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Effects of cadmium on some morphological and physiological traits of amaranth (*Amaranthus caudatus* L.) and oilseed rape (*Brassica napus* L.)

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Abstract

In order to investigate cadmium (Cd) effects on some morphological and physiological traits of amaranth (*Amaranthus caudatus* L.) and oilseed rape (*Brassica napus* L.) on experiment as Randomized Complete Block Design with six replications was carried out in greenhouse. Treatments were 10, 50 and 100 mg/kg Cd with control. Results showed that Cd Treatments decreased morphological traits such as number, area and dry weight of green leaves in both oilseed rape and amaranth. Also, shoot and root dry weight and plant height of oilseed rape reduced. Different Cd concentrations had a significant effect on F_0, chlorophyll fluorescence, chlorophyll a, b and total and carotenoid contents in amaranth. At concentration of 100 mg/kg Cd destructive effects on pigment content and chlorophyll fluorescence was observed in amaranth. Cd had no significant effect on chlorophyll fluorescence and pigment content in oilseed rape. Chlorophyll a and total had significantly negative correlation with SLA for amaranth. Anthocyanin content and chlorophyll a/b ratio were not affected by Cd stress in both amaranth and oilseed rape.

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Introduction

Cadmium (Cd) as a heavy metal cause environmental pollution that produced by industries, melting metals and consumption of some chemical fertilizers (Stritsis and Steingrobe, 2012). When Cd concentration increases to 100 mg/kg in agricultural soils, growth of plants extremely reduce (Salt, 1995). Plant families of Chenopodiaceae, Brassicaceae, Solanaceae, Asteraceae, Amaranthaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae accumulate heavy metals in their aboveground tissues included stem, leaf and grain (Kuboi *et al.*, 1986; Prasad and Freitas, 2003). For example *Brassica napus* is used for phytoextraction because it accumulates high concentration of Cd in their shoot (Turan and Esringu, 2007). Some studies reported that plant biomass decrease by Cd in contaminated soil (Melo *et al.*, 2011; Moteshare Zadeh *et al.*, 2008). Chen *et al.*, (2011) resulted that both shoot and root dry weight of pakchoi (*Brassica campestris* ssp. chinnensis) and mustard (*Brassica juncea* Czernajew) decreased with increasing Cd concentration. Amaranth (*Amaranthus* spp.) is used to remove radioactive and heavy metals from contaminated silos as Cs (Dushenkov *et al.*, 1999) and Ni (Bosiacki and Wojciechowska, 2012).

Cd is absorbed by plants, accumulated in different parts which cause growth inhibition and change in morphological, physiological and biochemical characteristics of plants (Milone *et al.*, 2003; Ren *et al.*, 2006). Cd decreases root and shoot growth (Eshghi *et al.*, 2010), leaf area expansion (Skorzynska-Polit *et al.*, 1998) and number of green leaves (Ghani, 2010) by inhibition cell division and the growth of cells or both of them (Pál *et al.*, 2006). Also, Cd limited uptake and distribution of necessary elements in plants (Gussarson *et al.*, 1996). Cd affect morphological and physiological traits related to photosynthesis and finally impress plant biomass. Therefore the plants get weak and its tolerance to biotic and abiotic stresses is declined. Decreased transpiration and increased temperature occur in plant by reduction leaves area. Cd general toxic effects are decreasing number of chloroplasts per cell, chlorophyll and carotenoid contents (Baryla *et al.*, 2001). Low Cd concentrations might increase Chlorophyll content (Drazkiewicz *et al.*, 2003).

There are different opinions about the primary site of the Cd damage effect on active pigments (Greger and Ogren, 1991), photosystem II water splitting enzymes (Pagliano *et al.*, 2006), Calvin's cycle enzymes (*Sheoran et al.*, 1990) and electron transport (Siedlecka and Baszynski, 1993). Therefore, exposure of plants to Cd, increase fluorescence and decrease PSII photochemical efficiency (Ouzounidou *et al.*, 1997).

