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Research Article

Response of Sunflower (*Helianthus annuus* L.) Plant at Early Growth Stage to Cadmium Exposure

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Abstract

The study carried out in greenhouse conditions at daylight in order to evaluate the effects of increasing Cd exposure on growth and physiological characteristics of sunflower at early growth stage as well the accumulation of some metal nutrient ions. Accordingly, the soil treated with six levels of Cd (0, 50, 100, 250, 500, and 1000 µM $CdCl_2.H_2O$). The plant growth and root elongation unfavorably affected by Cd exposure and detrimental effect of Cd on plant growth was appeared shoot more than roots. Growth tolerance index (GTI), relative water content (RWC), the contents of chlorophyll (Chl *a*+*b*) and carotenoids (Car), the uptakes of zinc (Zn), potassium (K), and calcium (Ca) in shoot and root, and total accumulation rate (TAR) of Zn were decreased by Cd exposure as well as bioaccumulation and translocation of Cd. Furthermore, increasing Cd doses augmented the concentrations and uptakes of Cd in shoot and root, net accumulation of Cd via roots, the TAR of Cd, membrane permeability (MP) and the rate of Car/Chl caused by its toxic effects. This study demonstrated that Cd exposure cause a reduction in growth due to affecting morphological and physiological characteristics of sunflower in the initial stages of plant development.

Key words: bioaccumulation, cadmium, growth tolerance index, metal nutrientions, translocation

Erken Gelişme Döneminde Ayçiçeği (*Helianthus annuus* L.) Bitkisinin Kadmiyum Maruziyetine Tepkisi

Öz

Çalışma, erken gelişme döneminde ayçiçeği bitkisinin büyüme ve fizyolojik özellikleri yanında bazı metal besin iyonlarının birikimine artan kadmiyum (Cd) maruziyetinin etkilerini değerlendirmek amacıyla sera koşullarında ve gün ışığı altında yürütülmüştür. Bu amaçla, toprağa artan düzeylerde Cd (0, 50, 100, 250, 500 ve 1000 µM $CdCl_2.H_2O$) uygulanmıştır. Bitki gelişimi ve kök uzaması Cd maruziyetinden olumsuz etkilenmiş ve Cd'un bitki büyümesi üzerindeki zararlı etkisi köklerden daha fazla gövdede görülmüştür. Cd maruziyeti, gelişim tolerans indeksi (GTI), nispi su içeriği (RWC), klorofil (Chl *a*+*b*) ve karotenoid (Car) içerikleri, gövde ve kökte çinko (Zn), potasyum (K) ve kalsiyum (Ca)'un alımları ve Zn'nun toplam akümülasyon oranı (TAR) ile birlikte Cd'un biyoakümülasyonu ve translokasyonunu azaltmıştır. Ayrıca, gövde ve kökteki Cd konsantrasyonları ve alımları, kökler aracığıyla net Cd akümülasyonu, Cd'un toplam akümülasyon oranı, membran geçirgenliğini (MP) ve Car/Chl oranı artan Cd dozlarının toksik etkisiyle artmıştır. Bu çalışma, ayçiçeği bitkisinin morfolojik ve fizyolojik özelliklerini etkileyerek Cd maruziyetinin bitki gelişiminin ilk dönemlerinde büyümede bir azalmaya neden olduğunu göstermiştir.

Anahtar kelimeler: *biyoakümülasyon, kadmiyum, gelişim tolerans indeksi, metal besin iyonları, translokasyon*

Introduction

Cadmium, one of the most toxicant non-essential elements for plants, animals, and humans, has been main contaminant in land and aquatic surroundings for last decades. Although Cd occurs naturally in soils, recent advancements in farming and industry have gone to an increment of Cd quantity in agricultural soils. Cadmium enters agrarian soil mainly through several anthropogenic emissions, such as the intensive using of phosphate fertilizers, wastewater, sewage sludge, manure, burning of fossil fuels, mining activities, metallurgical and cement industry and urban traffic (Singh and Agrawal, 2007; Sanitá di Toppi and Gabbrielli, 1999). Increasing Cd concentrations in the terrestrial and aquatic environment have brought about serious concern, because in the form of Cd^{2+} cation it is highly mobile in soil and toxic to plants, animals and humans (Zembala et al., 2010). Cd is easily taken up into plant cells in different plant parts by membrane transporters of essential elements (Clemens, 2006), and competes with the uptake, transport and physiological function of macro and micronutrients (Rivetta et al., 1997). Accumulated Cd ions in plants seem to compete for the same transmembrane carriers with most nutrients, such as K, Ca, Mg, Zn, and iron (Fe) across the same transmembrane carriers (Sanitá di Toppi and Gabbrielli 1999; Rivetta et al., 1997).

