Journal of Applied Botany and Food Quality 96, 41 - 47 (2023), DOI:10.5073/JABFQ.2023.096.005

[1]Government College Women University Faisalabad Pakistan, Faculty of Sciences, Department of Botany, Faisalabad, Pakistan
[2]University of Agriculture Faisalabad, Pakistan, Faculty of Biological Sciences, Department of Botany, Faisalabad, Pakistan

Concentrational study of nutrients related to chelators assisted sunflower (*Helianthus annuus* L.) from artificially cadmium-contaminated soil

Rumana Sadiq[1], Nazimah Maqbool[2*], Areej Javaid[1], Mewish Naseer[1], Anam Zikrea[1], Tanzila Rafique[1]

(Submitted: December 3, 2022; Accepted: April 1, 2023)

Summary

The present study investigates the appliance of cadmium (Cd) noxiousness and damage to nutrient concentration in sunflower hybrids, FH-533 and Hysun-33, as well as the role of natural and synthetic chelator's oxalic acid (OA) and ethylene triamine tetraacetic acid (EDTA) respectively in reducing the Cd toxicity in sunflower plant. Sunflower, known as heavy metal phytoextractor, was grown in soil artificially contaminated with soluble salt $CdCl_2$ at 250 ppm and 450 ppm. Pot experiment was conducted in a complete randomized design with three replicates for cadmium metal, chelating agents and sunflower hybrids at seedling, vegetative and reproductive stages. Application of Cd of 250 ppm and 450 ppm imposed the negative effect on shoot and root dry biomass by directly or indirectly disturbing the nutrient uptake. Maximum growth and nutrient uptake were observed when chelators (EDTA and OA) were applied alone respectively. In Cd contaminated soil the application of EDTA proved helpful in ameliorating the toxic effect of Cd_{250} on nutrient content of both sunflower hybrids as compared to Cd_{450}, the Hysun-33 showed better results in terms of K, Ca, Fe, Zn, P, N as compared to FH-533, so it can be cultivated on Cd adulterated soil for better yield. The presented results offer a novel solution for optimization of sunflower cultivation on soil affected by Cd, as well as for increased health-promoting nutrients of sunflower.

Keywords: Sunflower, Cd, Chelators, OA, EDTA

Introduction

Augmented emission of heavy metals inflowing into the biosphere is due to mining, smelting and synthetic fertilizers (Haider et al., 2021). The release and accumulation of metals in readily available forms as a result of human activities may cause severe harm or modify the properties of natural and artificial ecosystems (Shahid et al., 2015). Globally the metal stress has become a foremost problem in several terrestrial ecosystem and imparted damaging effects on soil texture and ultimately reduces plant productivity by disturbing various plant activities either physiological or molecular (Panuccio et al., 2009; Hassan et al., 2017).

Some metals like Cu, Co, Mn, Zn, Mo and Ni in nano quantity are essential for vital biological processes (Salla et al., 2011; Shahid et al., 2015). However, the macro concentrations of these metals as well as Lead (PB), Mercury (Hg), Arsenic (As) and Cadmium (Cd) can greatly influence the plants productivity (Xiong et al., 2014; Pierart et al., 2015).

Among these metals, Cd is considered to be highly toxic metal in nature due to its solubility in soil solution, absorbed by the plants and accumulation in grains, carcinogenic to plants as well as human (Buchet et al., 1990) reported by The International Agency for Research on Cancer (IARC, 1993; Gianazza et al., 2007; Gill and Tuteja, 2011). Cd has various toxic effect on plants such as chlorosis (Xu et al., 2017), alternations in chloroplast ultrastructure, reduced photosynthetic activity (Gallego et al., 2012), altered sulfur (S) and nitrogen (N) metabolism (Nazar et al., 2012), lipid peroxidation as well as disturbed antioxidant activity (Abbas et al., 2017). Even in low concentration it can effect plant growth and productivity by altering the activities of crucial enzymes such as of Calvin cycle, CO_2 fixation and phosphorus and carbohydrate metabolism (Gallego et al., 2012; Gill and Tuteja, 2011). The nutrient concentration diminished with increasing Cd uptake as most transmembrane carriers involved in nutrient uptake are engaged by Cd (El-Beltagi et al., 2010; Abbas et al., 2017).

Heavy metals remediation is very essential for environment fortification and conservation (Glick, 2010). For this, various physiochemical and biological practices have been implemented, although the physiochemical practices are swift but they are expensive and are technically very complexed and also alter the physical and chemical properties of soil leading towards the secondary soil pollution (Ali et al., 2013, Ullah et al., 2015). Conversely, the biological remediation is the effective way for the removal of toxic metals because it is environmental friendly, cost effective and publically acceptable method (Sayqal and Ahmed, 2021).

Phytoremediation is one of such biological methods in which hyperaccumulator plants which are fast growing and have high biomass productivity are grown on metal affected soil so they absorb and accumulate high concentration of metal in below or above ground parts without hindering their own photosynthetic activities (Khalifa et al., 2000; Novistasari, 2017; Haider et al., 2021). In addition to hyperaccumulators, the remediation process can also be made effective by the involvement of chelating agents (Liu et al., 2018).

The present study was carried out to understand the inter-relation of Cd uptake and essential macro, micro elements and to improve the efficiency of sunflower hybrids with the assistance of chelating agents.

Materials and methods

Plant material and growth conditions

The air-dried garden loamy sand soil was used for a pot experiment to examine chelate assisted nutrients concentration of sunflower hybrids. Soil samples (10 kg) were placed in plastic pots (30 × 20 cm size) and ten surface sterilized seeds of Hysun-33 and FH-533 were sown in each pot, which were then thinned to five after germination. Soil was irrigated with equal amounts of tap water (based on 70% of the water holding capacity of the soil) twice per week. The climatic conditions were 30 ± 2 °C with 50% relative humidity at the time of experiment. After complete germination of both the sunflower hybrids, the soil in the pots were artificially contaminated with 250 and 450 mg Cd per kg of soil separately and treated in combination with chelating agents (EDTA and OA at 1 g per kg of soil) in

* Corresponding author

rooting medium. Control pots with Cd and chelating agents were included and all treatments were conducted with three replicates with two factors factorial arrangement (CRD Model).