A few investigations have been done about Cd effects on morphological and physiological traits in amaranth and oil seed rape. In this regard, the aim of this experiment was to determine level of Cd that has harmful effect on growth parameters, chlorophyll fluorescence and pigment content in amaranth and oil seed rape.

Materials and methods

Plant material

The soil used in the experiment was collected from the surface layer (0-20 cm) of Botanical Garden - Tabriz University which had no pollution of heavy metals. Physical and chemical properties of the soil were determined by soil test (Table 1). Air dried soil was sieved by 4 mm sieve and then 5.8 kg soil is put to each pot (23 cm in height and 21 cm in diameter). This experiment was laid out in Randomized Complete Block Design (RCDB) with 6 replications. Cd ($CdCl_2.2.5H_2O$) treatments included 0, 10, 50 and 100 mg/kg. The moisture of soil was kept at 80% FC for 30 days then 20 seeds of amaranth (*Amaranthus caudatus* L.) and 10 seeds of oilseed rape (*Brassica napus* L.) planted at 1 and 3 cm depth in each pot, respectively. Optimal pant density which calculated 2 plants per pot was gained by hand thinning at 4 leaves stage. Greenhouse temperature, relative humidity, light intensity and day light were set at 24±6 °C, 60%, 600 µmol/m²/s/, and 16 h, respectively. Shoot and root dry weight, plant height, number, area and dry weight of green leaves, leaf area, special leaf area (SLA), Chlorophyll fluorescence (F_0, Fv and Fv/Fm), chlorophyll (a, b and total), carotenoid and anthocyanin content measured 60 days after emergence.

Pigments

Pigment content Leaf tissue sample as 0.1 g was powdered by liquid nitrogen. Then, 6 ml of solution of 85% acetone and 15% Tris HCl stock buffer added to the powdered leaf tissue to extract pigments. The extract was centrifuged at 12000 g for 3 min. spectrophotometer (WPA model S2100) with 663, 647, 537 and 470 nm of the wavelength used to determine chlorophyll a and b, carotenoid and anthocyanin content of tissue (Sims and Gamon, 2002).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured on the upper surface of the second leaf by a portable chlorophyll fluorometer (OS-30, OPTISCIENCIE, USA). For measurement of fluorescence, plants were maintained at least 30 min to dark adaptation and 25°C (Room temperature) (Moustakas *et al.*, 1994). The measured traits were included F_0 (minimal fluorescence yield of dark-adapted state), Fm (maximal fluorescence yield of dark-adapted state), Fv (variable fluorescence) and Fv/Fm (maximal quantum yield of PSII photochemistry) (Schreiber, 1983).

Statistical analysis

Analysis of variance was performed to determine the influence of Cd concentration on measured traits in both amaranth and oilseed rape by the statistical software SPSS for windows, version 16. Means were compared according to Duncan's multiple range test at 0.05 probability levels. Pearson's correlation coefficients were used to measure the relationship between the morphological and physiological traits. The figures were plotted by the Microsoft Office Excel 2003. All results are presented as arithmetic means with standard errors attached.

Results

Morphological traits

Different concentrations of Cd had no significant and significant effects on the shoot and root dry weight of amaranth and oilseed rape ($P<0.01$), respectively. Shoot dry weight of oilseed rape significantly decreased at 50 and 100 mg/kg Cd compared with control (25.74% and 20.72%, respectively), while 10 mg/kg Cd had no significant difference with control (Fig. 1A). There were no significant difference among 10, 50 and 100 mg/kg Cd on root dry weight of oilseed rape. There treatments decreased root dry weight as 51.4%, 60.52% and 60.32% related to control, respectively (Fig. 1B).

Table 1. Some physical and chemical properties of Soil.