Chlorophyll biosynthesis, electron transport and carbon assimilation in different species impaired by direct and indirect interactions of Cd with different components of the photosynthetic apparatus (Moradi and Ehsanzadeh, 2015; Shi et al., 2010; Mobin and Khan, 2007). Studies reported that Cd-induced negative effects can result in the inhibition of photosynthesis, transpiration, the decrease of biomass production and root growth, the reduction of chlorophyll content instability of mineral and water nutrition, and effects on membrane structure and permeability (Çikili et al., 2016; De Maria et al., 2013; Gomes et al., 2012; Rivelli et al., 2012; Shi et al., 2010; Mobin and Khan, 2007; Sanitá di Toppi and Gabbrielli 1999).

In the last decades, sunflower (*Helianthus annuus* L.), one of the most important oilseed crops, is an progressively important source of vegetable oil and biomass, industrial purposes and energy and, also acquired growing interest for phytoremediation of heavy metals and organic pollutants. Although sunflower usually regarded as a highly tolerant crop, which can cope with elevated heavy metal concentrations in soil, impairment of growth at initial stages of plant development may result in a poor crop establishment (Groppa et al., 2008). For this reason, the present study designed to understand the effects of different Cd concentrations on growth, physiological characteristics, and accumulation of some metal nutrient ions at early growth stage in sunflower seedlings, as well various components being an indicator in phytotoxicity of heavy metal stress.

Materials and Methods

Growth Conditions

The experimental study were performed on sunflower (*Helianthus annuus* L., cv. Sirena) plants grown in pots lined with polyethylene bags filled with 2 kg of soil, under daylight conditions at an ambient temperature in a greenhouse (lat 40°53' 40'' N, long 31°02'55'' E). The climatic conditions in the greenhouse during the experiment were air temperature 17/27 °C (night/day) and relative humidity 62%.

The experimental soil was loam in texture (sand/clay; 35.8/21.7, by dry weight), consisting of 0.625% organic carbon (Walkley-Black), total nitrogen (Kjeldahl method) 0.086%, lime ($CaCO_3$) 1.73%, and has a pH (1:2.5 soil/water) of 7.34, and 520 µS cm^{-1} electrical conductivity (EC) in saturation extract. The concentrations of ammonium acetate (CH_3COONH_4)-extractable K, Ca, and Mg were as follows (mg kg^{-1}); 100, 2151, and 124, respectively. Sodium bicarbonate ($NaHCO_3$)-available phosphorus (P) concentration was 12.43 mg kg^{-1} and the concentration of hot water extractable boron (B) was 1.64 mg kg^{-1}. The concentration of diethylene triamine penta acetic acid (DTPA)-extractable manganese (Mn), Fe, Zn, copper (Cu), and Cd were 65.3, 24.3, 2.09, 1.17, and 0.04 mg kg^{-1}, respectively. All characteristics of experimental soil were determined in accordance with analysis methods detailed in Page et al. (1982).

Experimental Layout

Six levels of Cd (0, 50, 100, 250, 500, and 1000 µM) as cadmium chloride ($CdCl_2.H_2O$) were applied to the soil and replicated three times in a completely randomized design. For basal fertilization; nitrogen (N) as ammonium nitrate (NH_4NO_3), P and K as potassium dihydrogen phosphate (KH_2PO_4), and potassium sulphate (K_2SO_4), was applied at 150, 75, and 150 mg kg^{-1}, respectively. Before the sowing and completely mixing, the entire supplemental component added into the soil by spraying. Before planting, equal-sized and healthy sunflower seeds were surface sterilized in sodium hypochlorite (NaOCl) solution 0.2% (w/v) for 20 minutes, and afterwards rinsed out ten times with purified water. For 24 hour, sunflower seeds soaked in water and sown in proportion as three seeds to every one pot. After well-growing of seedlings, they thinned out to one seedling per pot. The plants irrigated daily

with tap water up to 70% of field capacity of soil in pots.

Physiological Assay

Youngest fully expanded leaves taken from as fresh leaf sample before harvest, and it used in the biochemical assays as fresh matter. Before harvest, membrane permeability (MP), photo-synthetic pigments, and relative water content (RWC) with fresh leaf sample measured. Membrane permeability determined by EC method (Yan et al., 1996). The fresh matter (200 mg) break into pieces and homogenized with 10 mL of acetone (90% v/v) in a homogenizer for determining photosynthetic pigments. The absorbance of filtered homogenates determined at 663, 645, and 470 nm by utilizing a spectrophotometer. In accordance with the formula of Lichtenthaler (1987), the concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) were figured out.

