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The Effect of Exogenous Zn Application on Photosynthetic Pigments, Electrolyte Leakage and Carbohydrate Metabolism in Soybean Plants Subjected to Cd Stress

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Abstract
This pot experiment was designed to study interaction between cadmium(Cd)- a toxic element and zinc (Zn)- an essential micronutrient, in soybean. Soybean plants were treated with Cd (0.3 and 0.6mM) alone and in combination with Zn (0.3 and 0.8mM). Zn only (0.3 and 0.8 mM) treatments were also given for comparison. Cadmium had a deleterious effect on the leaf pigments (chlorophyll and carotenoids) and relative leaf water content (RLWC). Cd application caused a decrease in chlorophyll (25-31%), carotenoids (24-30%) and RLWC (3-7%) in comparison to the control plants. The plants showed accumulation of starch (39-53%), total sugars (42-49%) and reducing sugars (62-83%) and a higher leakage of electrolytes (25-42%), apparently caused by Cd-induced oxidative stress. Zn supplementation in combination improved the water status and photosynthetic pigments level of the crop. Further, Zn application was also counteractive in ameliorating heavy metal toxicity through reduced carbohydrates accumulation and prevention of ion leakage through membrane, thus, playing an antagonistic role in Cd stress. Zn alone treated plants did not show significant difference in the studied parameters, indicating that the Zn concentrations chosen in study were non-toxic.

Keywords: Carbohydrates, Heavy metal, Legume, stress, Water deficit

1. Introduction

Cd has been recognized as the most ecotoxic heavy metal that adversely affects all biological processes of a plant, animal and humans (Cuypers et al., 2010). According to US Environmental Protection Agency (EPA), Cd ranked third major contaminant after Hg and Pb (Jamers et al., 2013) that has contaminated around 20 million hectares of cultivable land around the globe (Liu et al., 2015). Cd enters different ecosystems through natural (Mahmood et al., 2012) and anthropogenic activities like mining, fertilizers, sewage sludge and wastewater irrigation, etc. (Baghaie, 2021; Wuana and Okieimen, 2011). The cause of concern is its long residual time and non-biodegradable nature (Smolders et al., 1999). Plants readily absorb Cd through the soil and transportit to edible parts leading to an accumulation(McLaughlin et al., 2006). Various metabolic processes get disturbed due to the production of reactive oxygen species (ROS) causing oxidative stress (Chen et al., 2019). ROS damages cellular organelles like mitochondria and chloroplast and other components such as membrane lipids, nucleic acid, proteins andenzymes leading to cell death and losses in yield (Gill and Tuteja, 2010). Many studies have indicated the losses in photosynthetic pigments mainly chlorophyll and carotenoids under Cd stress (Baszynski et al., 1980; Katoch and Singh, 2014). The reduced formation of chlorophyll precursors and disrupted thylakoid membrane were linked to Cd caused oxidative stress (Alyemeni et al., 2018; Imonova et al., 2007), activation of chlorophyllase enzyme responsible for its catabolism (Hashem et al., 2019). Cd was also reported to slow down the activity ofRibulose-1,5-bisphosphate carboxylase-oxygenase (RuBPCase) by substituting magnesium(Mg) ions needed as a cofactor in carboxylation reactions (Siedlecka et al., 1998).

Cadmium ions are responsible for the damage to cell membrane lipids and proteins, enhanced electrolyte leakage and water deficit conditions (Prasad, 1995).Thiswas attributed to Cd caused oxidative stress restricting water and nutritional uptake by the roots (Dinakar et al., 2009), photosynthesis and transpiration inhibition (Shi et al., 2010)and nutrientim balance (Ouariti et al., 1997)]. Amongst the various strategies adopted, the overproduction of antioxidants and osmolytes maintains the cellular integrity of membranes in a stressed plant (Ahmad et al., 2015). Carbohydrate metabolism provides significant protection by maintaining the integrity of membranes during oxidative stress (Jha and Dubey, 2004; Rosa et al., 2009).

Zinc (Zn) participates in the biosynthesis of chlorophyll, carotenoids, cytochrome C, Indole-3-acetic acid(IAA), carbohydrate metabolism, nitrogen metabolism and in the stabilization of ribosomal fractions (Broadley et al., 2007; Cherif et al., 2011). It also serves as a cofactor in many enzymatic and non-enzymatic reactions (van de Mortel et al., 2006) and protects the vital cell components like chlorophyll, lipids and –SH groups from oxidative and peroxidative damages (Bettger and O'Dell, 1981). Low water status during Zn deficiency increases membrane permeability causing cellular impairment (Cakmak, 2000).The optimum Zn levels in soil restrict the entry of Cd due to the antagonistic effect (Baghaie and Aghilizefreei, 2020; Rizwan et al., 2019).Zinc deficiency in the soil promotes Cduptake by the plants (Adiloğlu, 2002; Hassan et al., 2005). Conversely, there are reports also which show their synergistic behavior in soil (Almeida et al., 2019; Shen et al., 2006). Keeping in mind, the present study was aimed at Zn interaction lowering Cdcaused changes in photosynthetic apparatus, water content, electrolytes and carbohydrate metabolism in soybean crop.

2. Material and Methods

Soybean [*Glycine max* (L.) Merr.] *var.palam soya* seeds procured from CSK Himachal Pradesh Agriculture University, Palampur, India was surface sterilized in 0.1% $HgCl_2$, washed and soaked overnight in a thick slurry of rhizobium culture mixed with activated charcoal and acacia gum. Plants were raised in pots filled with river-washed sand. Five seeds were sown in each pot and maintained under natural daylight conditions in out-houses. Cadmium (0.3 and 0.6mM as $CdSO_4.7H_2O$) and zinc (0.3 and 0.8mM as $ZnSO_4.7H_2O$) treatments alone and in combinations were applied 8 DAS (days after sowing) along with the nutrient medium (Minchin and Pate, 1975), till first flowering at 20 days interval. Plants irrigated with nutrient medium served as control (C). The nutrient medium comprised of **(1)** Trace elements common to both the solutions- H_3BO_3 (3 mg/L), $FeCl_3$ (11.4 mg/L), $MnCl_2.4H_2O$ (1.7 mg/L), $NaMoO_4.2H_2O$ (0.02 mg/L), $CuCl_2$ (0.04 mg/L), $ZnSO_4.7H_2O$ (2.3 mg/L), $CaCl_2$ (0.01 mg/L); **(2)** Major elements of N^+ medium- KNO_3 (0.4 g/L), KH_2PO_4 (0.14 g/L), $MgSO_4.7H_2O$ (0.49 g/L), $Ca(NO_3)_2$ (0.66 g/L); **(3)** N^- medium- KH_2PO_4 (0.14 g/L), $MgSO_4.7H_2O$ (0.49 g/L), $CaCl_2$ (0.11 g/L), KCl (0.07 g/L).