Characteristic	Quantity	Nutrients and elements	Quantity
Soil texture	Sandy loam	Total nitrogen (%)	0.12
Clay (%)	13.00	Available P (mg/kg)	12.66
Silt (%)	21.00	Available K (mg/kg)	400
Sand (%)	66.00	Total Zn (mg/kg)	0.288
pH	7.80	Total Mn (mg/kg)	7.286
EC (dS/m)	0.70	Total Cu (mg/kg)	1.155
CEC (Cmol/kg)	17.00	Total Fe (mg/kg)	3.521
		Total Cd (mg/kg)	0.011

The plant height of oilseed rape significantly affected with Cd concentrations. It was similar for control and 10 mg/kg, but further increase in Cd concentration to 50 and 100 mg/kg reduced plant height, significantly ($P<0.05$) (Fig. 1C). Plant height of amaranth was not affected by Cd concentration.

The number of green leaves significantly reduced in both amaranth and oilseed rape under high Cd concentrations ($P<0.05$). At 50 and 100 mg/kg Cd, the number of green leaves in amaranth decreased by 12.38% and 11.46%, respectively (Fig. 1D). 100 mg/kg Cd decreased number of green leaves (17.74%) in oilseed rape, significantly (Fig. 1D).

All of Cd concentration (10, 50 and 100 mg/kg) reduced the area of green leaves in amaranth (19.05%, 19.72% and 23.64%, respectively) in comparison with control ($P<0.01$) (Fig. 1E). Green leaves area of oilseed rape was decreased by 50 and 100 mg/kg Cd as 16.86% and 23.15%, respectively

(P<0.01) (Fig. 1E). Also, Cd had negative effect on dry weight of green leaves of both plant species (P<0.01). At 10, 50 and 100 mg/kg Cd, dry weight of green leaves in amaranth decreased by 15.62%, 16.19% and 23.86%, respectively (Fig. 1F). At higher Cd concentrations included 50 and 100 mg/kg, dry weight of green leaves in oilseed rape decreased by15.96% and 19.39%, respectively (Fig. 1F). Cd had no significant effect on leaf area and SLA in both amaranth and oilseed rape.

Table 2. Correlations between morphological and physiological traits under Cd concentrations in amaranth.

Amaranth	Shoot dry weight	Root dry weight	Plant height	Number of green leaves	Green leaves area	Green leaves dry weight	leaf area	SLA	F_o	Fm	Fv/Fm	Chl. a	Chl. b	Total Chl.
Shoot dry weight	1													
Root dry weight	0.744**	1												
Plant height	0.000	-0.253	1											
Number of green leaves	-0.011	-0.230	0.389	1										
Green leaves area	0.037	-0.014	0.313	0.623**	1									
Green leaves dry weight	0.136	0.104	0.151	0.479*	0.871**	1								
Average leaf area	0.044	0.190	0.042	-0.109	0.706**	0.675**	1							
SLA	-0.174	-0.203	0.378	0.445*	0.558**	0.081	0.305	1						
F_o	-0.349	-0.227	0.230	-0.036	0.138	0.020	0.220	0.248	1					
Fm	-0.306	-0.451*	0.307	0.516**	0.519**	0.333	0.205	0.460*	0.604**	1				
Fv/Fm	-0.009	-0.298	0.065	0.583**	0.414*	0.373	0.001	0.170	-0.413*	0.464*	1			
Chl. a	0.334	0.365	-0.245	-0.036	-0.026	0.273	0.034	-0.502*	-0.452*	-0.311	0.159	1		
Chl. b	0.035	0.079	-0.072	-0.025	0.014	0.252	0.084	-0.383	-0.319	-0.149	0.233	0.897**	1	
Total Chl.	0.220	0.255	-0.185	-0.028	-0.008	0.272	0.054	-0.461*	-0.411*	-0.250	0.196	0.984**	0.961**	1
Carotenoids	0.161	0.035	0.144	0.203	0.068	0.051	-0.080	0.050	-0.362	-0.058	0.290	0.632**	0.632**	0.651**

*and** Significant at 5% and 1% levels, respectively.