A composite sample of leaf discs (1 cm) were used determined the RWC. Firstly, leaf discs weighed to record as fresh weight (FW). Weighed leaf discs was swelled out to determine turgid weight (TW) in purified water for 4 hour, and then it was dried to determine dry weight (DW) in oven at 70 °C for 48 hour. Relative water content figured out by Equation 1.

$$RWC = (FW - DW) / (TW - DW) \quad (1)$$

Harvest and Sampling

Sunflower plants harvested properly at 20 days after planting, and the fresh weight (FW) obtained by harvested shoots and roots separately. The shoot and root samples from each pot attentively taken out washed with tap water and then rinsed out by distilled water. The root samples firstly immersed into an aerated 0.5 mM calcium chloride ($CaCl_2$) solution for 15 minutes to remove adsorbed nutrients from root surface, and secondly rinsed out with purified water. The root length of each plant in pots measured using a centimeter scale. The shoot and root samples were oven-dried at 70°C and so dry weight (DW) weighted out. After that, all samples milled into powder with grinder for ion analysis.

Measurements and Calculations

Cadmium tolerance calculated as the growth tolerance index (GTI), which gives an opinion and/or effect of applied stress factor on plant grown and development. The GTI calculated by the Equation 2 (Rout et al., 1998).

$$GTI\ (\%) = 100 \times (\text{root or shoot DW in Cd treatment}) / (\text{root or shoot DW in the control}) \quad (2)$$

The dried samples incinerated according to dry-ashing method detailed in Miller (1998), and then liquefied in ten normal nitric acid (HNO_3). The concentrations of K, Ca, Zn, and Cd in shoot and root samples measured with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Ion uptake in roots and shoots figure out by Equation 3.

$$\text{Ion uptake in root or shoot } (\mu g\ plant^{-1}) = (\text{DW in root or shoot}) \times ([\text{ion}] \text{ in root or shoot}) \quad (3)$$

where: ([ion] in root or shoot = ion concentration of the root or shoot

The Cd distribution as %, bioconcentration factor (BCF), translocation factor (TF), total accumulation rate (TAR) as $\mu g\ g^{-1}$ DW day^{-1} and net accumulation of ion via roots (Net Acc) as $\mu g\ g^{-1}$ DW were figured out by Equation 4, 5, 6, 7, and 8, respectively (Çikili et al., 2016; Moradi and Ehsanzadeh 2015; Shi et al., 2010; Ait Ali et al., 2004).

$$Cd\ distribution = 100 \times ([Cd] \text{ in root or shoot} / ([Cd] \text{ in shoot} + [Cd] \text{ in root})) \quad (4)$$

$$BCF = [Cd] \text{ in root or shoot} / [\text{total Cd}] \text{ in soil} \quad (5)$$

$$TF = [Cd] \text{ in shoot} / [Cd] \text{ in root} \quad (6)$$

$$TAR = ([\text{ion}] \times DW) \text{ in shoot} + ([\text{ion}] \times DW) \text{ in root} / \text{growth day} \times (DW \text{ in shoot} + DW \text{ in root}) \quad (7)$$

$$Net\ Acc = \text{total amount of ion in whole plant } (\mu g) / \text{root DW } (g) \quad (8)$$

Statistical Analysis

The obtained data from the experiment analyzed by using F test procedure of JMP statistical software (SAS Institute Inc., Cary, NC). Values expressed as means ± standard error (SE) of three replicates. Significance level represented by * at $P \leq 0.05$, ** at $P \leq 0.01$, and ns: non-significant. Duncan's multiple range test to compare the differences of means among different Cd treatments at $\alpha = 0.05$ level of significance.

Results and Discussion

Plant Growth Parameters

Discernible symptom of Cd toxicity transpired as decrease of root length and plant growth. At the end of the experimental period, main roots in sunflower plants with the highest Cd treatments appeared discernible browning and thickened as well as wilted and yellowed leaves. It observed browning roots, reduction in number of roots, and growth inhibition of roots and shoots in the Cd-treated seedlings of sunflower as explained by Zou et al. (2008). Increasing Cd to the soil markedly reduced the FW and DW of shoot and root as well as root length (Table 1). These reductions, in comparison with the control, were significant and