2.1 *Biochemical Analysis*

2.1.1 *Photosynthetic pigments*

Photosynthetic pigments were extracted by grinding fresh leaves (200 mg) in 80% acetone, followed by filtration with What man's filter papers. The absorbance of the supernatant was measured at 480, 645 and 663 nm using Thermo scientic spectrophotometer. Total chlorophyll was calculated according to equation of Arnon (1949) and carotenoids according to equation of Kirk and Allen (1965).

2.1.2 *Relative Leaf Water Content (RLWC)*

The RLWC was measured according to the method of Chen et al. (2009). Fresh leaf (100 mg) was cut and their fresh weight (FW) was recorded. Further, the leaves were oven dried for 24 h at 110 ^{0}C in oven and weighed again (DW). RLWC was calculated as follows:-
Relative leaf water content (RLWC %) = [(FW – DW)/FW]* 100

2.1.3 *Membrane damage (as electrolyte leakage)*

Electrolyte leakage was used to assess permeability of cell membrane as described by Lutts et al. (1996). Leaves were collected, washed with deionized water and placed in test tubes containing 10 ml of deionized water and incubated over night at 25^{0}C. Electrical conductivity of the solution (C1) was measured after 24 h. Samples were then put in boiling water bath for 10–15 min and conductivity reading (C2) was measuring after cooling to 25^{0}C. The electrolyte leakage was calculated using the formula- EL% = C1/C2 x 100

2.1.4 *Total sugar*

Total sugars were determined using anthrone reagent method of Yemm and Willis (1954). Dried plant material was homogenized in 80% ethanol followed by centrifugation for 10 min. To 4.0 ml of chilled anthr one reagent, 1.0ml of ethanol extract was added. The test tubes were placed in boiling water bath for 10 min and then cooled in ice bath prior to determining absorbance at 625 nm.

2.1.5 *Reducing sugars*

Reducing sugars were measured according to the method given by Sumner (1935). Dried plant material was homogenized in ethanol (80%) and centrifuged for 10 min. To 1.0 ml of dinitro salicylic acid (DNSA) reagent, ethanol extract (1 ml) was added and the absorbance was read at 560 nm.

2.1.6 *Starch content*

Estimation of starch was done by the method of Mc Cready et al. (1950). The residual mass obtained after the extraction of soluble sugars was washed with 80% ethanol to remove any traces of sugars. Next, 5 ml distilled water and 6.5 ml of 52% perchloric acid was added to the residue followed by centrifugation for 20 minutes. The supernatant was decanted and collected and the whole process was repeated thrice. Supernatant of each step was then poured and the final volume was made 100 ml with distilled water. To 0.5 ml of diluted extract, 4.5 ml of distilled water and 10 ml of cold anthrone reagent were added in ice bath. The mixture was heated for 8 min at 100°C and cooled to room temperature and the absorbance was read at 630nm.

2.2 *Statistical analysis*

All values were in triplicates and represented as mean ± SE (standard error). Data were statistically analyzed using one-way ANOVA in SPSS-16 by taking the probability level of 5%. In the post hoc test, Fisher's least significant difference (LSD) was used to separate the means of various treatments.

3. Results

3.1 *Chlorophyll Content*

Zn alone treatments promoted chlorophyll content by 7.20% ($Zn_{0.3mM}$) and 12.68% ($Zn_{0.8mM}$), while, it dropped by about 25.52% ($Cd_{0.3mM}$) and 31.32% ($Cd_{0.6mM}$) to that of control with Cd application. Zn supplementation to Cd treatments prevented this drop and raised chlorophyll levels 7.29% ($Cd_{0.3}$+$Zn_{0.3mM}$), 27.17% ($Cd_{0.3}$+$Zn_{0.8mM}$)

and 3.06% ($Cd_{0.6}$+$Zn_{0.8mM}$) more than the control. Further, the chlorophyll loss in $Cd_{0.6}$+$Zn_{0.3mM}$ treatment was checked to 7.81% (Fig 1).

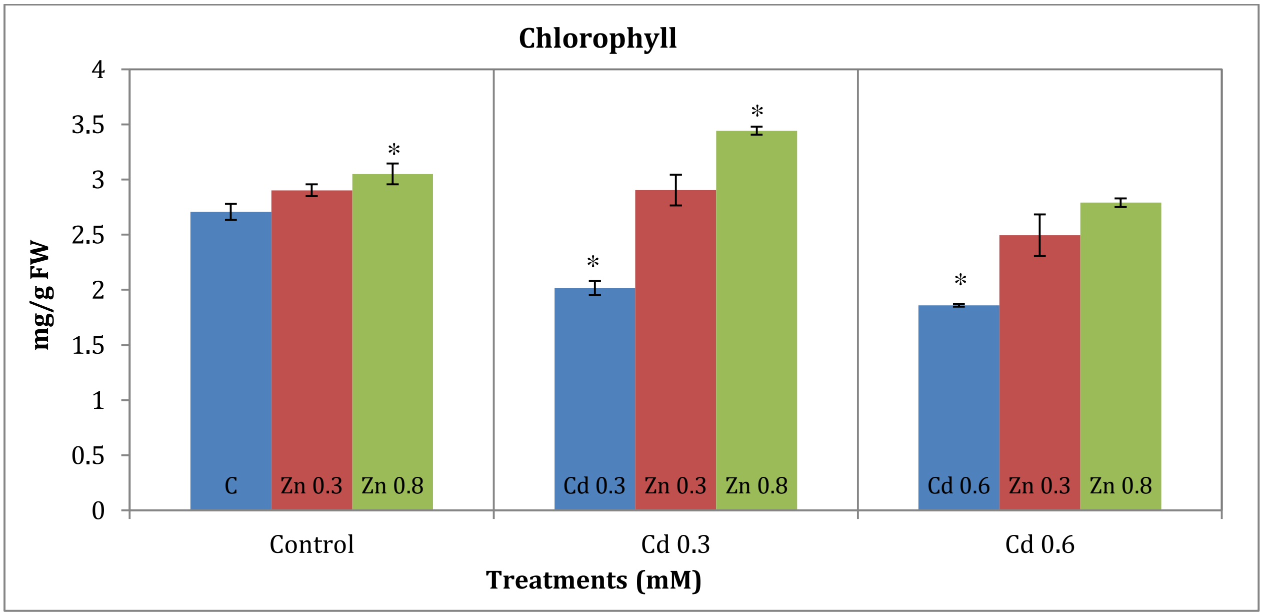

Fig 1: Effect of Cd and Zn alone and in combination on chlorophyll content in soybean plants. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=0.27).

