 ± 0.13 a	3.57 ± 0.06 a	547 ± 2.15 a	157 ± 4.53 a
	10 μM	8.35 ± 0.12 b	2.63 ± 0.11 b	513 ± 3.47 b	114 ± 5.29 b
	50 μM	7.16 ± 0.08 c	1.71 ± 0.08 b	464 ± 5.72 c	89.74 ± 3.54 c
	100 μM	3.21 ± 0.12 d	1.13 ± 0.02 c	415 ± 9.91 d	64.81 ± 1.97 d
	500 μM	1.12 ± 0.08 e	0.59 ± 0.03 d	373 ± 6.81 e	23.46 ± 1.11 e
20	control	12.66 ± 0.31 a	3.71 ± 0.15 a	565 ± 5.41 a	177 ± 8.14 a
	10 μM	9.49 ± 0.22 b	2.50 ± 0.12 b	515 ± 3.11 b	118 ± 5.49 b
	50 μM	7.46 ± 0.12 c	1.42 ± 0.06 c	458 ± 6.64 c	81.77 ± 3.40 c
	100 μM	2.57 ± 0.25 d	0.95 ± 0.03 d	4063 ± 3.00 d	54.44 ± 1.31 d
	500 μM	0.81 ± 0.03 e	0.44 ± 0.02 e	340 ± 16.75 e	20.13 ± 0.87 e

Note. Values with different letters differ significantly from each other ($p < 0.05$, *t*-test); vertical bars denote standard error, n = 10.

concentration in leaves of *C. sativus* treated with cadmium used in the present investigation during cadmium stress when compared with control (Table 2).

Cadmium had most toxic effects on intercellular CO_2 concentration in leaves exposed to 500 μM Cd in comparison with control and the other treatment groups. The stomatal conductance in control leaves was the highest during 20 day treatment. After cadmium treatment, the stomatal conductance in all cadmium treatment groups was inhibited with increasing cadmium concentration except for the group exposed to cadmium for 5 days (Table 2). The stomatal conductance in 500 μM Cd treatment was the lowest during periods of treatment and decreased with prolonged during of treatment.

Discussion

The results in the present investigation indicate that cadmium inhibits the seedling growth of *C. sativus* significantly ($P < 0.05$) at the concentrations of cadmium from 10 to 500 μM during the whole experiment except for the groups exposed to 10 and 50 μM Cd for 5 days and 10 μM Cd for 10 days. Chlorosis leaf rolls and stunting are the main and easily visible symptoms of cadmium toxicity in plants (Benavides et al., 2005). We observed that the leaves exposed to cadmium appeared chlorotic with slight green around veins. In the group of plants treated with 500 μM Cd, the leaves showed chlorotic symptoms like the plants with iron-deficiency. These phenomena might result from pigment and cell destruction in leaves. Çanakci and Karaboğa (2013) found that the chlorophyll content was reduced in cadmium-treated *C. sativus* plants, indicating that the increase in photosynthetic pigment destruction was a typical consequence of heavy metal toxicity.

Normally, cadmium ions are mainly retained in the roots, and only small amounts are transported to the shoots except for hyperaccumulators (Benavides et al., 2005). *C. sativus* has the ability to accumulate cadmium primarily in its roots, and transport and concentrate it in its stems and levels in much lesser concentrations. This distribution is due to the mobilization of the protective mechanisms of plants, which inhibits the transport to further tissues and organs. These differences in root and shoot accumulation can possibly be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role (Zou et al., 2008). Poor translocation of cadmium to the shoots could be due to sequestration of most of the cadmium in the vacuoles of the root cells to render it nontoxic, which might be a natural toxicity response of the plant (Tewari et al., 2008). Most recognized standard criteria based on metal concentrations in aboveground tissue of plant material were sampled from its natural habitat. Hyperaccumulators have been known to accumulate cadmium above 0.01% dry tissue (100 μg g^{-1}); whereas the normal range of cadmium concentrations in leaf tissue (dry weight) of some species is 0.05 to 0.2 μg g^{-1} (Bao et al., 2011). *C. sativus* could not be an efficient phytoextraction plant with considerable ability to accumulate cadmium.