linear in the shoot FW and DW as from 100 µM Cd treatment. Similarly, a reduction observed the root FW and DW; however, this reduction was only significant for 1000 µM Cd treatment. The root length increased with 50 µM Cd treatments and then remarkably decreased with increasing Cd levels compared with the control (Table 1). These results showed that the shoot and root growth were negatively affected Cd contamination. Supporting of these results, the decrement of plant growth parameters caused by Cd treatment has been showed in sunflower (Zou et al., 2008) and other plants, including safflower (Shi et al., 2010), soybean (Shamsi et al., 2010), and tomato (Haouari et al., 2012). Piršelová et al. (2015) also reported that Cd phytotoxicity caused a reduction over 50% in root elongation of maize, barley, bean, pea and soybean. The reduction the FW and DW of shoot and root because of increasing Cd concentrations might ascribed to noticeable decrements in root length and the rate of net photosynthesis. Cadmium declines photosynthesis rates due to affecting different pathways such as the disarranged chloroplast ultra-structure, the inhibited synthesis of chlorophyll, the restrained enzyme activities of the Calvin cycle, the obstructed electron transport, and CO_2 deficiency caused by stomatal closure, and these metabolic changes resulted from Cd lead to deceleration of plant growth and development (Haouari et al., 2012; Mobin and Khan, 2007). Many researchers also revealed that biomass production severely reduced due to excess Cd in plants as a result of a serial of stress symptoms including chlorosis, wilting, necrotic lesions, disturbances in carbohydrate metabolism and mineral nutrition (Azevedo et al., 2005).

Growth tolerance index, which is a summary assessment of effect of stress factor on plant growth and development, calculate based on morphological parameters (Haouari et al., 2012). The GTI of sunflower plants affected markedly with Cd treatments, and showed inhibition in growth of shoot and root and in biomass production (Table 1). The GTI in shoot and root of sunflower plants decreased with increasing Cd treatments, reaching to levels as high as 19.6% and 25.8%, respectively. These decreases for the GTI of shoot were significant as from 100 µM Cd level; however, the reduction in the GTI of root was significant at highest Cd level. On the other hand, the GTI of root was higher than those of shoot. Similar results in earlier studies reported in tomato (Haouari et al., 2012) and varieties of pea (Metwally et al., 2005).

Table 1. The effects of Cd exposure on FW and DW of shoot and root, root length, and GTI of sunflower plants.

Treatments	Shoot (g plant^{-1})		Root (g plant^{-1})		Root length (cm)	GTI (%)	
Cd (µM)	FW	DW	FW	DW		Shoot	Root
0	$16.2^{\pm1.37}$a	$1.57^{\pm0.12}$a	$6.36^{\pm0.34}$a	$0.39^{\pm0.030}$a	$17.7^{\pm0.46}$b	100.0a	100.0a
50	$16.3^{\pm1.09}$a	$1.41^{\pm0.12}$ab	$6.71^{\pm0.41}$a	$0.41^{\pm0.023}$a	$19.8^{\pm0.66}$a	89.9ab	104.0a
100	$12.8^{\pm0.51}$ab	$1.16^{\pm0.04}$bc	$6.16^{\pm0.25}$a	$0.36^{\pm0.023}$a	$16.6^{\pm0.18}$bc	74.0bc	92.6a
250	$11.5^{\pm0.68}$b	$1.15^{\pm0.11}$bc	$6.33^{\pm0.58}$a	$0.38^{\pm0.045}$a	$15.6^{\pm0.13}$cd	73.3bc	96.0a
500	$11.1^{\pm1.98}$b	$0.95^{\pm0.17}$c	$5.06^{\pm1.20}$a	$0.29^{\pm0.075}$a	$15.2^{\pm0.47}$d	60.5c	74.1a
1000	$3.0^{\pm0.13}$c	$0.31^{\pm0.04}$d	$1.67^{\pm0.12}$b	$0.10^{\pm0.011}$b	$9.0^{\pm0.20}$e	19.6d	25.8b
F-test	**	**	**	**	**	**	**

Cadmium Accumulation

The effects of the increasing Cd treatments on Cd concentration in shoot and root of sunflower plants and their uptakes and distribution given on Table 2. The Cd concentrations in both shoot and root markedly augmented with increasing Cd treatment as comparison with control. The Cd concentrations in shoot and root of sunflower plants enhanced in response to increasing Cd levels, attaining to levels as high as 174.1 and 657.3 µg g^{-1}, respectively. Evidently, the shoot Cd concentrations were much lower than those of the roots, concerning Wu (1990) explained that about 70-85% of the absorbed Cd by various plants remains in the roots. In addition, toxic metals, like Cd, substantially accumulated by the roots in many species including sunflower (Groppa et al., 2008). The high Cd concentration found mainly in roots and old leaves of sunflower by virtue of Cd toxicity as reported De Maria et al. (2013), who elucidated that sunflower tend to avoid toxicity in the physiologically most active parts of the plants by decreasing Cd translocation to the epigeous part, and by promoting the re-translocation of toxic metals from shoots to the roots. This is in line with results of Çikili et al. (2016) in *Solanaceae* plants, Haouari et al. (2012) in tomato, Shamsi et al. (2010) in soybean, and Shi et al. (2010) in two safflower cultivars.