3.2 *Carotenoids Content*

Similarly, carotenoids content was enhanced by 4.93% and 6.76% with Zn alone treatments (0.3 and 0.8mM), while, it declined by about 24.35% and 30.82% with Cd (0.3 and 0.6mM) in comparison to the control. In the combination treatments, Zn effectively raised the levels of carotenoids 0.83% ($Cd_{0.3}$+$Zn_{0.3mM}$) and 18.35% ($Cd_{0.3}$+$Zn_{0.8mM}$) more than the control, while, pigment drop in $Cd_{0.6}$+$Zn_{0.3mM}$ and $Cd_{0.6}$+$Zn_{0.8mM}$ treatments was restricted to 15.43% and 1.49%, respectively(Fig 2).

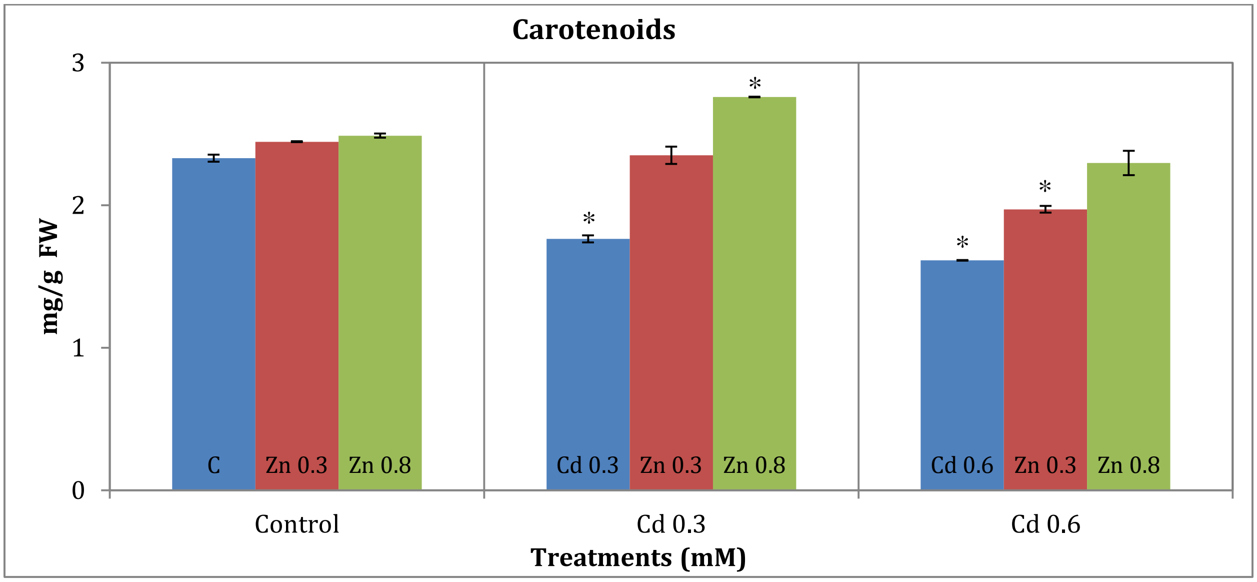

Fig 2: Effect of Cd and Zn alone and in combination on carotenoids content in soybean plants. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=0.24).

3.3 *Relative Leaf Water Content (RLWC)*

RLWC enhanced by 0.7% (0.3mM) and 1.85% (0.8mM) with Zn alone application, while its loss in Cd treatments was 3.96% (0.3mM) and 7.37% (0.6mM) in comparison to control. In the combination treatments, such losses were lowered to 2.27% ($Cd_{0.3}+Zn_{0.3mM}$), 0.41% ($Cd_{0.3}+Zn_{0.8mM}$), 5.76% ($Cd_{0.6}+Zn_{0.3mM}$) and 2.66% ($Cd_{0.6}+Zn_{0.8mM}$) to that of control (Fig. 3).

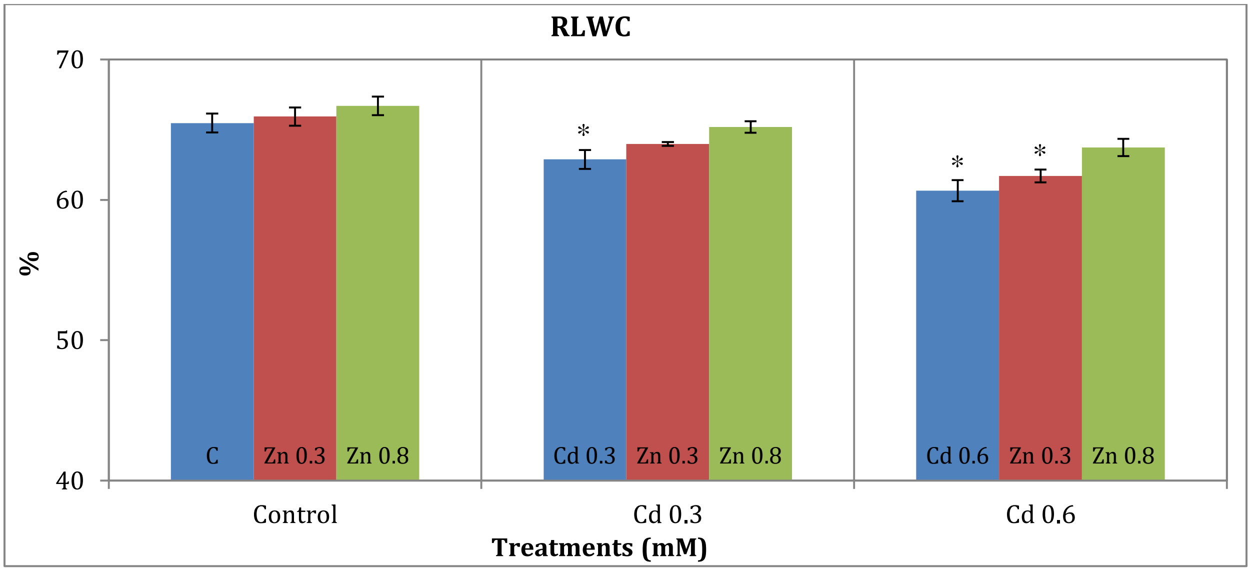

Fig 3: Effect of Cd and Zn alone and in combination on RLWC in soybean plants. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=1.74).

3.4 *Electrolyte Leakage*

In Zn alone treatments (0.3 and 0.8 mM) electrolyte leakage was 5.68% and 9.14% lesser in comparison to control. A sharp rise in electrolyte leakage was noticed with $Cd_{0.3mM}$ (25.46%) and $Cd_{0.6mM}$ (42.31%) treatments. In combination treatments such rise in electrolyte leakage was lowered to 13.83% ($Cd_{0.3}$+$Zn_{0.3mM}$), 4.57% ($Cd_{0.3}$+$Zn_{0.8mM}$), 31.41% ($Cd_{0.6}$+$Zn_{0.3mM}$) and 18.11% ($Cd_{0.6}$+$Zn_{0.8mM}$) to that of control (Fig. 4).