Pigments

Different Cd treatments had no significant effect on pigment content of oilseed rape but had significant effect (P<0.01) on Chlorophyll (a, b and total) and carotenoid content of amaranth leaves. Chlorophyll a, b and total of amaranth leaves decreased at 100 mg/kg Cd by 23.46%, 22.06% and 22.28% compared to control, respectively (Figs. 2A, B, C). There was negligible in chlorophyll a, b and total content at 10 and 50 mg/kg Cd in comparison with control, but it was no significant. The highest and lowest carotenoid content was observed at 10 and 100 mg/kg Cd, respectively (Fig. 2D). At 100 mg/kg Cd had significant reduction in comparison with control. Anthocyanin content and chlorophyll a/b ratio of amaranth had no significant change under stress Cd.

Chlorophyll fluorescence

Cd-stress significantly increased the F_o of amaranth at 100 mg/kg by 26.76% in comparison with control (P<0.05) (Fig. 3). Different Cd concentrations had no effect on the Fm. 10 and 50 mg/kg Cd did not show significant change the ratio of Fv/Fm of amaranth. At 100 mg/kg Cd, impact on the ratio of Fv/Fm (9.57%) was observed (P<0.05) (Fig. 4). In contrast, Cd concentration had no significant effect on the ratio of Fv/Fm, F_o and Fm in oilseed rape.

Table 3. Correlations between morphological and physiological traits under Cd concentrations in oilseed rape.

Canola	Shoot dry weight	Root dry weight	Plant height	Number of green leaves	Green leaves area	Green leaves dry weight	leaf area	SLA	F_o	Fm	Fv/Fm	Chl. a	Chl. b	Total Chl.
Shoot dry weight	1													
Root dry weight	0.763**	1												
Plant height	0.280	0.435*	1											
Number of green leaves	0.457*	0.480*	0.501*	1										
Green leaves area	0.783**	0.503*	0.427*	0.544**	1									
Green leaves dry weight	0.899**	0.678**	0.349	0.419*	0.762**	1								
leaf area	0.577**	0.214	0.104	-0.142	0.749**	0.585**	1							
SLA	-0.486*	-0.396	0.000	0.022	-0.027	-0.650**	-0.071	1						
F_o	-0.165	-0.030	-0.294	-0.477*	-0.404	-0.198	-0.084	-0.110	1					
Fm	-0.542**	-0.335	-0.359	-0.301	-0.407*	-0.437*	-0.257	0.190	0.348	1				
Fv/Fm	-0.349	-0.227	-0.022	0.184	-0.063	-0.260	-0.253	0.288	-0.575**	0.527**	1			
Chl. a	0.608**	0.237	-0.056	0.079	0.667**	0.684**	0.744**	-0.312	-0.103	-0.316	-0.317	1		
Chl. b	0.531**	0.226	0.066	0.102	0.703**	0.633**	0.766**	-0.188	-0.221	-0.239	-0.168	0.931**	1	
Total Chl.	0.584**	0.237	0.001	0.091	0.696**	0.674**	0.768**	-0.261	-0.158	-0.286	-0.255	0.986**	0.979**	1
Carotenoids	0.539**	0.133	-0.011	0.022	0.650**	0.543**	0.782**	-0.105	-0.083	-0.420*	-0.397	0.882**	0.799**	0.860**

*and** Significant at 5% and 1% levels, respectively.

Discussion

Our study showed, root in comparison with shoot of oilseed rape strongly affected by Cd because of high accumulation of Cd in root. Root dry weight of oilseed rape was significantly and positively correlated with shoot dry weight, plant height, number, area and dry weight of green leaves and it seems that Cd had destructive effect on root growth, so that water and nutrients uptake and ultimately shoot growth decreased (Table 2). Lefèvre *et al.*, (2009) reported that shoot and root dry weight in *Atriplex halimus* decreased by Cd stress after 24 days. Reduction of root growth of oil seed rape under Cd stress was reported in some researches (Larsson *et al.*, 1998; Nouairi *et al.*, 2006).