Table 2. The changes in concentrations and uptakes of Cd in shoot and root of sunflower plants as affected by Cd exposure.

Treatments	Cd concentration (µg g^{-1} DW)		Cd distribution (%)		Cd uptake (µg $plant^{-1}$)	
Cd (µM)	Shoot	Root	Shoot	Root	Shoot	Root
0	$0.93^{\pm 0.07}$e	$1.67^{\pm 0.24}$e	36.5	63.5	$1.46^{\pm 0.11}$c	$0.66^{\pm 0.14}$c
50	$14.4^{\pm 1.73}$d	$104.2^{\pm 9.14}$d	12.1	87.9	$20.3^{\pm 2.92}$b	$42.0^{\pm 1.95}$bc
100	$16.4^{\pm 1.25}$d	$98.7^{\pm 0.53}$d	14.2	85.8	$19.0^{\pm 1.39}$b	$35.7^{\pm 2.38}$bc
250	$33.0^{\pm 2.43}$c	$298.4^{\pm 14.4}$c	10.0	90.0	$38.4^{\pm 6.63}$a	$113.2^{\pm 18.1}$a
500	$59.6^{\pm 0.70}$b	$412.1^{\pm 13.9}$b	12.7	87.3	$56.3^{\pm 9.62}$a	$118.5^{\pm 29.0}$a
1000	$174.1^{\pm 4.32}$a	$657.3^{\pm 32.3}$a	21.0	79.0	$53.4^{\pm 6.32}$a	$67.1^{\pm 10.5}$b
F-test	**	**			**	**

Furthermore, the Cd uptake of the shoot and root significantly enhanced increments of Cd supply in comparison with the control (Table 2). The Cd uptake in shoots has been about 2-fold more than the roots at the control level, whereas this case entirely reversed with increasing of supplied Cd in growth medium and for example, the roots accumulated about 2-fold more than the shoots at the levels of both 50 and 500 µM Cd (Table 2). Similar results were found by, Gomes et al. (2012), who notified an increment of the Cd uptake for Pfaffia glomerata with increasing of supplied Cd. The root tissue acts as a defensive response of plants to protect the above-ground parts from excess Cd and a barrier to Cd translocation to the shoot. Having different mechanisms of plant for uptake of Cd and for accumulation in the different parts has been reported previously (Zhao et al., 2002).

Bioconcentration and Translocation of Cd

BCF in shoot and root notably decreased all for Cd treatments, and BCF in shoot and root are ranged from 1.03 to 2.53 and 5.83 to 18.40 under 50-1000 µM Cd soil condition, respectively (Table 3). The results revealed that BCF was much more in roots than in shoots. Moreover, BCF in both shoot and root surpassed the crucial level for a Cd hyperaccumulator plant, at present acknowledged as BCF > 1 (Baker, 1981). Hyperaccumulators, efficient plants root-to-shoot transport system and indicating enhanced capacity for detoxification, have an inherent capacity to absorb metal at levels 50-500 times greater than normal plants (McGrath and Zhao 2003). The BCF levels mostly tend to diminish as increasing concentrations of supplied metal in grown media, indicating a decreasing efficiency of heavy metal accumulation with increasing exposure (Zhao et al., 2003). The BCF is typically greater than one in metal-accumulating species; whereas that of excluding species is often lower than it (Baker, 1981). This result showed that sunflower seedlings could be described as Cd-hyperaccumulator plant.

The partitioning of Cd to different plant organs plays substantial role in toxicity of Cd to plants, and translocation factor can indicated as the translocation of heavy metals in plants. TF determined 0.59 at the control level, whereas TF significantly diminished with 50-1000 µM Cd treatments as compared to the control and ranged from 0.11 to 0.27 (Table 3). TF in sunflower was lesser than crucial degree (TF > 1). Apparently, at the control level (0 µM Cd), the metal accumulation due to that immobilizes Cd in the cells by phytochelatins has emerged as the most likely initial response of plants. However, the uptake and the translocation of Cd might decrease and/or block by plants with increasing Cd treatments. This is in accord with our findings of Shi et al. (2010) in safflower, and De Maria et al. (2013) in sunflower.