Cadmium can alter the uptake of minerals by plants through its effects on the availability of minerals from the soil, or through a reduction in the population of soil microbes (Benavides et al., 2005). Cadmium toxicity may result from disturbance in plant metabolism as a consequence of disturbance in the accumulation and translocation of mineral nutrients. Availability of manganese to plants decreases in the presence of cadmium in soil (Nazar et al., 2012). The manganese-mediated amelioration of cadmium-induced root growth inhibition in maize seedlings was shown to be associated with parallel reductions in cadmium accumulation (Pal'ove-Balang et al., 2006). The results from this investigation showed that manganese accumulation was reduced significantly ($P < 0.05$) in *C. sativus* treated with different cadmium concentrations and progressively decreased with an increase in cadmium concentration. The decrease of manganese concentration in leaves under cadmium stress was the key reason for the restraint of leaf photosynthesis (Nazar et al., 2012). Dong et al. (2006) through regression analysis showed that there was a significantly negative correlation between cadmium and manganese, implying the antagonistic effect of cadmium on manganese absorption and translocation. It was reported that chlorosis might reduce manganese transport (Benavides et al., 2005). In the presence of cadmium, adding manganese to the solution significantly improved the plant growth and reduced the concentrations of cadmium in all organs of the plant (Peng et al., 2008). Iron accumulation in the groups treated with cadmium was greater than in control plants in the present investigation. This is in agreement with the findings of Wang et al. (2007), where iron in maize plants was higher than in control plants after treatment with cadmium.

In plants there are protective enzymatic mechanisms and non-enzymatic mechanisms to scavenge ROS (reactive oxygen species) and alleviate their deleterious effects. ROS can be extremely harmful to organisms at high concentrations. ROS can oxidize proteins, lipids, and nucleic acids, often leading to alterations in cell structure and mutagenesis (Scandalios, 2005). Chakraborty et al. (2014) indicated that cadmium accumulation could induce oxidative stress characterized by H_2O_2/O_2^- production, lipid peroxidation and protein oxidation. Heavy metals cause oxidative damage to plants either directly or indirectly through the formation of ROS, which cause further severe oxidative damage to different cell organelles and biomolecules (Nagajyoti et al., 2010). To resist oxidative stress, plants can induce a series of detoxification reaction catalyzed by antioxidative enzymes, including SOD, POD and CAT (Zhang et al., 2005). SOD is the cell's first line of defence against ROS as the superoxide radical is a precursor to several other highly reactive species so that control over the steady state of superoxide concentration by SOD constitutes an important protective mechanism (Zhang et al., 2009). The results showed that the SOD activity in leaves and roots exposed to cadmium was noted to be high significantly ($P < 0.05$). High SOD activity has been associated with stress tolerance in plants. This may be attributed to the increased production of superoxide, resulting in the activation of existing enzyme pools or increased expression of genes encoding SOD (Zhang et al., 2009). POD is known to play a significant role in oxidative stress conditions, and it has been shown that POD activity can be used as a potential biomarker for sublethal metal toxicity in examined plant species (Gao et al., 2008). POD is also an important enzyme, able to scavenge H_2O_2, which is a major substance degraded by SOD. Our results indicated that cadmium induced POD activity in roots and leaves significantly during the whole

experiment time except for the group in roots exposed to 500 μM Cd at 20th day and the groups in leaves treated with cadmium at 5th day. Increase in POD activity suggested that cadmium directly caused excessive production of H_2O_2 in seedlings and/or increased H_2O_2 was due to SOD. Thus, increased POD activity, in turn, scavenged excessive H_2O_2 and damage was limited. CAT is the most universal oxidoreductase, which scavenges H_2O_2 to O_2 and H_2O. The CAT activity was found to be inhibited significantly ($P < 0.05$) in leaves and roots in the present investigation. As far as sensitivity to heavy metal stress was concerned, CAT was the highest. So the activity of CAT was inhibited firstly under heavy metal stress conditions, which led to H_2O_2 clearing blocked (Lin et al., 2012). In this present investigation, CAT activity decreased at the high concentrations of cadmium, indicating that the CAT to eliminate ROS was limited. Cadmium induced higher SOD and POD activities than CAT activity, suggesting that SOD and POD provided a better defence mechanism against cadmium-induced oxidative damage in *C. sativus*.