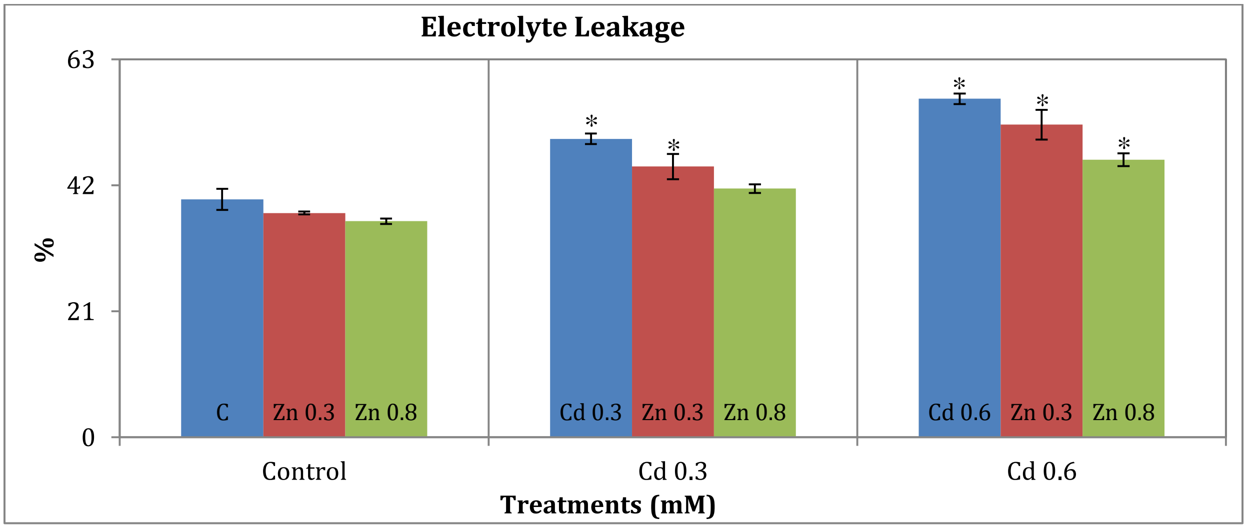

Fig 4: Effect of Cd and Zn alone and in combination on electrolyte leakage in soybean plants. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=4.09).

3.5 *Starch content*

In Cd treated plants there was a sharp increase of 39.36% ($Cd_{0.3mM}$) and 53.71% ($Cd_{0.6mM}$), whereas, in Zn alone treated plants this increase was 10.07% ($Zn_{0.3mM}$) and 15.58% ($Zn_{0.8mM}$)in comparison to control. In combination treatment the accumulation was lowered to 16.54% in $Cd_{0.3}$+$Zn_{0.3mM}$, 9.47% in $Cd_{0.3}$+$Zn_{0.8mM}$, 31.09% in $Cd_{0.6}$+$Zn_{0.3mM}$ and 14.33% in $Cd_{0.6}$+$Zn_{0.8mM}$ treatments vis-à-vis control (Fig 5).

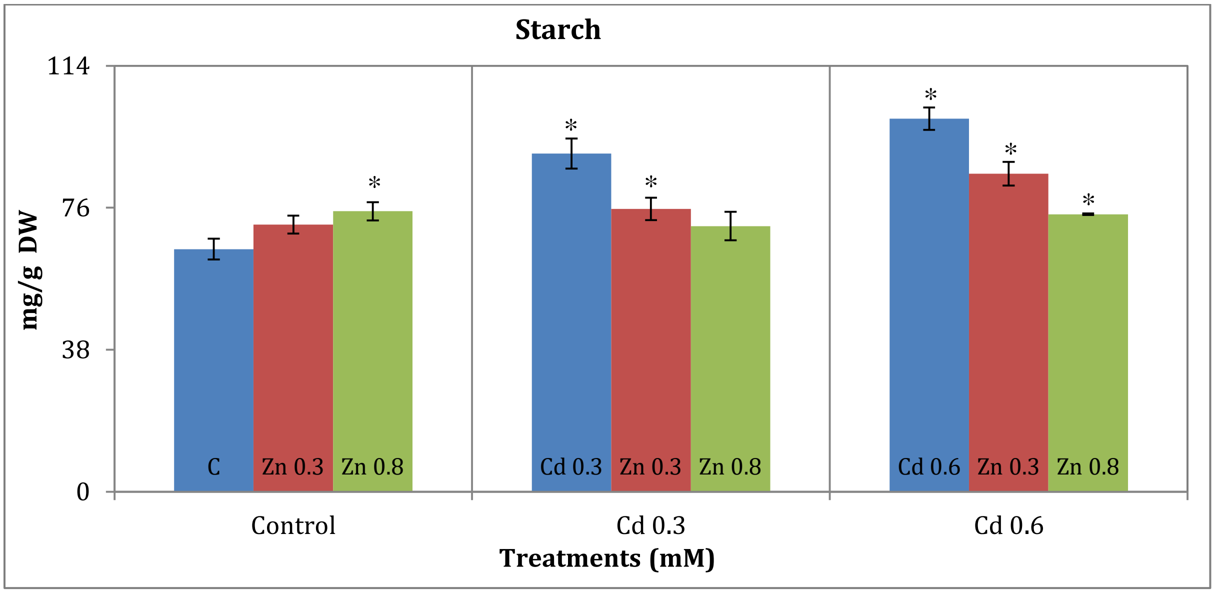

Fig. 5: Effect of Cd and Zn alone and in combination on starch content in soybean plants. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=5.34).

3.6 *Total Sugars*

Total sugars content also showed a significant increase of 42.98% ($Cd_{0.3mM}$) and 49.86% ($Cd_{0.6mM}$) in Cd treated plants, whereas, in Zn only treated plant the increase was 4.55% ($Zn_{0.3mM}$) and 13.25% ($Zn_{0.8mM}$) in comparison to control. Zn addition in the combination was able to reduce the buildup of sugars to 27.76% in $Cd_{0.3}$+$Zn_{0.3mM}$ and 9.56% in $Cd_{0.3}$+$Zn_{0.8mM}$; to 33.24% in $Cd_{0.6}$+$Zn_{0.3mM}$ and 17.94% in $Cd_{0.6}$+$Zn_{0.8mM}$ treatments vis-à-vis control (Fig. 6).