The total accumulation rate is an index parameter, which has greatly used in bioaccumulation studies and is a measure of heavy metal uptake by plants. TAR of Cd and net accumulation of Cd via roots notably increased by Cd treatments according to control; however, TAR of Zn reduced linearly (Table 3). Sharma and Agrawal (2006) were obtained similar results, and explained that TAR and Cd uptake in carrot notably accrued at level of excess Cd. Furthermore, Moradi and Ehsanzadeh (2015) stated that it is found an increment in net accumulation of Cd via roots in safflower genotypes subjected to increasing concentrations of Cd. Also, the reduction in the TAR of Zn could be explained by being an antagonistic relationship between Cd and Zn. The presence of a binary metal affect the uptake of another metal as reported several studies. Both Zn and Cd intake the root cell via a common transport membrane protein (Sharma and Agrawal, 2006).

Table 3. The changes in BCF in shoot and root, TF, TAR of Cd and Zn, and net accumulation of Cd via roots in sunflower plants as affected by Cd exposure.

Treatments	BCF		TF	TAR of Cd	TAR of Zn	Net Acc of Cd
Cd (µM)	Shoot	Root		(µg g^{-1} DW day^{-1})		(µg g^{-1} DW)
0	$23.33^{\pm1.67}$a	$41.63^{\pm5.98}$a	$0.59^{\pm0.01}$a	$0.21^{\pm0.01}$b	$6.77^{\pm1.01}$a	$5.4^{\pm0.20}$e
50	$2.53^{\pm0.31}$b	$18.40^{\pm1.61}$b	$0.14^{\pm0.00}$bc	$5.69^{\pm0.74}$b	$5.60^{\pm0.89}$ab	$155.0^{\pm18.0}$d
100	$1.43^{\pm0.11}$b	$8.77^{\pm0.05}$c	$0.17^{\pm0.01}$bc	$4.16^{\pm0.14}$b	$3.80^{\pm0.17}$bc	$151.7^{\pm6.36}$d
250	$1.17^{\pm0.09}$b	$10.60^{\pm0.51}$c	$0.11^{\pm0.01}$c	$11.78^{\pm2.50}$a	$3.76^{\pm0.44}$bc	$401.2^{\pm12.5}$c
500	$1.03^{\pm0.01}$b	$7.33^{\pm0.25}$c	$0.15^{\pm0.00}$bc	$11.65^{\pm3.86}$a	$2.62^{\pm0.92}$c	$621.7^{\pm44.7}$b
1000	$1.53^{\pm0.04}$b	$5.83^{\pm0.29}$c	$0.27^{\pm0.01}$b	$2.45^{\pm0.11}$b	$0.36^{\pm0.04}$d	$1211.0^{\pm84.4}$a
F-test	**	**	**	**	**	**

Membrane Permeability and Water Status

The MP in shoots diminished with excess Cd level and for instance, the MP has been about 2-fold more the highest Cd level than the control level; however, these chances were not significant (Figure 1). The RWC measured with a view to evaluate Cd on the water status of sunflower plants. The RWC in shoots affected by increasing Cd treatment, and significantly decreased from 86.6% in control to 80.2% in 1000 µM Cd level (Figure 1). Cd stress can alter water balance disturbances in plants, most likely due to the effects on stomatal conductance, water transport and cell wall elasticity (Barceló et al., 1986). Gomes et al. (2012) reported that the water status of *Pfaffia glomerata* reduced by the high Cd exposure relating to decreases in stomatal areas associated with increasing stomatal densities. In addition, Rivelli et al. (2012) reported that the effect of Cd on osmotic potential could be derived from dysfunctions of the membrane integrity by virtue of displacement of Ca from the cell surface by Cd. The RWC of sunflower plants reduced with 1000 µM of Cd concentration as revealed by Groppa et al. (2007). Moreover, De Maria et al. (2013) reported that increasing the levels of Cd in the soil decreased leaf osmotic potential, whereas the changes in RWC were not significant.

