

Full Length Research Paper

## Cadmium effects on sunflower growth and mineral nutrition

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A hydroponic greenhouse study was carried out to evaluate the effects of increasing cadmium (Cd) concentration on plant growth, mineral nutrition and Cd distribution of H-250 sunflower genotype. Exposure to increasing Cd concentrations reduced plant biomass by 40, 34, 47 and 42% of the total, leaves, stem and roots dry weights as compared to the control. Regardless of the treatment most of Cd uptake by the genotype was allocated in the root, followed by leaf and stem. The higher bioconcentration factors values in both above ground and underground plant tissues and low transfer factor value indicated that this genotype may be an alternative for use in phytostabilization programs. The results also showed that increasing Cd concentration disrupted plant homeostasis as it increased the concentration of some nutrients and had adverse effect on others, impacting plant growth. In this context, the results suggest that the low magnesium, iron and manganese concentrations in the leaves were the main cause for plant biomass reduction and leaf chlorosis and necrosis, as each one of these elements plays a key role on the chlorophyll molecule and on photosynthesis process.

**Key words:** Bioremediation, bioaccumulation factor, heavy metal stress, *Helianthus annuus*, transfer factor.

### INTRODUCTION

Cadmium (Cd) is a non-essential trace metal that presents potential threat for humans, plants, microorganisms and environment (Dong et al., 2007). It is considered to be one of the most toxic heavy metals in soil and water environments in the world (Järup and Åkesson, 2009). Exposure of humans to Cd is an

important issue. Based on the combined considerations of frequency, toxicity and potential for human exposure, Cd is ranked as the fourth metal in the List of Hazardous Substances (ATSDR, 2014). Environmental Cd contamination is a serious concern due to its high solubility in water Cd and because it can easily enter in

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the food chain mainly by plant uptake and accumulated in plants and animals to concentrations that may pose carcinogenic, teratogenic, and mutagenic effects (Nazar et al., 2012). The Food and Agriculture Organization/World Health Organization (FAO/WHO, 1972) established a maximum weekly intake of 7  $\mu\text{g}$  Cd per kg of body weight. Human activities, such as metallic industries, herbicides, concentrated phosphate fertilizers and industrial and municipal waste have contributed to the increase of Cd concentrations in the environment (Benavides et al., 2005).

To cope with metal toxicity or to maintain the level of essential metal within physiological ranges, plants have evolved complex cellular mechanisms of accumulation or exclusion to regulate uptake of nonessential metals. The main characteristic of the plants that accumulate metals is to translocate most of them to the aboveground tissue. These plants present bioconcentration factor - BCF (the ratio of metal concentration in shoot to soil), and transfer factor - TF (the ratio of metal concentration in shoot to root) greater than one. In contrast, excluders plants limit soil-root and root-shoot transfers, and therefore BCF and TF are lower than one (Malik et al., 2010; Yoon et al., 2006).

Exposure of non-accumulator plant species to Cd can inhibit plant growth and can cause plant stunting, chlorosis, apical meristem necrosis, brownish colour and plant death (Smeets et al., 2005). The metal toxicity may also alter many plant physiological processes including the nitrogen and carbohydrates metabolism, photosynthesis, mineral nutrients assimilation, and the plant water relationship (Gajdos et al., 2012). Cadmium induced plant tissue toxicity may be accompanied with reduced uptake of essential plant mineral nutrients. Increasing Cd concentration, in the external solution, may reduce the plant uptake of  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$  due to competition for the same membrane transporters. Some of these nutrients are also enzyme cofactors, involved in plant defense against oxidative stress, increasing the rate of biochemical reactions and physiological processes in plants (Nazar et al., 2012; Sarwar et al., 2010).

Sunflower (*Helianthus annuus* L.), a fast growing crop (Gajdos et al., 2012), is the fourth largest oil producer after soybean, canola and cotton (USDA-FAS, 2015) and presents a great economical potential for biodiesel production. The genotype diversity of sunflower confers to the specie a great plasticity in response to various growth environmental conditions (Capone et al., 2012) including the environmental stress. Sunflower has been cultivated as food and feed crop as well as for bioenergy production. The ability of the specie to remove organic and metal pollutants has also been evaluated in phytoremediation studies (Lopes Júnior et al., 2014; Zou et al., 2008). Selection of Cd-tolerant and Cd-accumulator sunflower genotypes can be achieved evaluating the Cd concentration in their organs. The

pattern of metal uptake and distribution of the specie vary with the level of contamination (Simon et al., 1998) and cultivars (Ansari et al., 2009; Zou et al., 2008). It is hypothesized that sunflower can be cropped up to a maximum Cd available concentration. The present study was undertaken, aiming better understanding in the growth, metal accumulation and partitioning, and mineral nutrition of H-250 sunflower genotype exposure to increasing Cd concentration. This information is relevant to indicate the maximum Cd concentration for this genotype cultivation.