Biomass analyses

Shoots and roots were sampled at seedling, vegetative and reproductive stages for all treatments washed with tap water and rinsed with deionized water. Then plant tissues were dried in fan forced oven at 70 °C for 48 h for dry matter measurements and further analysis (Almaroai et al., 2013).

Element analyses

The total contents of K, Ca, Fe, Zn, P, N and Cd in shoots and roots of Hysun-33 and FH-533 were determined after digestion with concentrated HNO_3 at hot plate (250 - 300 °C) in a fume hood chamber. The filtrate (50 mL) was analyzed for Iron (Fe), Zinc (Zn) and Cadmium (Cd) using Atomic Absorption Spectrophotometer (Analyst-330, Perkin Elmer and Germany). The Potassium (K) and Calcium (Ca) were estimated using Flame Photometer (Jenway PPP7, UK). Total Nitrogen (N) was determined following the protocol of Nelsona and Sommers (1973). Total Phosphorus (P) was analyzed in shoots and roots using the protocol of Raun et al. (1987).

Statistical analyses

Analysis of data was conducted through the computer software Statistix (Version 8.1, USA) and subjected to two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to measure statistical differences ($p \leq 0.05$).

Results and discussion

Dry biomass of root and shoot

The below ground biomass is as important as above ground biomass because stronger and healthier roots determine the vigor and growth of above ground plant. Secondly plants store nutrients in shoots and roots also or nutrients are required by all the cells of the plant body either it is above ground or below ground. The root dry biomass helps to determine the nutrients levels in plants as compared to fresh biomass as it is devoid of water contents, which are the fresh weight of the plant.

Statistical analysis of root ad shoot dry biomass showed significant differences ($P < 0.05$) among hybrids, treatments and non-significant for interactions (Tab. 1).

Application of EDTA and OA alone increase the root dry biomass from seedling to reproductive stage as compared to all other treatments except control. Among hybrids, Hysun-33 had more increase in root dry biomass as compared to FH-533. The application of EDTA and Cd combination increased the root dry biomass of Hysun-33 as compared to Cd treatments alone at all growth stages (Fig. 1).

The highest shoot dry biomass was recorded for Hysun-33 control at seedling, vegetative and reproductive stages as compared to all other treatments as well as FH-533. The comparison of Cd and chelators combination applications showed EDTA and OA alone had greater shoot dry biomass as compared to Cd_{250} and Cd_{450} at all growth stages for both hybrids. The chelator EDTA improved the shoot dry biomass of Hysun-33 in the presence of Cd_{250} and Cd_{450} while chelator OA improved the shoot dry biomass of FH-533 in the presence of Cd_{250} and Cd_{450} respectively (Fig. 1).

The maximum shoot fresh and dry weight and root fresh and dry weight and Cd metal accumulation was reported in HySun-33 sunflower when treated with 5 mM EDTA (Bakht et al., 2020). The collaboration of EDTA with Cd acceleration the Cd accumulation in sunflower root (Sadiq and Maqbool, 2016). Sinegani et al. (2010) reported that application of EDTA at sowing reduced the seedling emergence and their dry weight while its application at harvested shoots increased Cd accumulation in *Helianthus annuus*.

Tab. 1: Two way (ANOVA) variance analysis of different combinations of chelators and Cd concentrations with sunflower hybrids.

Root	Hybrids (H)	Treatments (T)	H × T	Error
Dry weight (DW)	88.68***	13.72***	1.04 ns	3.52
Cd contents	8407.87***	45839. 44***	678.16*	309.15
N contents	529.64***	273.11***	0.25 ns	14.76
P contents	101.19***	318.36***	4.37 ns	3.81
K contents	187.84***	171.07***	2.16 ns	2.42
Fe contents	64.92***	50.46***	0.72 ns	0.81
Zn contents	16.76***	19.18***	0.15 ns	0.33
Ca contents	201.30***	12.25***	0.74 ns	1.46
Shoot				
Dry weight (DW)	252.87 ***	39.52***	0.22 ns	3.22
Cd contents	8043.19***	24664.33***	931.36***	214.66
N contents	284.49***	185.99***	5.04 ns	7.70
P contents	86.32***	36.39***	3.91**	1.19
K contents	84.89***	95.22***	3.93**	1.18
Fe contents	3.03***	3.16***	0.07 ns	0.07
Zn contents	10.90***	7.78***	0.86***	0.16
Ca contents	59.45***	15.41***	1.54 ns	0.84

***(very highly significant), ** (highly significant) or * (significant) represent the significance $P < 0.05$; ns means non-significant
The values represent the MS (means square) for hybrids, treatments and their interactions

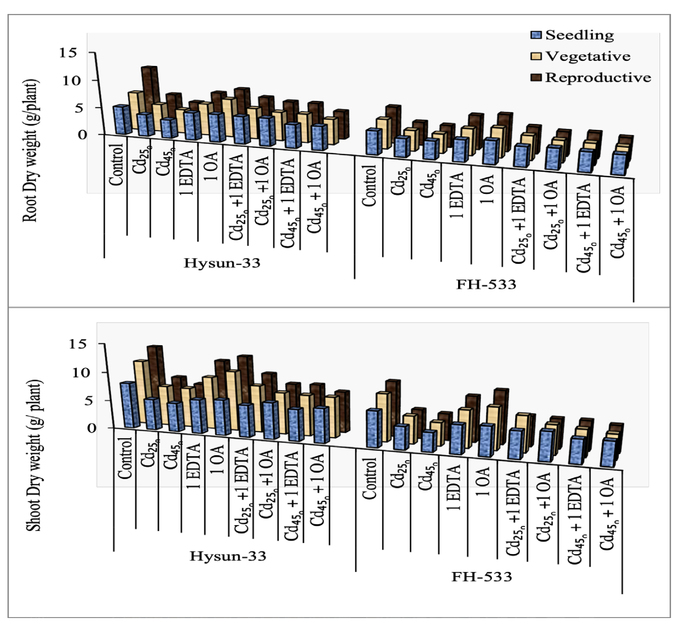

Fig. 1: Effect of different combinations of Cd_{250} and Cd_{450} and cheltors (EDTA and OA @ 1 g /kg) on root and shoot dry biomass of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

Cadmium (Cd) contents in root and shoot

Statistical analysis of hybrids, treatments and interactions all were highly significant for root and shoot Cd contents (Tab. 1).