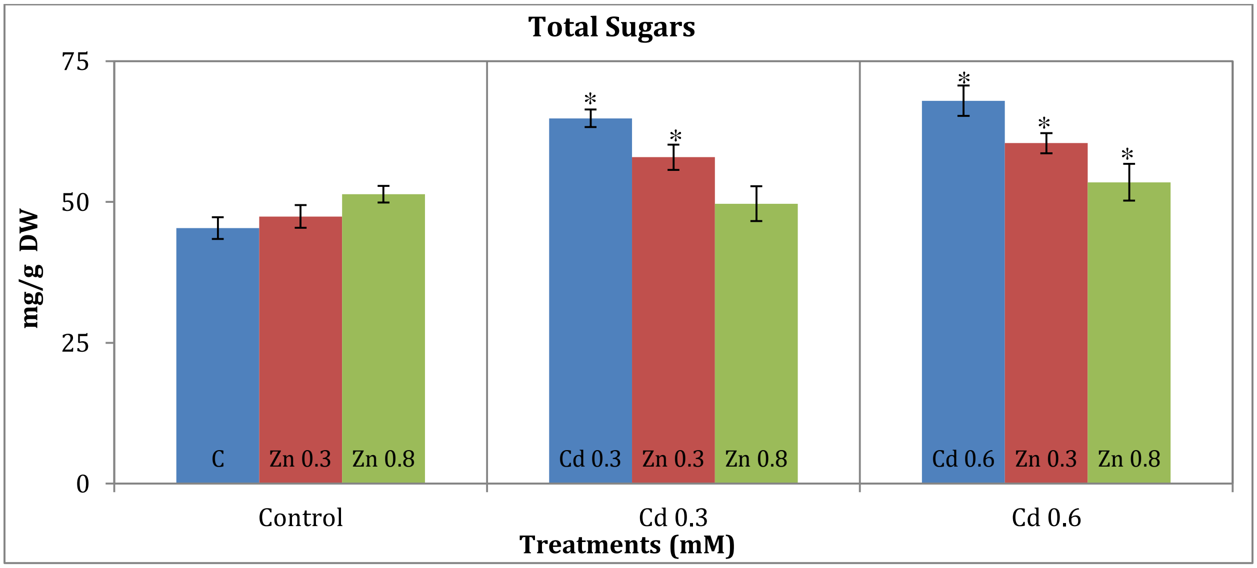

Fig. 6: Effect of Cd and Zn alone and in combination on total sugars content in soybean. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=6.11).

3.7 *Reducing Sugars*

Similarly, reducing sugars content increased significantly by 62.66% ($Cd_{0.3mM}$) and 83.73% ($Cd_{0.6mM}$) in Cd treated plants, whereas, the increase was 9.61% ($Zn_{0.3mM}$) and 18.11% ($Zn_{0.8mM}$) in Zn alone treated plants, compared to the control. Zn supplementation with heavy metal Cd reduced the buildup to 36.04% in $Cd_{0.3}+Zn_{0.3mM}$ and 14.97% in $Cd_{0.3}+Zn_{0.8mM}$; to 62.66% in $Cd_{0.6}+Zn_{0.3mM}$ and 41.22% in $Cd_{0.6}+Zn_{0.8mM}$ treatments to that of control (Fig. 7).

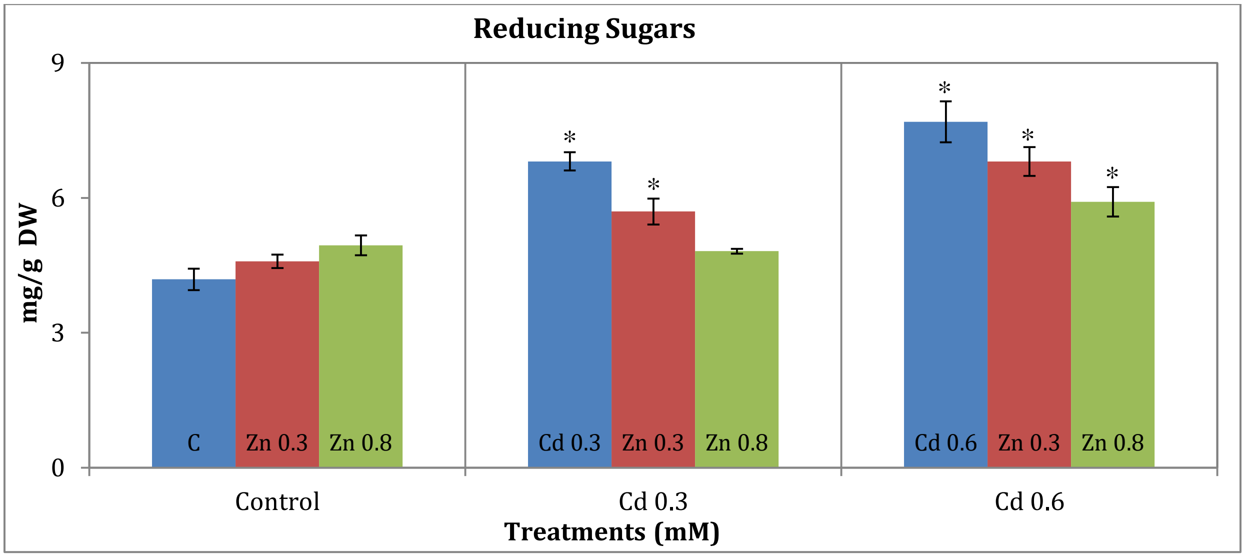

Fig. 7: Effect of Cd and Zn alone and in combination on reducing sugars content in soybean. Each bar represents Mean ± SE of three replicates and asterisk denotes significant difference to the control ($LSD_{0.05}$=0.58).

4. Discussion

Our observations have revealed the counteractive role of Zn in restoring Cd depleted leaf pigments like chlorophyll and carotenoids. Cd toxicity inhibits the biosynthesis of chlorophyll by interfering with sulfhydryl groups (-SH) of δ-aminolevulinic acid (ALA) dehydratase and proto-chlorophyll idereductase, lowering the production of ALA, the first common precursor of tetrapyrrole rings (Gadallah, 1995; Prasad and Strzalka, 1999). Loss of pigments due to Cd can also be attributed to the overproduction of ROS that restrictsthe formation of chlorophyll precursor, disrupting chlorophyll lamellae, PS I and PS II-mediated electron transport system and thylakoid membrane (Imonova et al., 2007; Laspina et al., 2005). Zn or Mg ions convert ALA to porphobilinogen through enzyme ALA dehydratase **(**Beale, 1999; Lebedev and Timko, 1998).The binding of Zn to -SH group of protein moiety also protects them from thiol oxidation (Cakmak, 2000). Importantly, Zn detoxifies ROS being a co-factor of the SOD enzyme, as the first line of defense (Weisany et al., 2012). The effectiveness of Zn has been proved in maintaining the content of leaf pigments during stress in many crop species such as tomato (Cherif et al., 2011), mustard (Ahmad et al., 2017), tamarind (Venkatachalam et al., 2017)and rice (Adil et al., 2020). Zn promotes the synthesis of chlorophyll, photosynthetic rate, photochemical reaction and electron transport through PS-II (Roach and Liszkay, 2014).