## MATERIALS AND METHODS

### Experiment setup

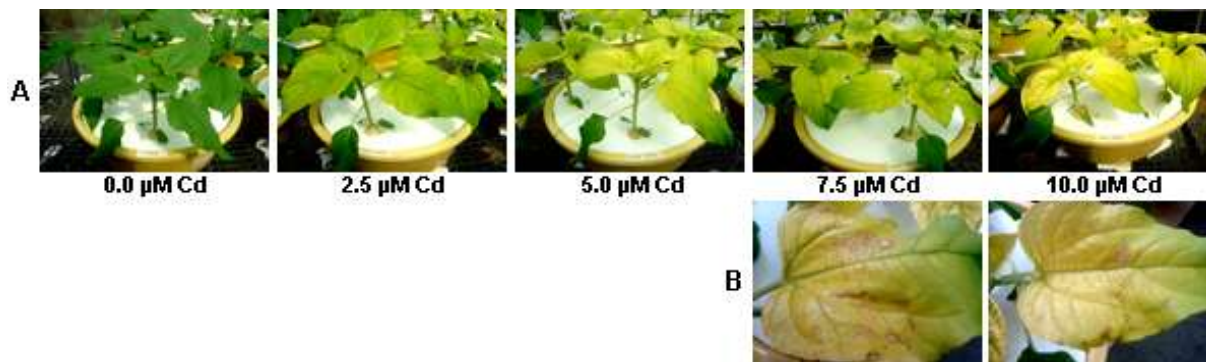
Seeds from H-250 sunflower genotype were sown in 200 mL plastic cups containing washed sand and irrigated daily with distilled water. Five-day-old seedlings selected for uniformity in size and form were acclimatized in pots containing half-strength Hoagland and Arnon (1950) nutrient solution at  $\text{pH } 6.0 \pm 0.5$ . Seven days later, they were transferred to containers containing 10 L of this same nutrient solution containing five different cadmium doses (0; 2.5; 5.0; 7.5 or 10.0  $\mu\text{M}$ ), from here on referred to as  $\text{Cd}_0$ ,  $\text{Cd}_{2.5}$ ,  $\text{Cd}_{5.0}$ ,  $\text{Cd}_{7.5}$  and  $\text{Cd}_{10}$ , applied as  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ . Preliminary studies using various Cd concentrations (from 0 to 50  $\mu\text{M}$ ) have shown that concentrations of up to 10  $\mu\text{M}$  are sublethal, but induce significant effects on short-term exposure. The nutrient solutions were aerated for 15 minutes every three hours by an air compressor, model GF180 (Resun Group CO, Shenzhen, Guangdong, CN). The volume of nutrient solutions was completed daily with distilled water. The mean values of temperature, air relative humidity and photosynthetic active radiation (at noon) in greenhouse were 27°C, 65% and 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. The plants were harvested seven days after transplanting.

### Growth measurements

The harvested plants were washed thoroughly with tap water, and then rinsed with deionized water. Sunflower plants were separated in leaf, stem and root and their fresh weight determined. Plant material was oven dried at 65°C for 72h and then the leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) were determined. Leaf succulence ( $L_{\text{SUC}}$ ) was calculated according to Mantovani (1999) as  $L_{\text{SUC}} = (\text{LFW} - \text{LDW}) / \text{LA}$ , where LFW is the leaf fresh weight (g), LDW the leaf dry weight ( $\text{g plant}^{-1}$ ), and LA the leaf area ( $\text{cm}^2 \text{ plant}^{-1}$ ). Leaf area was measured using a WinDIAS image system, model W-C110-PC (Delta-T Devices Ltd, Cambridge, Cambs., UK). The dried plant material was ground to 20-mesh fineness using a Wiley mill, model STAR FT-50 (Fortinox, Piracicaba, SP, BR)

### Cadmium and nutrient analysis

The plant parts were digested with 3.5 mL  $\text{H}_2\text{SO}_4$  (96-98% w/w) and 2.0 mL 30%  $\text{H}_2\text{O}_2$ , as described by Jones (2001) for the analysis of the elements. The digested material was adjusted to 100 mL volume with deionized water. The total concentration of Cd, K, Ca, Mg, Mn, Fe, Zn and Cu in the solution were analyzed by Optical Inductively Coupled Plasma Spectrometry, model Optima 3300XL (Perkin Elmer, Norwalk, MA, USA). The determinations of N and P were performed by spectrophotometry by the methods of phenol-



**Figure 1.** Sunflower leaves chlorosis (A) and necrosis (B) growing under increasing cadmium exposure concentration, in nutrient solution.

hypochlorite and molybdo-vanadate, respectively, as described by Faithfull (2002). The accuracy was confirmed using the standard reference material NIST-SRM-1572 (Gaithersburg, MD, USA). Cadmium bioconcentration factors of aboveground (BCF) and underground ( $BCF_R$ ) plant tissues, and transfer factor (TF) were calculated using the following equations (Žaltauskaitė and Šliumpaitė, 2013):

$$BCF = Cd_{shoot} / Cd_{solution}$$

$$BCF_R = Cd_{root} / Cd_{solution}$$

$$TF = Cd_{shoot} / Cd_{root}$$

Where  $Cd_{shoot}$  and  $Cd_{root}$  are the cadmium concentrations in aboveground and underground tissues, respectively, and  $Cd_{solution}$  is the cadmium concentration in nutrient solution.

### Statistical analysis

The treatments were arranged as a completely randomized design with five doses of cadmium and four replications. All data are expressed as the mean of all replicates. Treatment effects were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System (SAS, 2003) at  $p < 0.05$ . Regression equations were then adjusted to the significant data.

