International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(9),** 40-47, September **(2013)** *Int. Res. J. Biological Sci.*

Phytotoxic Effects of Cadmium on Seed Germination, Early Seedling Growth and Antioxidant Enzyme Activities in *Cucurbita maxima* Duchesne

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Available online at: www.isca.in
Received 24th May 2013, revised 6th June 2013, accepted 4th July 2013

Abstract

The environment is constantly being polluted by the accumulation of heavy metal contaminants. This is a matter of serious concern as it adversely affects the health of humans and animals. These contaminants also pose a major threat to Agricultural sector. Cadmium is an important heavy metal extensively used in electronic and other industries. The present investigation was conducted to determine the phytotoxic effect of heavy metal cadmium on seed germination, early seedling growth and antioxidant enzymes activity of Cucurbita maxima Duchesne. *The different dilutions of cadmium (25ppm; 50ppm; 100ppm; 250ppm and 500ppm) were used. Various parameters considered for the study includes percent seed germination, root and shoot elongation, seedling size, fresh and dry weight, root/shoot ratio, dry weight/fresh weight ratio, seedling vigor index, inhibition of seedling growth, tolerance index, activity of antioxidant enzymes like catalase, peroxidase and superoxide dismutase. All the tested concentrations were found inhibitory in action with respect to all the parameters, with the exception of dry weight/fresh weight ratio and activities of antioxidant enzymes which were found to gradually increase as the concentration of cadmium increased. However a slight decline in antioxidant enzyme activities was recorded due to acute cadmium stress at higher level (s). The intensity of inhibitory effect on all other parameters was directly proportional to the concentration of cadmium solution employed and inhibition was more prominent at 250ppm and 500ppm.*

Keywords: Cadmium toxicity, *Cucurbita maxima*, Seed germination, SVI and TI, Antioxidant enzymes.

Introduction

One of the main concerns of agricultural productions is the heavy metal pollutants. Industrialization has mainly resulted in the heavy metal contamination of agricultural soil. This poses a threat to primary and secondary consumers which finally affects the humans. The extent of metal toxicity and sensitivity are dependent on the concentration of metal pollutant, period of exposure and the various biological processes[1]. Some heavy metals are required for plant growth; however it becomes toxic to plants when the concentration is at higher level. It was reported that crop growth is affected when the concentration of the heavy metal solution is increased. The growth retardation may be attributed to the alterations in biochemical process like inhibition of protein penetration, enzyme activity and impaired nutrition[2].

In the recent past, extensive studies on heavy metals are being carried out throughout the world. There are reports that excess concentrations of some heavy metals in soil such as cadmium, chromium, copper, nickel and zinc have caused the disturbance of both natural aquatic and terrestrial ecosystems[3]. The toxic effects of heavy metal on the biological systems have been reported by various authors. Many plant species readily take up Cd^{2+}ions via the roots and it is transported to the leaves in the same way as the essential micronutrient metal ions[4], but Cd^{2+} are not essential for plant growth. The Cd^{2+} concentration is high in soil where Cd-containing fertilizers, sewage sludge and city waste are directly applied[5]. Leaf chlorosis, stunted growth and alteration in the activity of many key enzymes of various metabolic pathways are the prominent effects of Cd^{2+} toxicity in plants[6]. The presence of heavy metals in soil can function as stresses which causes physiological and biochemical constraints thereby decreasing plant vigor and inhibiting plant growth[7]. The aim of present study is to assess the effect of different concentrations of heavy metal cadmium (Cd^{2+}) on the seed germination percent, seedling growth and antioxidant enzymes activities in *Cucurbita maxima* Duchesne.

Material and Methods

Healthy seeds of *Cucurbita maxima* Duchesne were collected from Kerala Agricultural University, Vellayani, Thrissur in Kerala. Then seeds and germination trays were surface sterilized with 30% dilute solution of sodium-hypochlorite to prevent any fungal contamination. Filter papers were also sterilized in an autoclave to reduce the chances of fungal growth. Heavy metal test solutions were prepared from metal cadmium (Cd^{2+}) in five different concentrations of 25ppm, 50ppm, 100ppm, 250ppm and 500ppm. The chemical was of analytical reagent grade. Distilled water was used as control. After soaking the selected seeds in respective treatment solutions for 24 hours, seeds were subjected to standard germination test[8] using four replications of 30 seeds each. Seeds were placed on two sheets of Whatman No.1 filter paper contained in Trays (30x20cm size). Solutions of heavy metal (30 ml) and distilled water as control were

subsequently added to the corresponding treated seeds-containing Trays to wet the filter paper and to maintain the contaminated environment. Each treatment was replicated four times. The Trays containing treated seeds were incubated at room temperature of 28 ±2°C and the experiment lasted for 12 days. Germination was considered to have occurred when radicles were 2mm long. Germination percent was recorded every 24 h, till the end of the experiment. The following parameters were analyzed for the study.