Decrease in number of leaves can be attributed to reduction of oilseed rape plant height. Early senescence and leaves shedding with enhancing Cd concentration (50 and 100 mg/kg Cd) could be the reason for reduction of leaves at higher Cd concentrations. Plant height and leave numbers of oilseed rape had significantly positive correlation (Table 3). Wang and Su (2005) reported that Cd accumulation in older leaves of oilseed rape and Indian mustard (*Brassica juncea* L.) was higher than those of younger leaves.

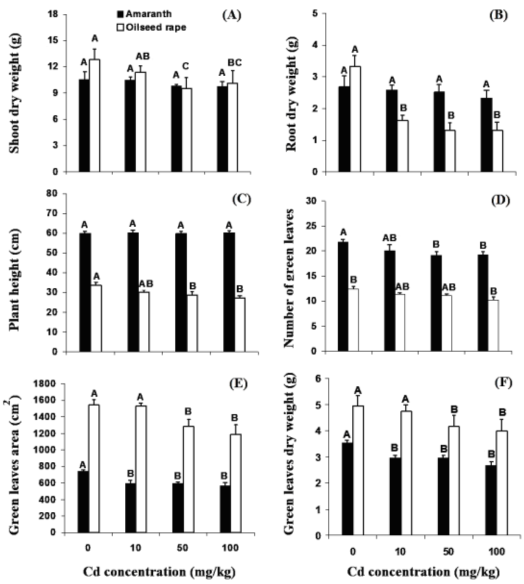

Fig. 1. Effects of Cd concentrations on shoot dry weight (A), root dry weight (B), plant height (C), number of green leaves (D), green leaves area (E) and green leaves dry weight (F) of oilseed rape and amaranth (mean ± SE, n = 6). Different letters shows significant difference between treatments based on Duncan's multiple range test, $P<0.05$.

The reduction of area and dry weight of green leaves was related to reduction of the green leaves number and area in both plants that reduced photosynthesis area and plant growth (Table 2 and 3). Cd-stress increased senescence and so death of older leaves and reduced green leaves number (Ghani, 2010). SLA did not change because of the dry weight and area green leaves reduction. Larsson *et al.*, (1998) reported that Cd-stress in oilseed rape had no effect on SLA.

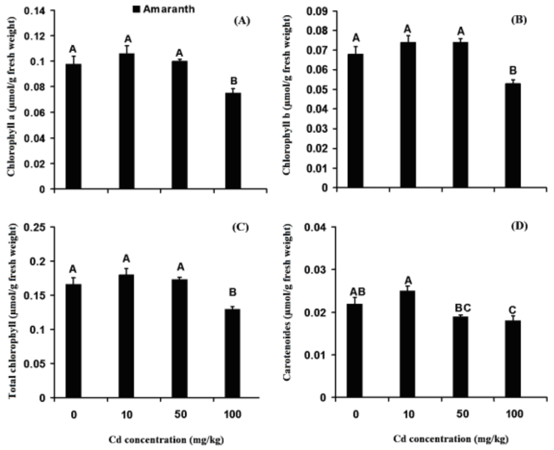

Fig. 2. Effects of Cd concentrations on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) content of amaranth (mean ± SE, n = 6). Different letters shows significant difference between treatments based on Duncan's multiple range test, P<0.05.

Under low concentration of Cd may increase chlorophyll and carotenoid content (Jia *et al.*, 2012). The chlorophyll content reduction is due to inhibiting different stages of chlorophyll biosynthesis by Cd (Hegedus *et al.*, 2001). It inhibits Chlorophyll product by disturbing synthesis of 5-aminolacvulinic acid and the protoehlorophyllide reductase (Stobart *et al.*, 1985). Also, Chlorophyll content reduction can be due to the content of essential nutrients reduction by Cd-stress (Ouzounidou *et al.*, 1997). Chlorophyll a and total had significantly negative correlation with SLA (Table 2). This result showed that deceasing of SLA and increasing of cell thickness under low concentration of Cd increased chlorophyll content in leaves. Also, this increase might be due to water loss that increased intracellular solute concentration. The carotenoids responded to Cd-stress faster than chlorophylls because carotenoids protect chlorophyll pigments from damage by free radicals (Anderson and Robertson, 1960). In oilseed rape, different Cd concentrations had no significant effect on the pigment content. The chlorophyll a/b ratio had no change in both amaranth and oilseed rape. The pigments of oilseed rape showed resistance to Cd destructive effects. Our results are inconsistent with others result (Larsson *et al.*, 1998; Barlya *et al.*, 2001; Vatehová *et al.*, 2012).