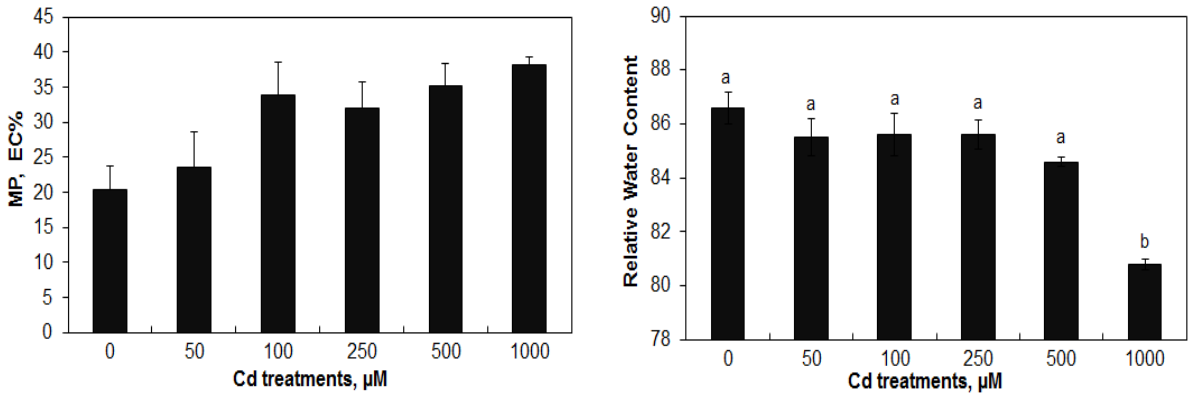

Figure 1. The changes in membrane permeability (MP) and relative water content (RWC) in sunflower plants as affected by Cd exposure.

Photosynthetic Pigments

The content of photosynthetic pigments (Chl *a*, *b*, and Car) of leaves in sunflower plants were decreased dramatically by increasing of supplied Cd to the soil (Table 4). For instance, the reduction of Chl *a*, Chl *b*, Chl *a+b* and Car content by 64.6%, 77.0%, 65.0%, and 58.2%, respectively, in plant subjected to high level of Cd treatment in comparison with the control. It is well known that Cd restrains photosynthesis and decreases chlorophyll content. The decreases of chlorophyll content in sunflower plant subjected to Cd treatment is associated with decrease of thylakoid membrane integrity, chlorophyll degradation and/or disorders in chlorophyll biosynthesis (Sandalio et al., 2001). In support of these findings, the reduction of chlorophyll content with an increment in Cd concentration eventuated in *Solanaceae* plants (Çikili et al., 2016), sunflower (De Maria et al., 2013, Azevedo et al., 2005), safflower (Shi et al., 2010, Moradi and Ehsanzadeh 2015), and tomato (Haouari

et al., 2012). In addition, Chen et al. (2011) revealed that Car content of pakchoi (*Brassica campestris* ssp.) and mustard (*Brassica juncea* Czernajew) decreased by increasing of Cd concentrations. As shown in Table 4, highest level of supplied Cd affected more strongly Chl *a* than Chl *b*, and relative reduction of Chl *b* was greater than Chl *a*. Furthermore, an increment in the ratio of Chl *a*/*b* were observed due to this relative differences; but this increases was non-significant. The reduction in the Chl *b* caused by excess Cd could explain the occurrence of different mechanism as reported by Ito et al (1996), who has revealed these mechanism as greater degradation of Chl *b* or an increment of Chl *b* in conversion to Chl *a* or a reduction in conversion of Chl *a* to Chl *b*, and combination of these factors. Besides, Car/Chl remarkably increased with Cd treatments. Having an interaction of Cd levels and safflower cultivars on the ratio of Car/Chl as reported Shi et al (2010), who stated that this ratio increased notably in safflower cultivar, which exhibited the high photosynthetic performance at high Cd level. Moreover, excess of Cd decreased the ratio Chl (*a*+*b*)/Car in leaf sections differing in age of Zea mays seedlings as mentioned by Dresler et al. (2014).

Table 4. The changes in photosynthetic pigments (Chl *a*, *b*, and Car) of sunflower plants as affected by Cd exposure.

Treatments	Chl *a*	Chl *b*	Chl *a*+*b*	Car	Chl *a*/*b*	Car/Chl
Cd (µM)	(µg g^{-1} FW)					
0	$477^{\pm1.2}$a	$100^{\pm6.3}$a	$577^{\pm5.5}$a	$294^{\pm2.7}$a	$4.32^{\pm0.33}$	$0.511^{\pm0.002}$c
50	$450^{\pm1.6}$b	$101^{\pm1.5}$a	$551^{\pm1.7}$b	$279^{\pm1.1}$b	$4.43^{\pm0.16}$	$0.506^{\pm0.005}$c
100	$393^{\pm3.8}$c	$83^{\pm0.7}$b	$476^{\pm3.8}$c	$247^{\pm2.0}$c	$4.73^{\pm0.05}$	$0.519^{\pm0.001}$bc
250	$322^{\pm7.0}$d	$72^{\pm1.8}$c	$394^{\pm9.0}$d	$206^{\pm4.1}$d	$4.44^{\pm0.02}$	$0.523^{\pm0.001}$bc
500	$327^{\pm5.8}$d	$68^{\pm2.9}$d	$395^{\pm8.4}$d	$209^{\pm3.8}$d	$4.76^{\pm0.11}$	$0.528^{\pm0.002}$b
1000	$169^{\pm2.3}$e	$33^{\pm1.7}$e	$202^{\pm4.0}$e	$123^{\pm1.2}$e	$5.13^{\pm0.22}$	$0.608^{\pm0.006}$a
F-test	**	**	**	**	*ns*	**