## RESULTS AND DISCUSSION

Exposure to nonessential elements, such as Cd, impacts plant homeostasis with reflex on plant growth, element partitioning, nutrient uptake, water relations (Hossain et al., 2010), enzyme activity (Ouariti et al., 1997; Van Assche and Clijsters, 1990), photosynthesis and respiration metabolisms (Mobin and Khan, 2007; Shi et al., 2010; Vassilev and Yordanov 1997), nutritional deficiencies and imbalances (Clemens, 2006; di Toppi and Gabbriellini, 1999).

### Effect of Cd on sunflower development

After one week, youngest leaves of sunflower growing in

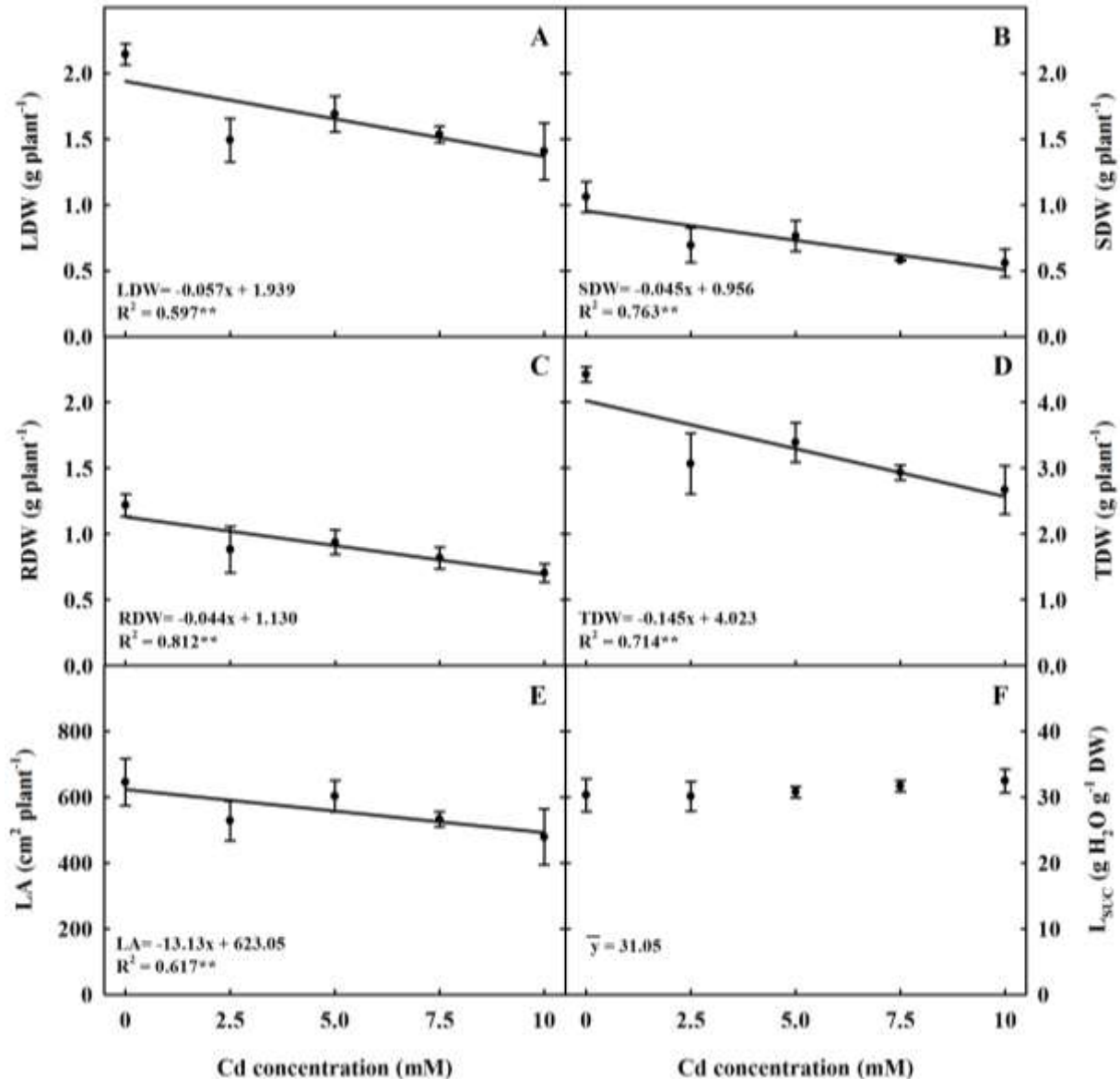
all Cd spiked solutions showed symptoms of toxicity, as indicated by chlorosis (Figure 1). Cadmium concentration in the solution had a major impact on plant growth indicators. Exposure to increasing Cd concentrations linearly reduced the sunflower LDW (Figure 2A). For instance, exposure of 10.0  $\mu\text{M}$  of Cd resulted in the reduction of LDW by  $\sim 0.73$  g, which is equivalent to 0.057 g DW per  $\mu\text{M}$  Cd in solution. This effect was also observed on the SDW ( $Cd_0$  1.06 to  $Cd_{10}$  0.56 g DW, a reduction of 0.045g DW  $\mu\text{M}^{-1}$  Cd-solution, Figure 2B); on RDW ( $Cd_0$  1.22 to  $Cd_{10}$  0.70 g DW or a reduction of 0.044 g DW  $\mu\text{M}^{-1}$  Cd, Figure 2C) and, consequently, on TDW ( $Cd_0$  4.42 to  $Cd_{10}$  2.93 g DW or a reduction of 0.145 g DW  $\mu\text{M}^{-1}$  Cd, Figure 2D). The LDW accounted for about 50% of the TDW. At the highest Cd-concentration (10  $\mu\text{M}$ ), the plant biomass reduction was higher in the stem (47%) and roots (42%) and lower in the leaves (34%) as compared to the control. Increasing Cd concentration did not alter the shoot to root ratio of the treatments.