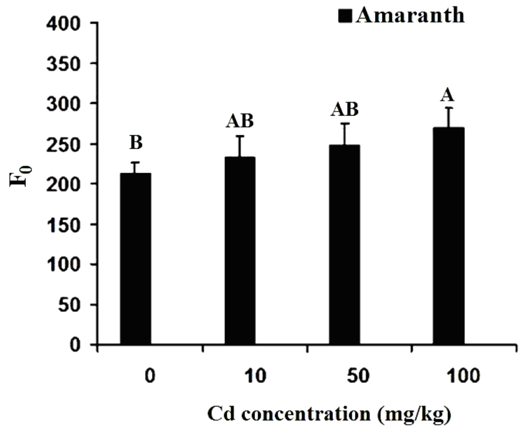

Fig. 3. Effects of Cd concentrations on F_0 of amaranth (mean ± SE, n = 6). Different letters shows significant difference between treatments based on Duncan's multiple range test, P<0.05.

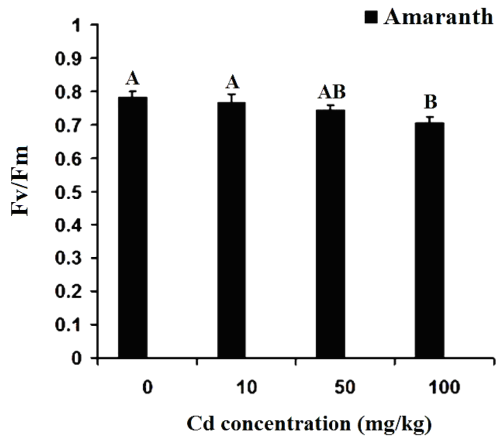

Fig. 4. Effects of Cd concentrations on Fv/Fm in amaranth (mean ± SE, n = 6). Different letters shows significant difference between treatments based on Duncan's multiple range test, P<0.05.

Cd effect is inhibiting protochlorophyllide reductase and the photosynthetic electron transport at the Photosystem II (PSII) reaction center (Van Assche and Clijsters, 1990). The ratio of Fv/Fm indicates the efficiency of the primary photochemistry in PSII, thus, decline in active pigments caused to decrease of PSII photochemical efficiency (Greger and Ogren, 1991; Ouzounidou *et al.*, 1997) and photosynthetic rate (Shi *et al.*, 2010). Increasing of fluorescence yield (F_0) caused to decrease in Fv and photochemical efficiency that was observed as significant negative correlation between F_0 and Fv/Fm (Table 2). This result indicated that the reaction center of PSII in amaranth leaves was affected by Cd-stress. Similar results have been obtained in barley seedlings under Cd-stress (Chen and Huerta, 1997).

Conclusion

Our study showed that Cd significantly reduced number and development of leaves in both amaranth and oilseed rape. High concentration of Cd (100 mg/kg) had significant effect on chlorophyll fluorescence and pigments content in amaranth but lower concentrations (10 and 50 mg/kg) non significantly increased chlorophyll concentration as compared with the control, while, Cd had no effect on oilseed rape. Oilseed rape biomass decreased by reduction of root growth, number of leaves, leaf area and photosynthetic area under Cd stress, while, amaranth biomass had no change that be due to lower reduction in number of green leaves and slight increasing of chlorophyll content (10 and 50 mg/kg) in compare is on with control. Cd contaminated soil greater than 50 mg/kg decreased chlorophyll a, b and total and carotenoid content of amaranth, significantly.

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