Among hybrids, Hysun-33 showed greater root Cd contents that increase from seedling to vegetative and reproductive stages as compared to FH-533. The combination of Cd and EDTA treatment had more root Cd contents as compared to Cd and OA in both the hybrids (Fig. 2). The present research also showed that as Hysun-33 at reproductive stage had greater shoot Cd contents as compared to FH-533 in the presence of Cd_{250} and Cd_{450} along with EDTA (Fig. 2).

EDTA increased the exchangeable or soluble fraction of metal available to the plant and thus enhanced Cd uptake (Sinhal et al., 2010). Six and Smolders (2014) researched that Cd uptake in plants increased proportionally with increasing Cd in soil. The application of EDTA enhanced the phyto-accumulation of Cd and Cr in sunflower (Bakht et al., 2020). The synergistic application of EDTA and Cd helps the plants to absorb Cd and transport it to the vegetative and reproductive parts. On the one hand chelators activate Cd in the soil, enhance its bioavailability and contribute to its chelation and complexation in root and shoot of the Chrysanthemum (Bai, 2017). Similarly, Clemens (2006) highlighted that Cd could be taken up into plant cells by Fe and Zn transporters and then stored in vacuoles by phytochelatins.

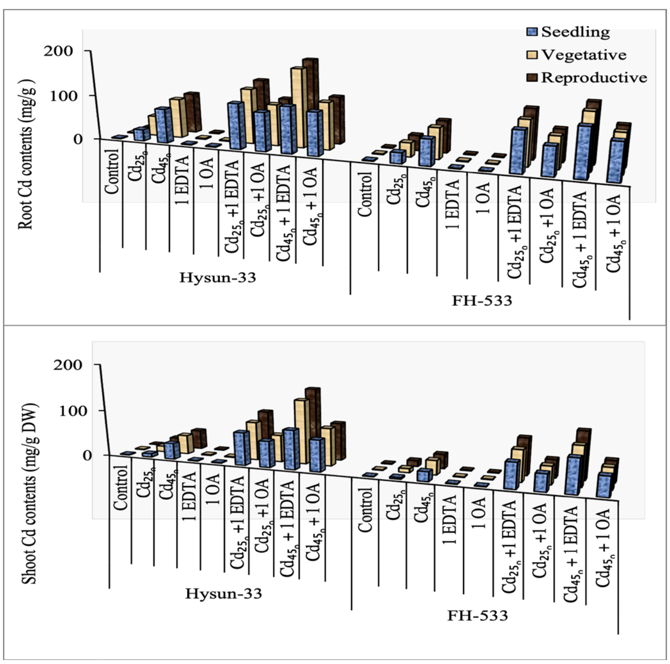

Fig. 2: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot Cd contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

Nitrogen (N) and phosphorus (P) contents in root and shoot

N contents of root and shoot had significant ($P < 0.05$) statistical differences among hybrids, treatments and non-significant for all interactions (Tab. 1). The application of Cd_{250} decreased the root N contents of both Hysun-33 and FH-533 and this decrease was further noticed for Cd_{450} treatment as compared to control. The application of EDTA and OA alone enhanced the root N contents in both hybrids at seedling, vegetative and reproductive stages respectively. The combined application of Cd and chelators showed slight increase in N contents at growth stages. However, EDTA supports the uptake of root N contents more than OA in the presence of Cd applications (Fig. 3).

The application of Cd_{250} reduced the shoot N contents of both Hysn-33 and FH-533 and this reduction was further noticed for Cd_{450} treatment as compared to control. The application of EDTA and OA alone increased the shoot N contents in both hybrids at seedling, vegetative and reproductive stages respectively. The combined application of Cd and chelators showed slight enhancement in N contents at growth stages. However, EDTA supports the uptake of shoot N contents more than OA in the presence of Cd applications (Fig. 3).

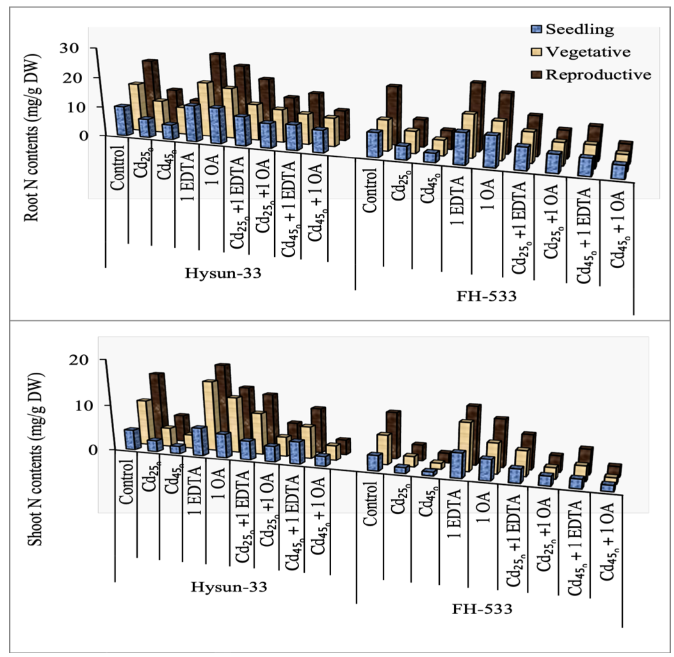

Fig. 3: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot N contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

Significant ($P < 0.05$) differences among sunflower hybrids and treatments as well as their interactions were measured for shoot P contents while non-significant for root interaction (Tab. 1).

The higher P contents were noticed in root of Hysun-33 as compared to FH-533 in the presence of EDTA and OA alone at seedling, vegetative and reproductive stages respectively. The application of Cd_{250} and Cd_{450} decreased the root P contents in both the hybrids. However, application of EDTA along with Cd_{250} showed greater root P contents as compared to OA in the presence of both Cd treatments (Fig. 4).