A drop in the RLWC was proportionately linked to a rise in Cd levels of soybean plants. A decrease in relative water content was reported in many Cd stressed crops like coon-tail (Aravind and Prasad, 2004), soybean (Thakur and Singh, 2012) and moth bean (Vijendra et al., 2016).Such a decrease in water absorption was also attributed to disrupted translocation owing to reduction in size and number of xylem vessels and hormonal imbalance (Poschenrieder and Barceló, 1999), modification of secondary root branching and their geotropic response (Costa and Spitz, 1997; Singh and Thakur, 2014), hydraulic conductivity with a partial blockage of xylem elements by cellular debris (Rucińska-Sobkowiak, 2016). Our results clearly pointed out the improved water status with elevated RLWC upon Zn supplementation of Cd stressed soybean plants. Zn application leads to better development of root system with more number of root tips promoting water uptake (Zaman et al., 2018).Zn improved vascular tissue formation and hence RLWC thus, preventing its destruction during unfavorable conditions (Gadallah and Ramadan, 1997). Improved water content, leaf pigments level, photosynthetic rate and reduced electrolyte leakage in mustard plants under Cd stressed was correlated with Zn supplementation (Ahmad et al., 2017).

In the present study, more electrolyte leakage (EL) was noticed with a rise in Cd concentration. Cd initiates the oxidation of NADPH to produce superoxide radicals ($O_2^{\cdot -}$) and accumulating more H_2O_2 (Kawano et al., 2001). The cell membrane is one of the primary targets of an oxidative burst in plants (Levitt, 1972), disrupting membrane lipids and proteins due to ROS generation (Smeets et al., 2005). According to Pireh et al. (2017),an increase in Cd levels of soybean relates to more EL accompanied by reduced photosynthesis and yield parameters. The role of Zn in maintaining the integrity of cell membranes was also highlighted which can protect lipids and proteins against ROS species (Cakmak and Marschner, 1988; Qin et al., 2018).In fact, Zn supplementation improved RWC to offset the negative effects of Cd-caused water deficit and also preventing leakage of ions with a better membrane stability index (MSI). The findings of Bashir et al. (2020) also supported our viewpoint that Zn supplementation to Cd stressed plants improves the content of leaf pigments like chlorophyll and carotenoid accompanied by reduced electrolyte leakage.

It was also seen that the buildup of sugars including starch and reducing sugars depleted with Zn supplementation in Cd stressed soybean crop. As reported by Rosa et al. (2009) abiotic stress adversely affects carbon assimilation including the source-sink mechanism of sugar translocation [23]. Plants raise the level of the soluble sugars in leaves to maintain osmotic homeostasis, water potential including the base metabolism (Verma and Dubey, 2001; Zoufan et al., 2020). Besides the role of sugars in osmo-protection, they also participate in many important processes like energy production, signaling, maintaining the integrity of cellular membranes and turgor pressure (Nayer and Reza, 2008). Interference of Cd with the enzymes of the Calvin cycle and carbohydrate metabolism also alters the antioxidative metabolism (Khan et al., 2009; Shi et al., 2010). The depletion of such osmolytes was reported with Zn application in stressed soybean cultivars (Gadallah, 2000; Karami et al., 2016). An increment in carbohydrate level with Zn application is linked to its direct participation in photosynthetic activity and activation of enzymes like starch synthetase (Jyung et al.,1975; Singh et al., 2014).

5. Conclusions

Thus, it can be stated based on current observations that Zn supplementation improves the photosynthetic capacity of soybean plants by raising its pigments level and water status, translocation of carbohydrates and preventing ion leakage through membranes. Zn has an antagonistic interaction with the heavy metal Cd to ameliorate its toxicity.

Conflict of interest

The authors declare no conflict of interest.

References

1. Adil, M.F., Sehar, S., Han, Z., Lwalaba, J.L.W., Jilani, G., Zeng, F., Chen, Z.H., and Shamsi, I.H(2020). Zinc alleviates cadmium toxicity by modulating photosynthesis, ROS homeostasis, and cation flux kinetics in rice, Environmental Pollution, 265, 114979.
2. Adiloğlu, A.(2002). The effect of zinc (Zn) application on uptake of cadmium (Cd) in some cereal species, Archives of Agronomy and Soil Science, 48(6), 553-556.
3. Ahmad, P., Ahanger, M.A., Alyemeni, M.N., Wijaya, L., Egamberdieva, D., Bhardwaj, R., and Ashraf, M.(2017). Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) Czern and Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content, Journal of Plant Interactions, 12(1), 429-437
4. Ahmad, P., Sarwat, M., Bhat, N.A., Wani, M.R., Kazi, A.G., and Tran, L.S.P.(2015). Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. and Coss.) by calcium application involves various physiological and biochemical strategies, Plos One, 10, 0114571.
5. Almeida, G.L., da Silva, M.D., Jorge, A.P., de Souza, K.R.D., Guilherme, L.R.G., and Alves, D.J.(2019). Synergy between cadmium and zinc in bean plants cultivated in multi contaminated soils, Acta Scientiarum Agronomy, 41, 35829.
6. Alyemeni, M.N., Ahanger, M.A., Wijaya, L., Alam, P., Bhardwaj, R., and Ahmad, P.(2018). Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system, Protoplasma, 255, 459-469.
7. Aravind, P. and Prasad, M.N.V.(2004). Carbonic anhydrase impairment in cadmium-treated *Ceratophyllum demersum* L. (free floating freshwater macrophyte): toxicity reversal by zinc, Journal of Analytical Atomic Spectrometry, 19, 52-57.
8. Arnon, D.I.(1949). Copper Enzyme in isolated chloroplast: Polyphenol oxidase in *Beta vulgaris.* Plant Physiology, 24, 1-15.
9. Baghaie, A.H.(2021). Indole-3-Acetic Acid and Humic Acid Increase the Bio-Degradation of Diesel Oil in Soil Polluted with Pb and Cd.Journal of Environmental Health and Sustainable Development, 6(2), 1302-10.
10. Baghaie, A.H. andAghilizefreei, A.(2020). Effect of zinc sulfate and salicylic acid on biological degradation of phenanthrene in the Cd polluted soil under sorghum cultivation inoculated with *Pseudomonas putida*,Journal of Chemical Health Risks, 10(1), 35-43.
11. Bashir, A., Rizwan, M., Ali, S., Adrees, M., Zia ur Rehman, M., and Qayyum, M.F.(2020). Effect of composted organic amendments and zinc oxide nanoparticles on growth and cadmium accumulation by wheat; a life cycle study. Environmental Science and Pollution Research, 10.1007/s11356-020-08739-8.
12. Baszynski, T., Wajda, L., Krol, M., Wolinska, D., Krupa, Z., and Tukendorf, A.(1980). Photosynthetic activities of cadmium–treated tomato plants, Physiologia Plantarum, 48, 365-370.
13. Bettger, W.J. and O'Dell, B.L.(1981). A critical physiological role of zinc in the structure and function of biomembranes, Life Sciences, 28, 1425-1438.
14. Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., and Lux, A. (2007). Zinc in plants, New Phytologist, 173, 677-702.