Cadmium increasing concentration also reduced sunflower LA ( $13.13 \text{ cm}^2 \mu\text{M}^{-1}$  Cd, Figure 2E). Cadmium stress did not alter the water status of the leaves as indicated by the leaf succulence ( $L_{SUC}$ ) that show no variation with increasing doses of cadmium in nutrient solution (Figure 2F). The leaf water concentration averaged  $31.05 \text{ g H}_2\text{O cm}^{-2}$  LA.

Cadmium promotes disturbances in vital physiological plant processes. Our data suggest that the photosynthetic activity restriction caused by chlorosis, necrosis and LA reduction may explain, at least in part, the Cd-induced plant growth reduction.

### Cadmium concentration and partition in the plant

Cadmium concentration in the plant parts changed with Cd-solution (Figure 3). In leaf, ranged from 0.01  $\mu\text{mol g}^{-1}$  DW at  $Cd_0$  to 0.71  $\mu\text{mol g}^{-1}$  DW at  $Cd_{10}$ ; in stem varied from 0.03  $\mu\text{mol g}^{-1}$  DW at  $Cd_0$  to 0.71  $\mu\text{mol g}^{-1}$  DW at  $Cd_{10}$ ; and in root ranged from 0.03  $\mu\text{mol g}^{-1}$  DW at  $Cd_0$  to

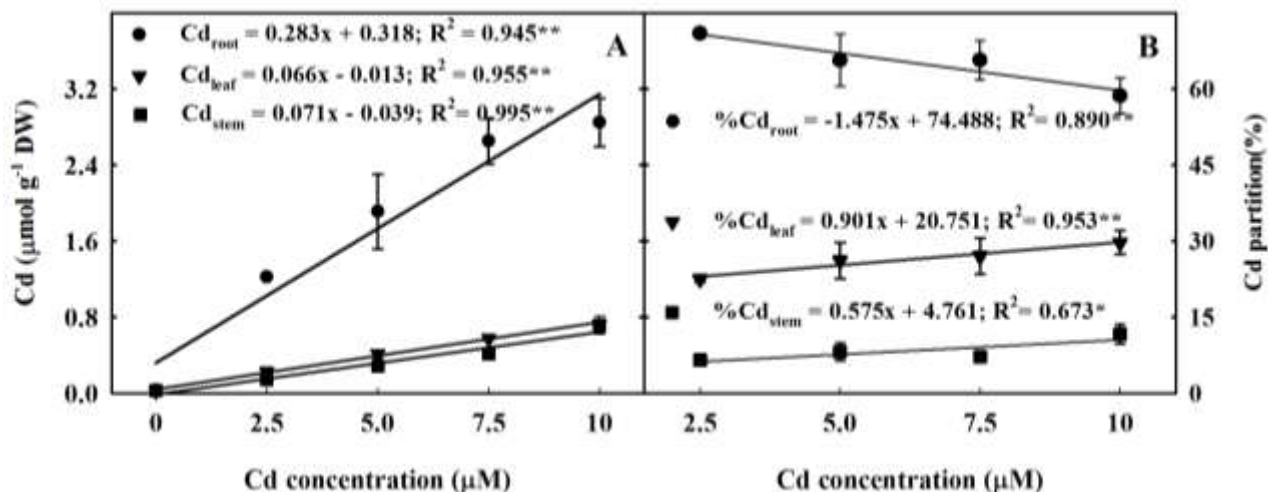


**Figure 2.** Leaf (LDW), stem (SDW), root (RDW) and total dry weights (TDW) leaf area (LA) and leaf succulence ( $L_{suc}$ ) of sunflower plants growing in nutrient solution containing different cadmium concentrations. Values indicate the mean of four replicates  $\pm$  S.D.