The application of EDTA markedly improved shoot P contents of Hysun-33 as compared to FH-533 and control at vegetative and reproductive stages. It was observed that both chelators failed to improve the shoot P contents of FH-533 when applied with Cd_{250} and Cd_{450} respectively (Fig. 4). Liphadzi et al. (2003) report that EDTA application had a little effect on uptake of Cu, Fe, Mn, Zn in sunflower grown at long term sewage-sludge farm.

Potassium (K) and calcium (Ca) contents in root and shoot

Statistical studies showed significant ($P < 0.05$) differences among hybrids, treatments while their interactions were non-significant for root K contents as shown in Tab. 1. The clear difference in chelators

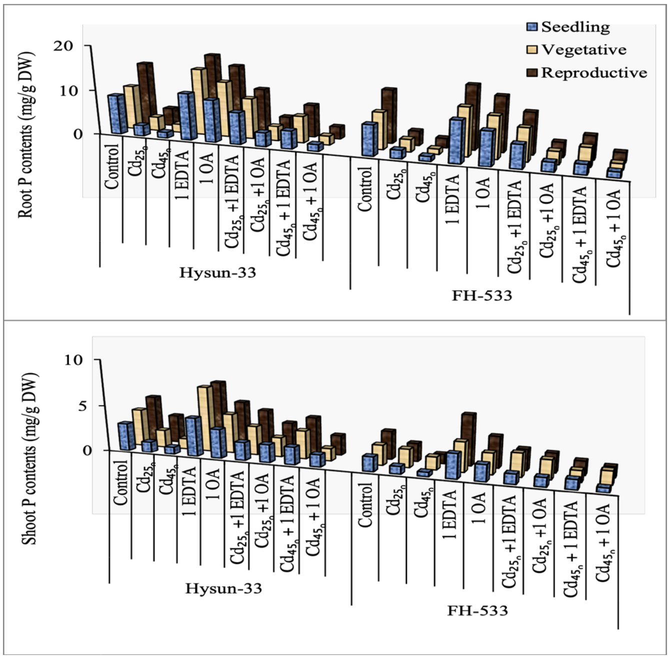

Fig. 4: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot P contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

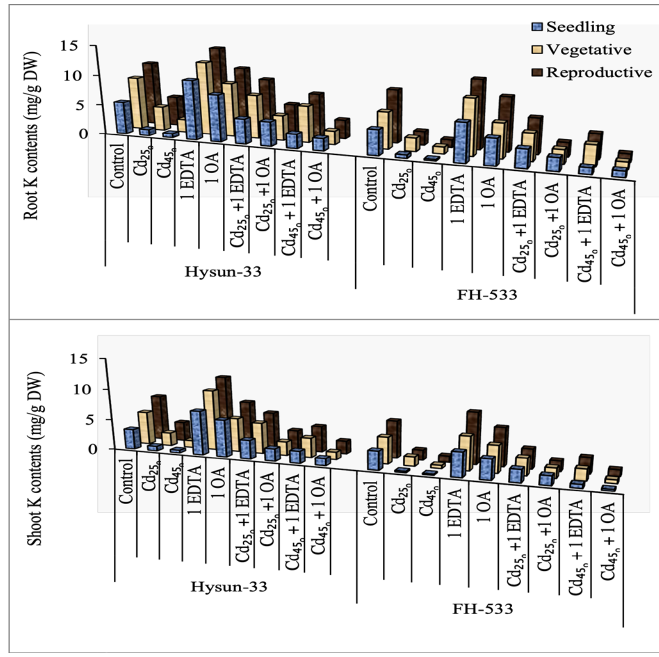

Fig. 5: Effect of different combinations of Cd levels Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot K contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

behavior was noticed for root K contents of Hysun-33 as compared to FH-533 in combination with Cd treatments as well as alone. The increased in the root K contents was markedly measured from seedling to vegetative and reproductive stages of Hysun-33 and FH-533 in the presence of EDTA and OA respectively. The Cd_{250} and Cd_{450} treatments decreased the root K contents of both the hybrids but it was found that application of EDTA improved K contents in roots of Hysun-33 (Fig. 5).
Shoot K contents showed significant ($P < 0.05$) statistical differences among hybrids, treatments and their interactions (Tab. 1). The application of both Cd treatments reduced K contents in shoot of FH-533 as compared to Hysun-33. The increase in shoot K contents was marked in both hybrids at seedling, vegetative and reproductive stages with the application of EDTA as compared to OA treatment. The combination of Cd_{250} along with EDTA showed higher shoot K contents in comparison to combination of Cd_{450} and EDTA for both hybrids. The further decrease in shoot K contents was noticed in both hybrids for combined treatment of OA and Cd respectively (Fig. 5).
The translocation of K, Ca, Mg from root to shoot was negatively affects by Cd during the growing cycle. The greater imbalance in nutrient contents was measured in root as compared to shoot (Rivelli et al., 2014).
Statistical significance ($P < 0.05$) was found for root Ca contents of hybrids and treatments while non-significance was recorded for their interactions as shown in Tab. 1. Maximum Ca contents were analyzed in Hysun-33 roots when treated with EDTA alone as compared to FH-533 roots as well as all other treatments. An increase in root Ca contents was recorded from seedling to vegetative and reproductive stages of both the hybrids. Both Cd treatments reduced the root Ca contents of both hybrids. While the application of EDTA and OA in combination with Cd treatments increased the Ca contents in roots as compared to Cd treatments alone (Fig. 6).
Statistical analysis of shoot Ca contents showed significant ($P < 0.05$) differences for hybrids and treatments while non-significant for their interactions (Tab. 1). Hysun-33 shoot showed greater Ca contents