MDA, one of the decomposition products of membrane polyunsaturated fatty acids, is regarded as a reliable indicator of oxidative stress (Zou et al., 2009). Evidence here demonstrates that cadmium toxicity enhances lipid peroxidation in plant cells, reflected by increased MDA concentration. The cadmium accumulation in *C. sativus* leads to various physiological and biochemical changes. The high contents of MDA in the investigation suggested that cadmium exposure might lead to more ROS in *C. sativus*. The elimination of ROS mainly depended on the antioxidative defence enzymes. The results further suggested that cadmium exposure might depress the activities of antioxidative enzymes.

The progressive decrease of photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration in association with increasing cadmium concentration was observed in the present study. It is supposed that the closure of stomata reduced the photosynthesis (Turner et al., 2007). Ying et al. (2010) indicated that the decrease of photosynthetic and gas exchange parameters, especially intercellular CO_2 concentration, stomatal conductance and transpiration rate, could be ascribed to the abnormality of stomata, like stomatal closure, less stoma density and high stomatal resistance, in high cadmium-exposed *Picris divaricata.* The parallel changes of photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate provide evidence that maintenance of photosynthetic rate mainly was attributed to the stomatal conductance, which later controls the transpiration. A reduction in photosynthesis in *C. sativus* in cadmium-treated plants was caused by a series of processes, including stomatal and non-stomatal limitations during cadmium stress. The reduction in the rate of photosynthesis denotes that in addition to depressed growth, the cadmium interferes with the photosynthetic activity by directly interfering in the process of photosynthesis. Ci et al. (2010) found a similar inhibiting effect in leaves of wheat. Inhibition of photosynthesis induced by cadmium may also result in lower chlorophyll content. Further studies are needed to clarify this effect.

Conclusion

Effects of cadmium (Cd) on cucumber (*Cucumis sativus* L.) were evaluated in this investigation by accumulation of cadmium and its effect on other minerals, antioxidant enzymes, MDA (malondialdehyde) content and leaf gas exchange and photosynthetic parameters. Cadmium was accumulated primarily in roots and it induced decrease of manganese and increase of iron. Cadmium induced higher SOD (superoxide dismutase) and POD (peroxidase) activities than CAT (catalase) activity, suggesting that SOD and POD provided a better defence mechanism against cadmium-induced oxidative damage in cucumber. Cadmium increased concentration of MDA. The net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) were reduced in the presence of cadmium. The information available in this work is an important step towards obtaining a better understanding of photosynthetic parameters and antioxidant defence system caused by cadmium.

Acknowledgements

This project was supported by the National Natural Science Foundation of China. The authors wish to express their appreciation to the reviewers for this paper.

Received 28 06 2014
Accepted 31 12 2014

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ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 102, No. 2 (2015), p. 193–200
DOI 10.13080/z-a.2015.102.025

Kadmio įtaka mineralų kaupimuisi, antioksidacinei sistemai ir dujų mainams agurkuose

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Santrauka

Hidroponiniu būdu augintuose agurkuose tirta įvairių koncentracijų (10, 50, 100 ir 500 µM) kadmio (Cd) įtaka mangano (Mn) bei geležies (Fe) kiekiui, lipidų peroksidacijai, antioksidacinių fermentų aktyvumui ir fotosintezei. Tyrimų rezultatai parodė, kad kadmis pirmiausia kaupiasi agurkų šaknyse. Didinant kadmio koncentraciją tirpale, jo kiekis šaknyse ir daiguose padidėjo, o mangano kiekis esmingai (P < 0,005) sumažėjo. Didinant kadmio koncentraciją tirpale ir poveikio laiką, geležies kiekis augaluose turėjo tendenciją didėti. Kadmio sukeltas oksidacinis stresas ir lipidų peroksidacija paskatino MDA (malondialdehido) koncentracijos didėjimą agurkuose. Palyginus su katalazės aktyvumu, kadmis labiau skatino superoksido dismutazės ir peroksidazės aktyvumą. Tai rodo, kad superoksido dismutazė ir peroksidazė yra svarbesnės siekiant pašalinti kadmio sukeltą oksidacinį stresą agurkuose. Palaisčius kadmiu, agurkuose sumažėjo fotosintezės bei garavimo intensyvumas, žiotelių laidumas ir tarpląstelinė CO_2 koncentracija.

Reikšminiai žodžiai: antioksidaciniai fermentai, fotosintezės parametrai, kadmis, lipidų peroksidacija, paprastasis agurkas.