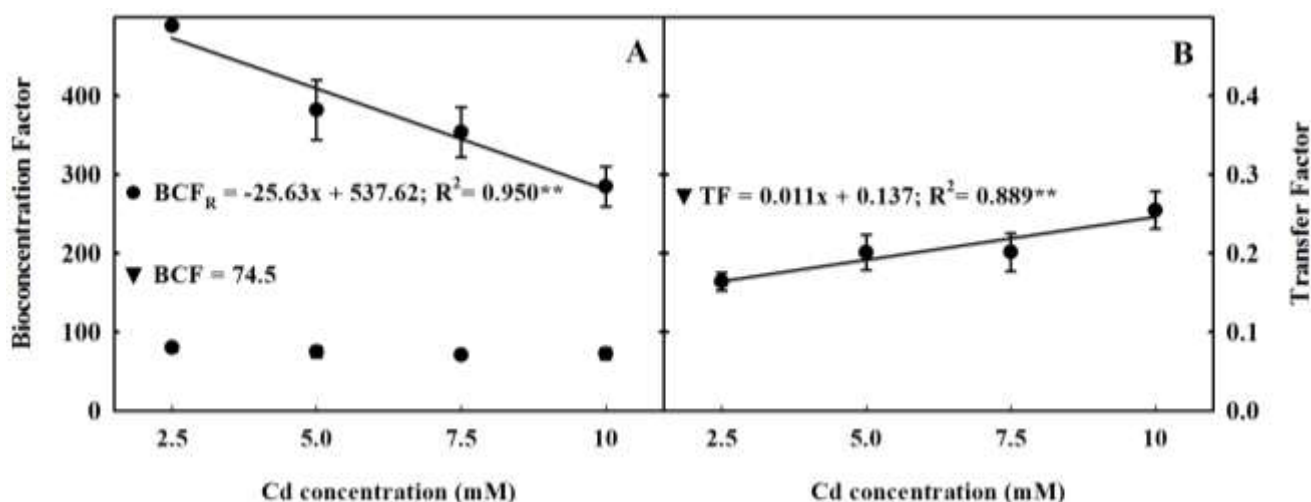
$2.85 \mu\text{mol g}^{-1}$  DW at Cd<sub>10</sub> (Figure 3A). To all plant parts it was observed a linear increase of Cd concentration with Cd solution (Figure 3A). Sunflower concentrated more Cd in the roots than in other plant parts in the Cd treatments. The highest difference in Cd root as compared to the other part of the plant occurred at Cd<sub>2.5</sub>. For instance, at Cd<sub>2.5</sub> 71.0, 22.5 and 6.5% of the Cd were allocated in the roots, leaves and stem, respectively, as compared with 58.7, 29.7 and 11.6%, respectively in Cd<sub>10</sub> (Figure 3B). The result of Cd partitioning in sunflower organs in this study was similar to others reported elsewhere (Lopes Júnior et al., 2014; Zou et al., 2008).

The higher concentration of Cd in the root was due to

the immobilization of the metal in the roots caused by its limited translocation from the roots to the shoot. The higher Cd retention in sunflower root as compared to the other plant parts is attributed to a complex system, which involve the element adsorption, chelation, and compartmentalization, which in turns limits Cd translocation from roots to the shoot (Nocito et al., 2011). Cadmium passes from the external solution to the interior of the plant through the apoplastic transport where it accumulates (Lux et al., 2011). The apoplast is a dense region of negative charge due to the presence of carboxylic groups, which are critical to the retention of cations such as Cd<sup>2+</sup> (Redjala et al., 2009). The roots act



**Figure 3.** Cadmium concentration (A) and partition (B) in the leaf, stem and root of sunflower plants grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates  $\pm$  S.D.



**Figure 4.** Bioconcentration Factor (BCF), Bioconcentration Factor of the root (BCFR) (A) and Transfer Factor (TF) (B) of sunflower plants grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates  $\pm$  S.D.

as a barrier for immobilization of toxic ions, preventing their translocation to the shoot (Azad et al., 2011). The stem retention is also considered as a tolerance mechanism, by preventing that toxic elements disrupt cell metabolism in the leaves (Higuchi et al., 2013). The results of Cd partition in the plants parts show that the stem retention was not an effective mechanism for regulating the Cd transport from roots to the leaves. The ability of the H-250 sunflower genotype to concentrate Cd in the shoot tissues relative to Cd-solution (BCF) was constant around 74.5, over the range of the Cd concentration tested (Figure 4A). The most of Cd in the plant was located in the root, therefore  $BCF_R$

values ranged from 538 at  $Cd_{2.5}$  to 281 at  $Cd_{10}$  (Figure 4A). The TF of the genotype were lower than one and ranged from 0.137 at  $Cd_{2.5}$  to 0.247 in the plants growing at  $Cd_{10}$  (Figure 4B).

The ratio of metal concentration in the roots to growing media, bioaccumulation factor (BCF) has been used to estimate the ability of the plant concentrate the metal from the solution. On the other hand, the ratio of metal concentration in the shoots to the roots (TF) has been used to estimate metal translocation from the roots to the shoots. BCF and TF greater than one indicate that the metal is concentrated in the shoot relative to the growing media or that the metal is accumulating preferentially in