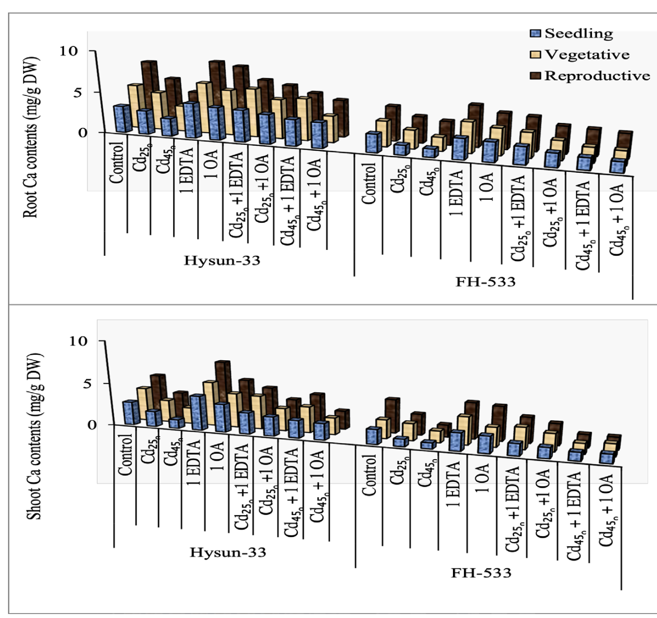

Fig. 6: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot Ca contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

with EDTA alone as compared to all other treatments and FH-533 shoot. Cd_{250} and Cd_{450} decreased the Ca contents of Hysun-33 and FH-533 shoot. The combination of EDTA and Cd_{250} had greater shoot Ca contents in Hysun-33 as compared to combination of Cd_{450} and EDTA, Cd_{250} with OA and Cd_{450} with OA respectively (Fig. 6).
The effect of Cd metal on the mineral nutrients in the roots and

leaves of rice varied with growing stages (LIU et al., 2003). KRAMER et al. (2007) devised the operation of transition metal transporters for the metal uptake and their partitioning to plant organs or cell types, their storage and remobilization. Cadmium replaces Ca with similar chemical behavior due to identical charge and ionic radius (KUBIER et al., 2019). CORGUINHA et al. (2015) reported the entry of Cd ions in guard cells through Ca channels.

Iron (Fe) and zinc (Zn) contents in root and shoot

Statistical significance ($P < 0.05$) was found for root Fe contents of hybrids and treatments while non-significance was recorded for their interactions as shown in Tab. 1. Maximum Fe contents were analyzed in Hysun-33 roots when treated with EDTA alone as compared to FH-533 roots as well as all other treatments. An increase in root Fe contents was recorded from seedling to vegetative and reproductive stages of both the hybrids. Both Cd treatments reduced the root Fe contents of both hybrids. While the application of EDTA and OA in combination with Cd treatments increased the Fe contents in roots as compared to Cd treatments alone (Fig. 7).

Statistical analysis of shoot Fe contents showed significant ($P < 0.05$) differences for hybrids and treatments while non-significant for their interactions (Tab. 1). Hysun-33 shoot showed greater Fe contents with EDTA alone as compared to all other treatments and FH-533 shoot. Cd_{250} and Cd_{450} decreased the Fe contents of Hysun-33 and FH-533 shoot. The combination of EDTA and Cd_{250} had greater shoot Fe contents in Hysun-33 as compared to combination of Cd_{450} and EDTA, Cd_{250} with OA and Cd_{450} with OA respectively (Fig. 7).

Root Zn contents were statistically significant ($P< 0.05$) among hybrids, treatments and non-significant for their interactions as shown in Tab. 1. The clear difference in chelators behavior was noticed for root Zn contents of Hysun-33 as compared to FH-533 in combination with Cd treatments as well as alone. The increased in the root Zn contents was markedly measured from seedling to vegetative and reproductive stages of Hysun-33 and FH-533 in the presence of EDTA and OA respectively. The Cd_{250} and Cd_{450} treatments decreased the root Zn contents of both the hybrids but it was found that application of EDTA improved Zn contents in roots of Hysun-33 (Fig. 8).

Shoot Zn contents showed significant ($P < 0.05$) statistical differences among hybrids, treatments and their interactions (Tab. 1). The application of both Cd treatments reduced Zn contents in shoot of FH-533 as compared to Hysun-33. The increase in shoot Zn contents was marked in both hybrids at seedling, vegetative and reproductive stages with the application of EDTA as compared to OA treatment. The combination of Cd_{250} along with EDTA showed more shoot Zn contents in Hysun-33 as compared to combination of Cd_{450} and EDTA while FH-533 showed same amount of Zn contents in shoot for all combinations of Cd and EDTA. The further decrease in shoot Zn contents was noticed in both hybrids for combined treatment of OA and Cd respectively (Fig. 8).

REVELLI et al. (2014) found significant interactions between Cd contamination and nutrient contents in shoot and root were found only for Cu, Zn and Fe. The Fe contents were higher in roots of sunflower under Cd application. Such results indicated the translocation of Cu, Zn and Fe from the root to the shoot of Cd affected plants. The translocation of nutrients is reduced when Cd is translocated to shoot. This interaction of translocation and absorption capacities of nutrients and Cd in plants was reported by HERRERO et al. (2010). A non-protein amino acid nicotinamide is lined with Fe, Cu and Zn homeostasis is present in almost all plants (WANG et al., 2013), seems to be involved also in Cd transport and chelation (SHARMA and DIETZ, 2006; KRAMER et al., 2007). The root plasma membrane of maize is not particularly specialized for Fe phytometallophores, which enables Cd transport in roots while Zn phytometallophores get easily absorbed by the maize roots (PANDEY et al., 2007). Several proteins from the ZIP family (ISMAEL et al., 2018; ZHENG et al., 2018) transport Cd from extracellular spaces and the lumen of organelles into the cytoplasm (VERT et al., 2009). The effect of Cd on nutrient elements in sunflower plants depends on Cd contamination level, nutrient considered, part portion and phenological stage (REVELLI et al., 2014).