15. Cakmak I.(2000). Possible roles of zinc in protecting plant cells from damage by reactive oxygen species, New Phytologist, 146, 185-205.

16. Cakmak, I. and Marschner, H. (1988). Increase in membrane permeability and exudation in roots of zinc deficient plants, Journal of Plant Physiology, 132, 356-361.
17. Chen, D., Chen, D., Xue, R., Long, J., Lin, X., Lin, Y., Jia, L., Zeng, R., and Song, Y.(2019). Effects of boron, silicon and their interactions on cadmium accumulation and toxicity in rice plants, Journal of Hazardous Materials, 367, 447-455.
18. Chen, J., Shiyab, S., Han, F.X., Monts, D.J., Waggomer, A.W., and Su, Z.Y.(2009). Bioaccumulation and physiological effects of mercury in *Pteris vitatta* and *Nephrolepis exaltata*, Ecotoxicology, 18, 110-121.
19. Cherif, J., Mediouni, C., Ben Ammar, W., and Jemal, F.(2011). Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (*Solanum lycopersicum*), Journal of Environmental Sciences, 23, 837-844.
20. Costa, G.and Spitz, E. (1997). Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinus albus*, Plant Science, 128, 131-140.
21. Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A.R., Munters, E., Artois, T.J., Nawrot, T., Vangronsveld, J., and Smeets, K. (2010). Cadmium stress: an oxidative challenge, Biometals, 23, 927-940.
22. Dinakar, N., Nagajyothi, P.C., Suresh, S., Damodharam, T., and Suresh, C.(2009). Cadmium induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in *Arachis hypogaea* L, Journal of Environmental Biology, 30, 289-294.
23. Gadallah, M. and Ramadan, T.(1997). Effects of zinc and salinity on growth and anatomical structure of *Carthamus tinctorius* L, Biologia Plantarum, 39, 411-418.
24. Gadallah, M.A.A.(1995). Effects of cadmium and kinetin on chlorophyll content, saccahrides and dry matter accumulation in sunflower plants, Biologia Plantarum, 37, 233-240.
25. Gadallah, M.A.A.(2000). Effects of indole-3- acetic acid and zinc on growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit, Journal of Arid Environments, 44(4), 451-467.
26. Gill, S.S. and Tuteja, N.(2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants,Plant Physiology and Biochemistry, 48, 909-930.
27. Hashem, A., Abd Allah, E.F., Alqarawi, A.A., Malik,J.A., Wirth, S., and Egamberdieva, D.(2019). Role of calcium in AMF-mediated alleviation of the adverse impacts of cadmium stress in *Bassia indica* [Wight] A. J. Scott, Saudi Journal of Biological Sciences, 26, 828-838.
28. Hassan, M.J, Zhang, G., Wu, F., Wei, K., and Chen, Z.(2005). Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice, Journal of Plant Nutrition and Soil Science, 168, 255-261.

29. Imonova, E.S., Henselova, M., Masarovicova, E., and Kohanova, J.(2007). Comparison of tolerance of *Brassica juncea* and *Vigna radiata* to cadmium, Biologia Plantarum, 51, 488-492.
30. Jamers, A., Blust, R., De Coen, W., Griffin, J.L., and Jones, O.A.(2013). An omics based assessment of cadmium toxicity in the green alga *Chlamydomonas reinhardtii*, Aquatic Toxicology, 126, 355-364.
31. Jha, A.B. and Dubey, R.S.(2004). Carbohydrate metabolism in growing rice seedling under arsenic toxicity, Journal of Plant Physiology, 161, 867-872.
32. Jyung, W.H., Ehniann, A., Schlender, K.K., and Scala, J.(1975). Zinc Nutrition and Starch Metabolism in *Phaseolus vulgaris* L, Plant Physiology, 55, 414-420.
33. Karami, S., Modarres Sanavy, S.A.M., Ghanehpoor, S., and Keshavarz, H.(2016). Effect of foliar zinc application on yield, physiological traits and seed vigor of two soybean cultivars under water deficit. Notulae Scientia Biologicae, 8(2), 181-191.

34. Katoch, K. and Singh, K.J.(2014). Role of calcium in antagonizing cadmium induced heavy metal toxicity in mungbean seedlings, Indian Journal of Plant Sciences, 3(3), 1-6.
35. Kawano, T., Kawano, N., Muto, S., and Lapeyrie, F.(2001). Cation-induced superoxide generation in tobacco cell suspension culture is dependent on ion valence, Plant, Cell and Environment, 24, 1235-1241.
36. Khan, N.A., Anjum, N.A., Nazar, R., and Iqbal, N.(2009). Increased activity of ATP-sulfurylase and increased contents of cysteine and glutathione reduce high cadmium-induced oxidative stress in mustard cultivar with high photosynthetic potential, Russian Journal of Plant Physiology, 56(5), 670-677.
37. Kirk, J.T.O. and Allen, R.L.(1965). Dependence of chloroplast pigment synthesis on protein synthesis effects of actilione, Biochemical and Biophysical Research Communications, 27, 523-530.
38. Laspina, N.V., Groppa, M.D., Tomaro, M.L., and Benavides, M.P.(2005). Nitric oxide protects sunflower leaves against Cd-induced oxidative stress, Plant Science, 169(2), 323-330.
39. Lebedev, N. and Timko, P.M.(1998). Protochlorophyllide photoreduction, Photosynthesis Research, 58, 5-23.
40. Liu, Y., Xiao, T., Baveye, P.C., Zhu, J., Ning, Z., and Li, H.(2015). Potential health risk in areas with high naturally-occurring cadmium background in southwestern China, Ecotoxicology and Environmental Safety, 112, 122-131.
41. Lutts, S., Kinet, J.M., and Bouharmont, J.(1996). NaCl-induced senescence in leaves of rice (*Oryza sativus* L.) cultivars differing in salinity resistance, Annals of Botany, 78, 389-398.
42. Mahmood, Q., Rashid, A., Ahmad, S.S., Azim, M.R., and Bilal, M.(2012). Current status of toxic metals addition to environment and its consequences. In: Anjum, N.A.et al. (eds) The plant family *Brassicaceae*: contribution towards phytoremediation, vol. 21, Environmental pollution. Dordrecht: Springer.
43. McCreddy, R.M., Guggolz, J., Silviera, V., and Owens, H.S.(1950). Determination of starch and amylase in vegetables, Analytical Chemistry, 22, 1156-1158.
44. McLaughlin, M.J., Whatmuff, M., Warne, M., Heemsbergen, D., Barry, G., Bell, M., Nash, D., and Pritchardet, D.(2006). A field investigation of solubility and food chain accumulation of biosolid-cadmium across diverse soil types, Environmental Chemistry, 3, 428-432.
45. Minchin, F.R. and Pate, J.S.(1975). Effects of water, aeration and salt regime on nitrogen fixation in a nodulated legume: definition of an optimum root environment, Journal of Experimental Botany, 26, 60-80.
46. Nayer, M. and Reza, H.(2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties, World Applied Sciences Journal, 3(3), 448-453.
47. Ouariti, O., Gouia, H., and Ghorbal, M.H.(1997). Responses of bean and tomato plants to cadmium: Growth, mineral nutrition and nitrate reduction, Plant Physiology and Biochemistry, 35, 347-354.
48. Pireh, P., Yadavi, A., and Balouchi, H.(2017). Effect of cadmium chloride on soybean in presence of arbuscular mycorrhiza and vermicompost, Legume Research, 40(1), 63-68.
49. Poschenrieder, C. and Barceló, J.(1999). Water relations in heavy metal stressed plants. in: Prasad, M.N.V. and Hagemeyer, J., (Eds.) Heavy Metal Stress In Plants: From Molecules To Ecosystems. Heidelberg, Springer-Verlag, (pp. 207-229).
50. Prasad, M.N.V.(1995). Cadmium toxicity and tolerance in vascular plants, Environmental and Experimental Botany, 35(4), 525-545.
51. Prasad, M.N.V. and Strzalka, K.(1999). Impact of Heavy Metals on Photosynthesis. In: Prasad, M.N.V. and Hagemeyer, J., Eds., Heavy Metal Stress in Plants, Springer, Berlin, 117-138.