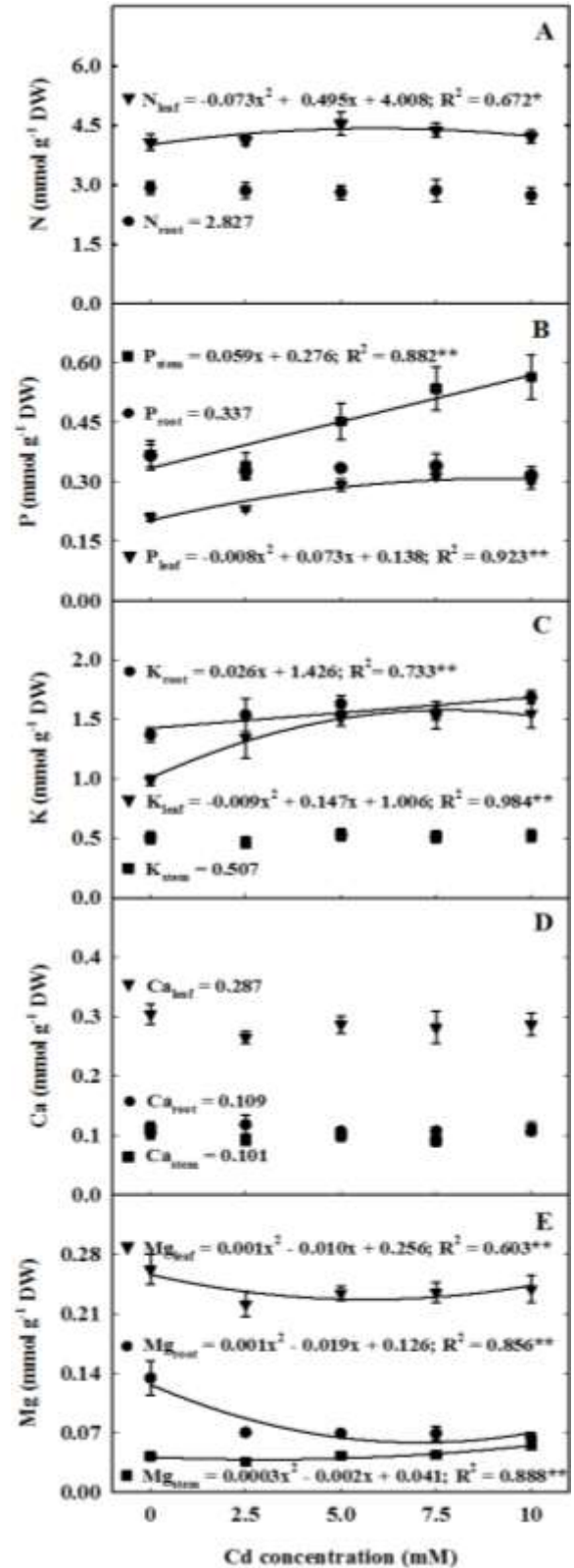
shoot relative to the root. The BCF and  $BCF_R$  values found in this study were high, in contrast to the TF values which were low, suggesting that the sunflower plants can be used for the phytostabilization purpose. The limitation of Cd transfer from the root to the shoot may be seen as a defense mechanism used by plants to minimize the deleterious effects of excessive nonessential metals in the shoot and according to Hossain et al. (2010) it is considered a tolerance mechanism. While non-tolerant species and tolerant excluders tend to accumulate nonessential metals, as Cd in roots, accumulator plants evolved a more efficient mechanism to translocate metals from root to shoot.

### Sunflower nutrient status

Cadmium toxicity alters plant macro and micronutrients uptake (Ciećko et al., 2004; Sarwar et al., 2010). The macronutrients N, P, K, Ca and Mg concentrations in sunflower leaf and root are shown in Figure 5. Increasing Cd concentration had a positive quadratic effect on leaf N (Figure 5A), P (Figure 5B) and K (Figure 5C). The estimated maximum sunflower leaf N ( $4.381 \text{ mmol g}^{-1} \text{ DM}$ ), P ( $0.325 \text{ mmol g}^{-1} \text{ DM}$ ) and K ( $1.577 \text{ mmol g}^{-1} \text{ DM}$ ) concentrations occurred at  $Cd_{5.9}$ ,  $Cd_{9.3}$  and  $Cd_{7.8}$ , respectively. Thus, the estimated N, P and K concentrations were 10, 53 and 57% higher than the control, respectively. The concentration of N in the stem was not evaluated. Cadmium in solution also had positive linear effect on P stem and K root. The concentration of Cd-solution has no effect in the N and P concentrations in the root, and in K concentration in the stem.

The increase of N in the leaves is a clear indicative that the symptom of chlorosis presented by the plant was not determined by the deficiency of this nutrient. There are reports in the literature that N uptake decreases with the Cd concentration in nutrient solution (Guimarães et al., 2008; López-Millán et al., 2009). However the synergisms and antagonisms existent between the uptake of N and Cd by the plants seem to be related to nitrogen source (Ciećko et al., 2004). Studies have shown the existence of antagonistic interactions between  $N-NH_4^+$  and  $Cd^{2+}$  uptake and synergistic interactions between  $N-NO_3^-$  and Cd (Sarwar et al., 2010). As in this study the  $N-NO_3^-:N-NH_4^+$  ratio in nutrient solution is 14:1, and  $N-NO_3^-$  is responsible for at least 50% of total anions absorbed by plants (Marschner, 2012), the higher  $N-NO_3^-$  plant uptake may have been used for balancing the electrical charge of the plant, thereby interfering with the regulation and absorption of other nutrients.

Although the Cd concentration did not impact the P concentration in the root, the P concentration in the sunflower leaves increased up to 53% as compared to the control as cadmium concentration in the nutrient solution increased. Similar result was found in a study with cedar plants (Paiva et al., 2001). Plants may maintain a higher P concentration in the leaves as a way



**Figure 5.** Concentration of nitrogen (A), phosphorus (B), potassium (C), calcium (D) and magnesium (E) in the root, stem and leaf of sunflower grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates  $\pm$  S.D.