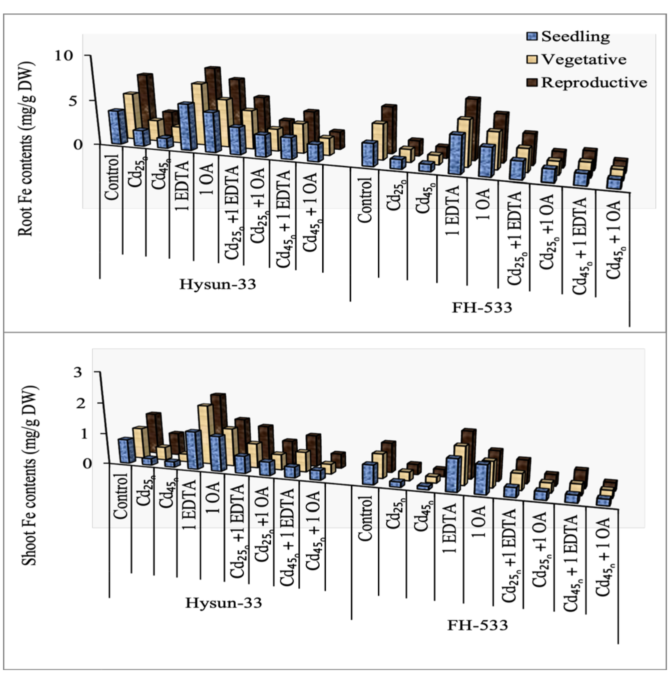

Fig. 7: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot Fe contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

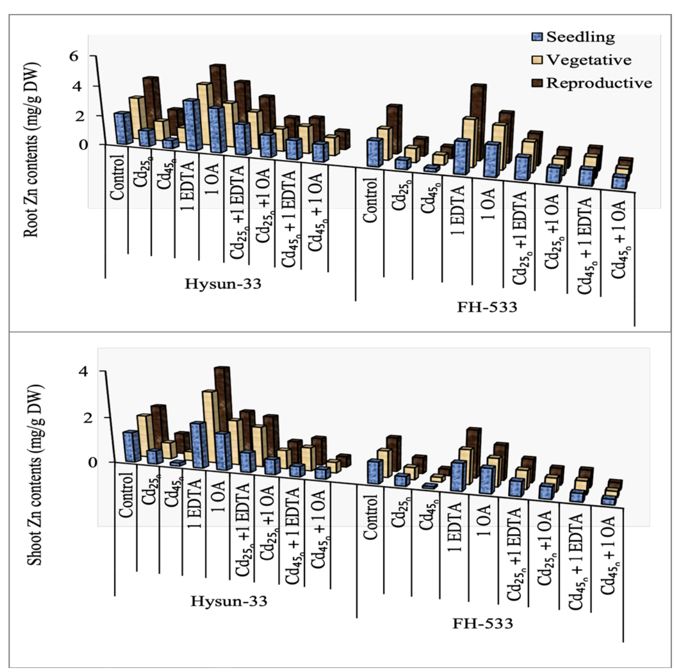

Fig. 8: Effect of different combinations of Cd_{250} and Cd_{450} and chelators (EDTA and OA @ 1 g /kg) on root and shoot Zn contents of Hysun-33 & FH-533 sunflower hybrids at seedling, vegetative and reproductive growth stages.
*The graphical bars represent the mean values of replicates

Tab. 2: Correlation coefficient (r) of root and shoot dry weight and Cd with nutrient contents of two sunflower hybrids.

Root		Cd	N	P	K	Fe	Zn	Ca
Hysun- 33	**DW**	-0.64 ns	0.81**	0.78*	0.74*	0.78*	0.74*	0.79*
	Cd		-0.69*	-0.63 ns	-0.61 ns	-0.65 ns	-0.66 ns	-0.59 ns
FH-533	**DW**	-0.75 ns	0.92***	0.91***	0.88**	0.91***	0.89**	0.87**
	Cd		-0.67*	-0.63 ns	-0.64 ns	-0.71*	-0.64 ns	-0.65 ns
Shoot								
Hysun- 33	**DW**	-0.61 ns	0.79*	0.73*	0.73*	0.74*	0.74*	0.74*
	Cd		-0.39 ns	-0.45 ns	-0.48 ns	-0.49 ns	-0.51 ns	-0.44 ns
FH-533	**DW**	-0.58 ns	0.83**	0.72*	0.88**	0.81**	0.87**	0.85**
	Cd		-0.44 ns	-0.55 ns	-0.53 ns	-0.51 ns	-0.53 ns	-0.61 ns

***(very highly significant), ** (highly significant) or * (significant) represent the significance of 'r' differ from 0 and the variables show some degree of correlation; ns means non-significant

Correlation

Root and shoot dry weight of Hysun-33 and FH-533 showed significant correlation with N, P, K, Ca, Fe and Zn, while non-significantly correlated with Cd contents. The correlation of Cd with shoot and root nutrients showed negative correlation which means that presence of Cd reduce P, K, Ca, Fe and Zn uptake especially N in both hybrids. Statistically significant negative correlation was found for N and Cd in Hysun-33 and FH-533 root (Tab. 2).

Conclusion

The present study demonstrated that Cd_{250} and Cd_{450} significantly decrease the dry biomass by lowering the uptake of essential N, P, K, Ca, Fe and Zn elements more in FH-533 than Hysun-33. Treatment with EDTA (natural chelator) is considered a profitable mean of boosting phytoextraction of Cd (heavy metal) in sunflower as compared to Oxalic acid (synthetic chelator). Although our results coincides with the literature, provide useful information about interaction of Cd and nutrients in sunflower and with what concentration of which chelator can boost the metal extraction at which growth stage, but still needs to be tested in open field.

EDTA increased the macro-micro nutrients of sunflower this supports it to complete life cycle under Cd stressed soil. Our results may contribute to enhance the yield and nutritional composition of sunflower (Hysun-33) cultivated in Cd affected areas as sunflower is also commercially important for its edible oil. It will helps in increasing sunflower cultivation in affected areas that will share the burden of economic demand of this crop and remediation of heavy metal contaminated areas as well.

Conflict of interest

No potential conflict of interest was reported by the authors.