52. Qin, F., Liu, G., Huang, G., Dong, T., Liao, Y., and Xu, X.(2018). Zinc application alleviates the adverse effects of lead stress more in female *Morus alba* than in males, Environmental and Experimental Botany, 146, 68-76.
53. Rizwan, M., Ali, S., Zia ur Rehman, M., and Maqbool, A.(2019). A critical review on the effects of zinc at toxic levels of cadmium in plants, Environmental Science and Pollution Research, 26, 6279-6289.
54. Roach, T. and Liszkay, A.K.(2014). Regulation of photosynthetic electron transport and photoinhibition, Current Protein and Peptide Science, 15, 351-362.
55. Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M., and Prado, F.E.(2009). Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants, Plant Signaling and Behavior, 4, 388-393.
56. Rucińska-Sobkowiak R.(2016). Water relations in plants subjected to heavy metal stresses, Acta Physiologiae Plantarum, 38, 257.

57. Shen, G., Lu, Y., and Hong, J.(2006). Combined effect of heavy metals and polycyclic aromatic hydrocarbons on urease activity in soil, Ecotoxicology and Environmental Safety, 63(3), 474-480.
58. Shi, G., Liu, C., Cai, Q., Liu, Q., and Hou, C.(2010). Cadmium accumulation and tolerance of two safflower cultivars in relation to photosynthesis and antioxidantive enzymes, Bulletin of Environmental and Contamination Toxicology, 85(3), 256-263.
59. Siedlecka, A., Krupa, Z., Samuelsson, G., Oquist, G., and Gardestrom, K.(1998). Primary carbon metabolism in *Phaseolus vulgaris* plants under Cd/Fe interaction, Plant Physiology and Biochemistry, 35, 951-957.
60. Singh, D., Yadav, S., and Nautiyal, N.(2014). Evaluation of growth responses in wheat as affected by the application of zinc and boron to a soil deficient in available zinc and boron, Communications in Soil Science and Plant Analysis, 45(6), 765-776.
61. Singh, K.J. and Thakur, A.K.(2014). Graviperceptional changes in the root tips of cadmium-treated soybean seedlings, Current Science, 107(8), 1294-1298.
62. Smeets, K., Cuypers, A., Lambrechts, A., Semane, B., Hoet,P., Laere, A.V., and Vangronsveld, J.(2005). Induction of oxidative stress and antioxidative mechanism in *Phaseolus vulgaris* after Cd application, Plant Physiology and Biochemistry, 43, 437-444.
63. Smolders, E., Brans, K., Foldi, A., and Merckx, R.(1999). Cadmium fixation in soils measured by isotopic dilution. Soil Science Society of American Journal, 63(1), 78-85.
64. Sumner, J.B.(1935). A more specific reagent for the determination of sugar in urine,Journal of Biological Chemistry, 69, 393.
65. Thakur, A.K. and Singh, K.J.(2012). Leaf temperature as thermal as thermal bio-indicator of heavy metal pollutants, Journal of Agricultural Science and Technology A, 2(1), 131-137.
66. van de Mortel, J.E., Villanueva, L.A., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland, P.D., van Themaat, E.V.L., Koornneef, M., and Aarts, M.G.M.(2006). Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*, Genetics, Genomics, and Molecular Evolution, 142(3), 1127-1147.
67. Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E.R., Sharma, N.C., and Sahi, S.V.(2017). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: a physiochemical analysis, Plant Physiology and Biochemistry, 110, 59-69.
68. Verma, S. and Dubey, R.S.(2001). Effect of cadmium on soluble sugars and enzymes of their metabolism in rice, Biologia Plantarum, 44, 117-123.

69. Vijendra, P.D., Huchappa, K.M., Lingappa, R., Basappa, G., Jayanna, S.G., and Kumar, V.(2016). Physiological and biochemical changes in moth bean (*Vigna aconitifolia* L.) under cadmium stress, Journal of Botany, 13 pages.
70. Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., and Golezani, K.G.(2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.), Plant Omics Journal, 5(2), 60-67.
71. Wuana, R.A. and Okieimen, F.E.(2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Notices,
72. Yemm, E.W. and Willis, A.J.(1954). The estimation of carbohydrates in plant extracts by anthrone, Biochemical Journal, 57, 508-514.
73. Zaman, Q.U., Aslam, Z., Rashid, M., Khaliq, A., and Yaseen, M.(2018). Influence of zinc fertilization on morpho-physiological attributes, growth, productivity and hematic appraisal of paddy rice, The Journal of Animal and Plant Sciences, 28(3), 778-790.
74. Zoufan, P., Azad, Z., Ghahfarokhie, A.R., and Kolahi, M.(2020). Modification of oxidative stress through changes in some indicators related to phenolic metabolism in *Malva parviflora* exposed to cadmium, Ecotoxicology and Environmental Safety, 187, 109811.