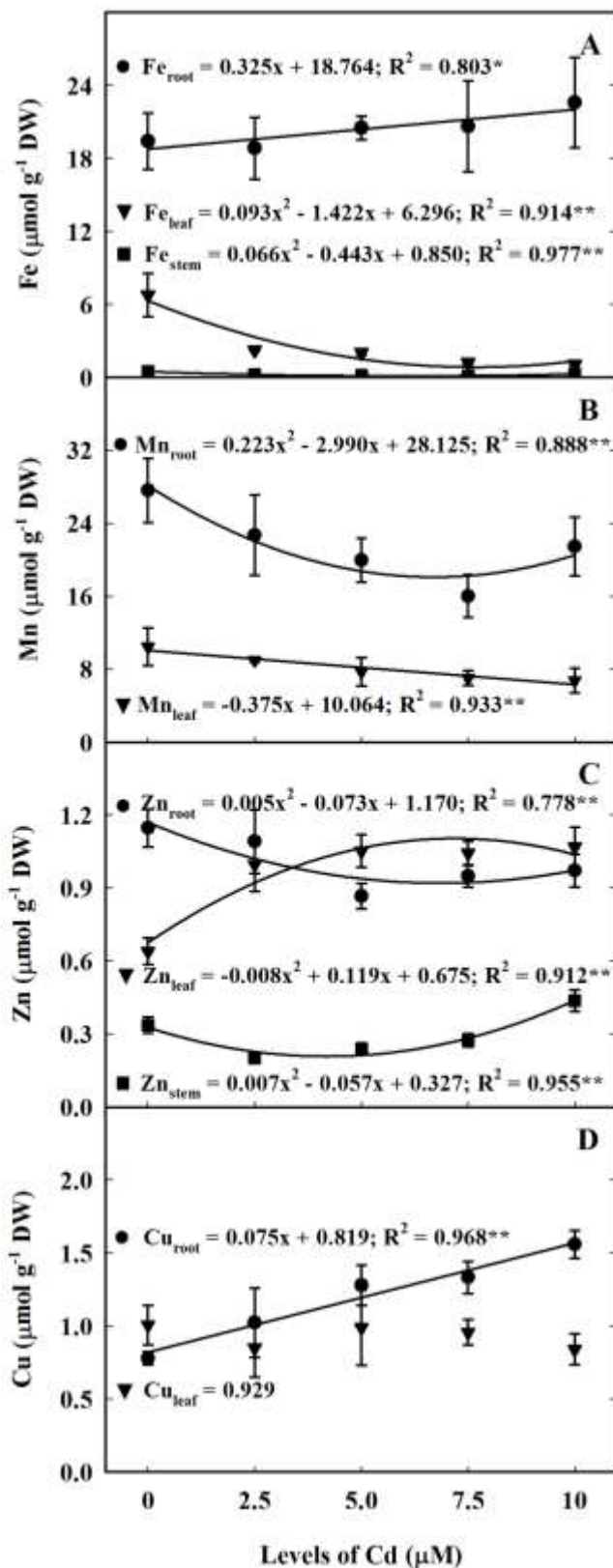
of reduce the free Cd and promoting the Cd-phosphate precipitate formation (Sarwar et al., 2010), which is sequestered in the vacuole and immobilized in the cell wall (Belleghem et al., 2006).

Potassium plays a central role in the water relations (Meurer, 2006), therefore the increase in leaf and root K concentrations is consistent with the need of the plant to keep the turgor, mainly in higher Cd concentrations. The increasing K concentration in sunflower roots may have helped the maintenance of water absorption by the roots and its flux to the shoot. Additionally, the increasing K concentration in the leaves may have favored the stomata opening at higher Cd concentrations, thereby maintaining the plant gas exchange during stress. The increase in K concentration in sunflower leaves obtained in this study differed from those obtained for sunflower, (Simon, 1998), cedar (Paiva et al., 2001) and maize roots, (Ciećko et al., 2004). However, the Cd concentration in the solution, the time of the exposure to Cd, and the plant age of these studies were higher than that tested in our study.

The concentration of Cd-solution has no effect in the Ca concentration in the root, stem and leaf (Figure 5D). In contrast, increasing Cd concentration has negative quadratic effect in plant Mg (Figure 5E). The estimated minimum concentration in leaf ( $0.231 \text{ mmol g}^{-1} \text{ DM}$ ), stem ( $0.038 \text{ mmol g}^{-1} \text{ DM}$ ) and root ( $0.036 \text{ mmol g}^{-1} \text{ DM}$ ) occurred, respectively, at  $\text{Cd}_{5.0}$ ,  $\text{Cd}_{3.3}$  and  $\text{Cd}_{9.5}$ . Iron, Mn, Zn, and Cu concentrations in sunflower leaves and roots are shown in Figure 6. Cadmium concentrations had positive effect on sunflower root Fe and Cu and leaf Zn but negative effect on leaf and stem Fe, leaf and root Mn, and stem and root Zn (Figure 6). The estimated minimum leaf Fe concentration ( $0.86 \mu\text{M g}^{-1} \text{ DW}$ ) was at  $\text{Cd}_{7.65} \mu\text{M}$  Cd, representing a reduction of 87% when compared with Fe concentration of the control ( $6.75 \mu\text{M g}^{-1} \text{ DW}$ ) (Figure 6A). Leaf Mn concentration decreased linearly ( $0.375 \mu\text{M g}^{-1} \text{ DW}$ ) with Cd solution (Figure 6B). Leaf Fe and Mn concentrations were present in concentration reverse to that of Cd. Sunflower leaf Zn concentration increased with Cd-solution (Figure 6C). The estimated maximum leaf Zn concentration  $1.12 \mu\text{M g}^{-1} \text{ DW}$  occurred at  $\text{Cd}_{7.4}$ . Leaf Cu concentration had no change ( $0.929 \mu\text{M g}^{-1} \text{ DW}$ ) with Cd-solution (Figure 6D). The concentrations of Mn and Cu in the stem were below the level of detection.