References

ABBAS, T., RIZWAN, M., ALI, S., ADREES, M., ZIA-UR-REHMAN, M., QAYYUM, M.F., OK, Y.S., MURTAZA, G., 2017: Effect of biochar on alleviation of cadmium toxicity in wheat (*Triticum aestivum* L.) grown on Cd-contaminated saline soil. Environ. Sci. Pollut. Res. 25, 25668-25680. DOI: 10.1007/s11356-017-8987-4

ALI, H., KHAN, E., SAJAD, M.A., 2013: Phytoremediation of heavy metals-concepts and applications. Chemosphere 91(7), 869-881. DOI: 10.1016/j.chemosphere.2013.01.075

ALMAROAI, Y.A., USMAN, A.R.A., AHMAD, M., KIM, K.-R., VITHANAGE, M., OK, Y.S., 2013: Role of chelating agents on release kinetics of metals and their uptake by maize from chromated copper arsenate contaminated soil. Environ. Technol. 34(6), 747-755. DOI: 10.1080/09593330.2012.715757

BAI, W., 2017: Effects of application of NTA and EDTA on accumulation of soil heavy metals in Chrysanthemum. IOP Conference Series, Earth and Environmental Science 113, 012175. DOI: 10.1088/1755-1315/113/1/012175

BAKHT, J., ULLAH, R., SHAFI, M., 2020: Effect of chelator EDTA on phytoremediation of cadmium, chromium and lead and their effect on growth of sunflower (*Helianthus annuus* L.). Pak. J. Sci. Indus. Res. Ser. B. Biol. Sci. 63B(1), 22-29. DOI: 10.52763/PJSIR.BIOL.SCI.63.1.2020.22.29

BUCHET, J.P., LAUWERYS, R., ROELS, H., BERNARD, A., BRUAUX, P., CLAEYS, F., 1990: Renal effects of cadmium body burden of the general population. Lancet. 336, 699-702. DOI: 10.1016/0140-6736(90)92201-r

CLEMENS, S., 2006: Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88, 1707-1719. DOI: 10.1016/j.biochi.2006.07.003

CORGUINHA, A.P.B., DE-SOUZA, G.A., GONCALVES, V.C., CARVALHO, C.D.A., DE-LIMA, W.E.A., MARTINS, F.a.D., YAMANAKA, C.H., FRANCISCO, E.A.B., GUILHERME, L.R.G., 2015: Assessing arsenic, cadmium, and lead contents in major crops in Brazil for food safety purposes. J. Food Compos. Anal. 37, 143-150. DOI: 10.1016/j.jfca.2014.08.004

EL-BELTAGI, H.S., MOHAMMAD, A.A., RASHED, M.M., 2010: Response of antioxidative enzymes to cadmium stress in leaves and roots of radish (*Raphanus sativus* L.) Notulae Sci. Biol. 2, 76-82. DOI: 10.15835/nsb.2.4.5395

GALLEGO, S.M., PENA, L.B., BARCIA, R.A., AZPILICUETA, C.E., IANNONE, M.F., ROSALES, E.P., ENAVIDES, M.P.B., 2012: Unrevealing cadmium toxicity and tolerance in plants, insight into regulatory mechanisms. Environ. Experi. Bot. 83, 33-46. DOI: 10.1016/j.envexpbot.2012.04.006

GIANAZZA, E., WAIT, R., SOZZI, A., REGONDI, S., SACO, D., LABRA, M., 2007: Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. Environ. Experi. Bot. 59, 179-187. DOI: 10.1016/j.envexpbot.2005.12.005

GILL, S.S., TUTEJA, N., 2011: Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal. Behav. 6, 215-222. DOI: 10.4161/psb.6.2.14880

GLICK, B.R., 2010: Using soil bacteria to facilitate phytoremediation. Biotechnol. Adv. 28(3), 367-374. DOI: 10.1016/j.biotechadv.2010.02.001

HAIDER, F.U., LIQUN, C., COULTER, J.A., CHEEMA, S.A., WU, J., ZHANG, R., WENJUN, M., FAROOQ, M., 2021: Cadmium toxicity in plants impacts and remediation strategies. Ecotoxicol Environ. Safety. 211, 111887. DOI: 10.1016/j.ecoenv.2020.111887

HASSAN, T.U., BANO, A., NAZ, I., 2017: Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field. Inter. J. Phytoremed. 19, 522-529. DOI: 10.1080/15226514.2016.1267696

HERRERO, E.M., LOPES-GONZALVEZ, A., RUIZ, M.A., LUCAS-GARCIA, J.A., BARBAS, C., 2010: Uptake and distribution of zinc, cadmium, lead and copper in *Brassica napus* var. oleifera and *Helianthus annuus* grown in contaminated soils. Inter. J. Phytoremed. 5, 153-167.

DOI: 10.1080/713610177

IARC, 1993: IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry, Vol. 58. Lyon: Inter. Agency Res. Cancer Publicat. 41-117.

Ismael, M.A., Elyamine, A.M., Moussa, M.G., Cai, M., Zhao, X., Hu, C., 2018: Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 11(2). DOI: 10.1039/c8mt00247a

Jali, P., Pradhan, C., Das, A.B., 2017: Effects of cadmium toxicity in plants: A review Article. Sch. Acad. J. Biosci. 4, 1074-1081. DOI: 10.21276/sajb.2016.4.12.3

Khalifa, F.M., Schneiter, A.A., Eltayeb, E.I., 2000: Temperature-germination responses of sunflowers (*Helianthus annuus* L.) genotypes. HELIA 33, 97-104. DOI: 10.1515/helia.2000.23.33.97

Krämer, U., Talke, I.A., Hanikenne, M., 2007: Transition metal transport. FEBS Letters. 581, 2263- 2272. DOI: 10.1016/j.febslet.2007.04.010

Kubier, A., Wilkin, R.T., Pichler, T., 2019: Cadmium in soils and groundwater, A review. App. Geochem. 108, 104388. DOI: 10.1016/j.apgeochem.2019.104388

Liphadzi, M.S., Kirkham, M.B., Mankin, K.R., Paulsen, G.M., 2003: EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long term sewage-sludge farm. Plant Soil 254, 171-182. DOI: 10.1023/A:1026294830323