Uptake of Metal Nutrients

The effects of increasing of supplied Cd to the soil on the uptakes of Zn, Ca, and K in shoot and root of sunflower plants given on Table 5. In response to increasing Cd doses with comparison to control, significant reductions in uptakes of Zn, Ca, and K in both shoot and root observed. These reductions for the uptake of Zn and K of shoot were significant as from 250 µM Cd level; however, the decrease in the uptake of Ca was significant as from 100 µM Cd level. In addition, the decreases in the uptake of Zn, Ca, and K of root notably found at the high Cd level (Table 5).

Cadmium competes with macronutrients (P, Ca, and Mg) and micronutrients (Zn, Fe, Mn, and Cu) for the same transmembrane carrier protein (Rivetta et al., 1997). As a result of this case, excess Cd affect the nutrient composition of plants caused by inhibiting the normal uptake and utilization of macro- and micro-nutrients (Krupa et al., 2002). Cadmium could rival with several nutrients elements, like K and Ca, changing their concentration in sunflower tissues as stated by Rivelli et al. (2012). Haouari et al. (2012) in tomato seedling, and Zembala et al. (2010) in wheat and rape seedlings declared the decreases in Ca and K contents of shoot and root with Cd applications. Herrero et al. (2010) revealed differences in translocation and absorption capacities of nutrient elements in sunflower and oil plants due to impact of interactions between nutrients elements and Cd. Liu et al. (2003) reported that impacts of Cd on the mineral nutrients in the leaves and roots of rice were usually considerable; however, changed with growing stages, organs and metal elements.

Table 5. The changes in the uptake of metal nutrients in shoot and root of sunflower plants as affected by Cd exposure.

Treatments	Zn uptake (µg plant $^{-1}$)		Ca uptake (mg plant $^{-1}$)		K uptake (mg plant $^{-1}$)	
Cd (µM)	Shoot	Root	Shoot	Root	Shoot	Root
0	$55.7^{\pm 8.64}$a	$12.8^{\pm 1.51}$a	$13.89^{\pm 1.07}$a	$0.48^{\pm 0.03}$a	$100.5^{\pm 8.5}$a	$21.2^{\pm 1.3}$a
50	$49.6^{\pm 5.15}$ab	$11.5^{\pm 0.80}$a	$11.04^{\pm 0.93}$ab	$0.45^{\pm 0.02}$a	$99.5^{\pm 8.4}$a	$20.7^{\pm 0.6}$a
100	$39.6^{\pm 2.10}$abc	$10.3^{\pm 0.46}$ab	$9.73^{\pm 0.28}$b	$0.46^{\pm 0.0}$ a	$79.5^{\pm 2.4}$ab	$20.0^{\pm 0.9}$a
250	$38.0^{\pm 1.51}$bc	$11.2^{\pm 1.32}$ab	$9.41^{\pm 0.97}$b	$0.48^{\pm 0.07}$a	$70.3^{\pm 5.1}$b	$18.2^{\pm 2.2}$a
500	$31.6^{\pm 7.33}$c	$7.4^{\pm 2.06}$bc	$8.00^{\pm 1.45}$b	$0.32^{\pm 0.09}$a	$63.3^{\pm 9.3}$b	$16.3^{\pm 4.0}$a
1000	$12.4^{\pm 1.52}$d	$5.2^{\pm 0.51}$c	$3.03^{\pm 0.35}$c	$0.12^{\pm 0.02}$b	$19.3^{\pm 2.5}$c	$5.4^{\pm 0.6}$b
F-test	**	**	**	**	**	**

Conclusion

Cadmium stress gradually reduced plant growth and root elongation in sunflower plants and noxious effect of Cd toxicity on plant development was emerged shoot more than roots. Also, GTI, RWC, the BCF of Cd, TF of Cd, the contents of photosynthetic pigments, and the uptakes of Zn, K, and Ca in shoot and root reduced by Cd stress as well TAR of Zn. Increasing Cd exposure increased MP, the rate of Car/Chl, the concentrations and uptakes of Cd in shoot and root, TAR of Cd, and net accumulation of Cd via roots due to its toxic effects. The present study evidently demonstrates that Cd exposure cause a reduction due to affecting morphological and physiological characteristics of sunflower at the initial stages of plant development.

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