Sunflower root concentration of Fe and Cu increased in 0.325 and 0.075  $\mu\text{mol g}^{-1} \text{ DW}$ , respectively for each unit of Cd-solution (Figures 6A and 6D). At  $\text{Cd}_{10}$  the root Cu concentration,  $1.57 \mu\text{mol g}^{-1} \text{ DW}$ , was twice the value of the control plants  $0.819 \mu\text{mol g}^{-1} \text{ DW}$ . In contrast, the root concentration of Mn and Zn decreased quadratically with Cd-solution (Figures 6B and 6C). The minimum Mn  $18.10 \mu\text{M g}^{-1} \text{ DW}$  and Zn  $0.90 \mu\text{M g}^{-1} \text{ DW}$  concentrations were observed at  $\text{Cd}_{6.7}$  and  $\text{Cd}_{7.3}$ , respectively.

The cadmium toxicity symptoms (chlorosis and necrosis) as presented especially in younger leaves of H-250 sunflower genotype exposed to increasing Cd



**Figure 6.** Concentration of Iron (A), manganese (B), zinc (C) and copper (D) in root, stem and leaf of sunflower grown in nutrient solution containing different cadmium concentrations. Values are mean of four replicates  $\pm$  S.D.

concentrations have been reported elsewhere (Chaves et al., 2011; Zou et al., 2008). The increase of Cd in the nutrient solution may have an antagonist effect on the concentration of several elements since the Cd seems to enter in the plants, mainly via  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  channels or via other divalent transporters such as  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Zn}^{2+}$  (Clemens, 2006; Sarwar et al., 2010). In the present study, the chlorosis and necrosis symptoms were attributed to a combined adverse effect of Cd on leaf Mg, Fe and Mn concentrations.

The decrease of Fe concentration in the leaf in conjunction with the increase in the root also suggests the occurrence of Cd-induced disturbances in the Fe translocation from shoot to the root. Iron is frequently referred as the most affected nutrient by Cd exposure. According to Pál et al. (2006), leaf chlorosis and plant growth inhibition are the most noticeable symptoms caused by Cd stress. Considering that Fe plays a key role in the chlorophyll biosynthesis (Benavides et al., 2005), our results suggest that the decrease in the Fe concentration was a determining factor for plant chlorosis and biomass reduction. Our results also show a decrease of Mn concentrations in both leaves and roots. Manganese is a key element in restoring the chlorophyll molecule structure (Sarwar et al., 2010). In addition, Mn plays key role in the activity of enzymes that mediate the catalysis of the water-splitting reaction to produce oxygen and to provide electrons for the photosynthetic electron transport chain (Goussias et al., 2002; Nickelsen and Rengstl, 2013), and its deficiency directly affects the photosynthetic rate. Therefore, it is likely that the reduction of Mn concentrations observed in this study may be an additional factor inducing chlorosis symptoms and reducing plant growth.

Cadmium and Zn tend to compete for accumulation in plant shoots (Nocito et al., 2011). Zinc is an important nutrient in maintaining the biomembranes. Zinc also plays a crucial role as a functional and/or structural component of a number of enzymes, besides being essential for activity, regulation, and stabilization of protein structure (Mason, 2013). In this study, Cd induced a slight reduction in Zn concentration in the roots, but increased Zn concentration in the leaves, suggesting that the Cd-induced growth reduction did not seem to be related to changes in concentrations of this nutrient in the plant.

Cadmium stress induced an increase of 100% in root Cu concentration but did not affect the concentration of this element in the leaves. The increased root Cu concentration in Cd-stressed plants has also been reported in other studies (López-Millán et al., 2009; Obata and Umebayashi, 1997; Sandalio et al., 2001). The increase in root concentrations of Cu and Fe transition metals observed in this study may lead to increased hydroxyl free radical production through the Fenton reaction (Silva et al., 2013). Therefore, it could be hypothesized that Cd-induced oxidative stress was more pronounced in the roots than in the leaves. The

observation that RDW was more affected than LDW by Cd-stress is an additional support to this hypothesis.

## Conclusions

Our results show that H-250 is a Cd-excluder sunflower genotype, since it preferentially concentrates the metal in the roots. Thus, this genotype may be an alternative for use in phytostabilization programs. The results also showed that the increasing Cd concentration disrupted the plant nutrient homeostasis as it increased the concentration of some nutrients and had adverse effect on others, impacting plant growth. In this scenario, the results indicate that the low Mg, Fe and Mn concentrations in the leaves were the main cause for plant growth reduction and leaf chlorosis and necrosis, as each one of these elements plays a key role on the chlorophyll molecule and on photosynthetic process.

## Conflict of Interests

The authors have not declared any conflict of interests.

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