Liu, J., Li, K., Xu, J., Liang, J., Lu, X., Yang, J., Zhu, Q., 2003: Interaction of Cd and five metal nutrients for uptake and accumulation in different rice cultviars and genotypes. Field Crop Res. 83, 271-281. DOI: 10.1016/S0378-4290(03)00077-7

Liu, L., Li, J.W., Yue, F.X., Yan, X.W., Wang, F.Y., Bloszies, S., Wang, Y.F., 2018: Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd- contaminated soil. Chemosphere. 194, 4975-503. DOI: 10.1016/j.chemosphere.2017.12.025

Nazar, R., Iqbal, N., Masood, A., Khan, M.I.R., Syeed, S., Khan, N.A., 2012: Cadmium toxicity in plants and role of mineral nutientsin its alleviation. Amer. J. Plant Sci. 3, 1476-1489. DOI: 10.4236/ajps.2012.310178

Nelson, D.W., Sommers, L.E., 1973: Determination of Total Nitrogen in plant materials. Agron. J. 56(1), 109-112. DOI: 10.2134/agronj1973.00021962006500010033x

Novistasari, B.P., 2017: The Influence of Zinc Concentration (Zn) in Soil is polluted to the Sunflower Absorption *(Helianthus annuus* L.*)* in the Vegetative Phase, Universitas Brawijaya, Department of Agricultural Engineering, Malang, Indonesia.

Pandey, S., Gupta, K., Mukherjee, A.K., 2007: Impact of cadmium and lead on *Catharanthus roseus* a phytoremediation study. J. Environ. Biol. 28(2007), 655-662.

Panuccio, M.R., Sorgona, A., Rizzo, M., Cacco, G., 2009: Cadmium adsorption on vermiculite, zeolite and pumice: batch experiment studies. J. Environ. Manage. 90, 364-374. DOI: 10.1016/j.jenvman.2007.10.005

Pierart, A., Shahid, M., Sejalon-Delmas, N., Dumat, C., 2015: Antimony bioavailability: knowledge and research perspectives for sustainable agricultures. J. Hazard. Mater. 289, 219-234. DOI: 10.1016/j.jhazmat.2015.02.011

Raun, W.R., Olson, R.A., Sander, D.H., Westerman, R.L., 1987: Alternative procedure for total phosphorus determination in plant tissue. Commun. Soil Sci Plant Anal. 18(5), 543-557. DOI: 10.1080/00103628709367840

Rivelli, A.R., Puschenreiter, M., De Mariz, S., 2014: Assessment of cadmium uptake and nutrient content in sunflower plants grown under cd stress. Plant Soil Environ. 60(2), 80-86. DOI: 10.17221/520/2013-PSE

Sadiq, R., Maqbool, N., 2016: Acceleration of cadmium phytoextractionby sunflower (*Helianthus annuus* L.) in collaboration of Ethlene diamine tetraacetic acid (EDTA). Amer-Eurasia. J. Agric. Environ. Sci. 16(3), 77-583. DOI: 10.5829/idosi.aejaes.2016.16.3.12891

Salla, V., Hardaway, C.J., Sneddon, J., 2011: Preliminary investigation of *Spartina alterniflora* for phytoextraction of selected heavy metals in soils from Southwest Louisiana. Microchem. J. 97, 207-212. DOI: 10.1016/j.microc.2010.09.005

Sayqal, A., Ahmed, O.B., 2012: Advances in heavy metal bioremediation: An overview. App. Bionics Biomech. 2021, 1609149. DOI: 10.1155/2021/1609149

Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., 2015: Heavy metal stress and crop productivity. In: Hakeem, K.R. (ed.), Crop Production and Global Environmental Issues. Cham: Springer International Publishing, 1-25.

Sharma, S.S., Dietz, K.J., 2006: The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J. Experi. Bot. 57, 711-726. DOI: 10.1093/jxb/erj073

Singani Safari, A. A., Fahime, K., 2010: Effect of EDTA, sheep manure extract, and their application time on Cd uptakeby *Helianthus annuus* from a alcareous mine soil. Soil Sediment Contam. 19(3), 378-390. DOI: 10.1080/15320381003695199

Sinhal, V.K., Sriastava, A., Singh, V.P., 2010: EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb, and Cd through marigold (*Tagetes erecta*). J. Eniron. Biol. 31, 255-259.

Six, L., Smolders, E., 2014: Future trends in soil cadmium concentration under current cadmium fluxes to European agricultural soils. Sci. Total Environ. 485-486, 319-328. DOI: 10.1016/j.scitotenv.2014.03.109

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S., Yang, X., 2015: Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. Environ. Experi. Bot. 117, 28-40. DOI: 10.1016/j.envexpbot.2015.05.001

Vert, G., Barberon, M., Zelazny, E., Segue, M., Briat, J.F., Curie, C., 2009: Arabidopsis IRT2 cooperates with the high-affinity iron uptake system to maintain iron homeostasis in root epidermal cells. Planta 229, 1171-1179. DOI: 10.1007/s00425-009-0904-8

Xiong, T., Leveque, T., Shahid, M., Foucault, Y., Mombo, S., Dumat, C., 2014: Lead and cadmium phytoavailability and human bio accessibility for vegetables exposed to soil or atmospheric pollution by process ultra-fine particles. J. Environ. Qual. 43, 1593-1600. DOI: 10.2134/jeq2013.11.0469

Xu, Z.M., Li, Q.S., Yang, P., Ye, H.J., Chen, Z.S., Guo, S.H., Wang, L.L., He, B.Y., Zeng, E.Y., 2017: Impact of osmoregulation on the differences in Cd accumulation between two contrasting edible amaranth cultivars grown on Cd-polluted saline soils. Environ. Pollut. 224, 89-97. DOI: 10.1016/j.envpol.2016.12.067

Zheng, X., Chen, L., Li, X., 2018: Arabidopsis and rice showed a distinct pattern in ZIPs genes expression profile in response to Cd stress. Bot. Stud. 59, 22. DOI: 10.1186/s40529-018-0238-6

Address of the corresponding author:
Nazimah Maqbool, University of Agriculture Faisalabad, Pakistan, Faculty of Biological Sciences, Department of Botany, Faisalabad, Pakistan