i. Germination percent, ii. Root length, Shoot length and Seedling size, iii. Seedling fresh and dry biomass: Seedling fresh and dry biomass was measured by using electronic balance. Seedling dry biomass was determined by placing the seedling in an oven at 80°C for 24 hours, iv. Seedling vigour index (SVI)[9]: Vigour index= seedling length (cm) x germination percent, v. The tolerance index (TI)[10]: The tolerance index (TI) was calculated by dividing the root length at the different metal concentrations by that obtained in the control solution: TI (%) = (root length in metal treatment)/ (root length in the control) x 100 and vi. Estimation of antioxidant enzymes: The following antioxidant enzymes were estimated.

a) Assay of Superoxide dismutase (SOD)[11]: The sample (0.5gm), were ground with 3.0ml of potassium phosphate buffer and centrifuged at 2000g for 10 minutes and the supernatants were used for the assay. Assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of Nitroblue tetrazolium (NBT), 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2 ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

b) Assay of Catalase (CAT)[12]: 20% homogenate was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay. Phosphate buffer (3.0 ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

c) Assay of Peroxidase (POD)[13]: 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the various parts of the plant, clarified by centrifugation and the supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430nm..

Results and Discussion

Seed germination percent: The effect of different concentrations of cadmium (Cd^{2+}) on the germination percent of *Cucurbita maxima* is shown in the table 1. The percent seed germination was highest when seeds were treated and grown in distilled water as control (93.33%); however Cd^{2+} treatments reduced seed germination of *C. maxima* at different concentrations. The inhibition of seed germination increased with increasing concentration of Cd^{2+}, only with the exception of 25ppm of Cd^{2+} where seed germination percent was not affected when compared with control. The germination percent was remarkably reduced in 500ppm of Cd^{2+} over control (84%). The results of the present study clearly revealed that metal Cd^{2+} unfavorably influenced the seed germination in *C. maxima*. This observation is in conformity with the previous findings of Seyed *et al*[14], who reported that germination rate was decreased in Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and Safflower (*Carthamus tinctorious*) with increase in concentration of Cd^{2+}. Reduction in seed germination may be due to the interference and alterations in the cell membrane permeability properties by Cd^{2+}, which resulted in decreased water absorption and transport as well as lowered water stress tolerance[15].

Root length, Shoot length and Seedling size: The data presented in the table 1 indicates a decrease in trend with respect to root length, shoot length and seedling size of Cd^{2+} treated seedlings over control, as the concentration of Cd^{2+} increases. The decline in root length ranged from 7.01% (with 25ppm Cd^{2+}) to 83.09% (with 500ppm Cd^{2+}) whereas shoot length declined from 1.53% (with 25ppm Cd^{2+}) to 79.13% (with 500ppm Cd^{2+}) at the end of experiment. Similarly seedling size declined from 3.96% (with 25ppm Cd^{2+}) to 80.95% (with 500ppm Cd^{2+}). The decrease in growth performance of *C. maxima* may be attributed to the reduction in meristamatic cells and the inhibitory effect on the activities of hydrolytic enzymes contained in the cotyledon and endosperms due to the toxicity of Cd^{2+}. Hydrolytic enzymes are essential for the mobilization of stored food materials to reach the radicle and plumule for favoring seedling growth[16]. The reduction in growth parameters of *C. maxima* can also be because of accelerated breakdown of stored food materials in seed by the application of cadmium[17].

Seedling fresh weight and dry weight: Effect of different concentrations of metal Cd^{2+} on the fresh weight and dry weight of germinated seedlings of *C. maxima* is shown in table 2.

Table- 1
Effect of different concentrations of Cadmium on Seed germination, Root and Shoot length and Seedling size of *Cucurbita maxima*

Treatment Cadmium (Cd) ppm	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling size (cm)
25	93.33±4.62	11.93±2.18	15.43±1.25	27.37±2.57
50	89.33±2.31	11.33±0.76	14.8±2.43	26.13±2.76
100	76.67±7.02	10.17±2.02	13.9±1.64	24.07±1.29
250	49.33±6.11	5.33±0.91	7.33±1.93	12.67±2.06
500	9.33±2.31	2.17±0.15	3.27±1.09	5.43±0.93
Control	93.33±2.31	12.83±2.08	15.67±1.62	28.5±2.17

Each data presented is an average of four replicates

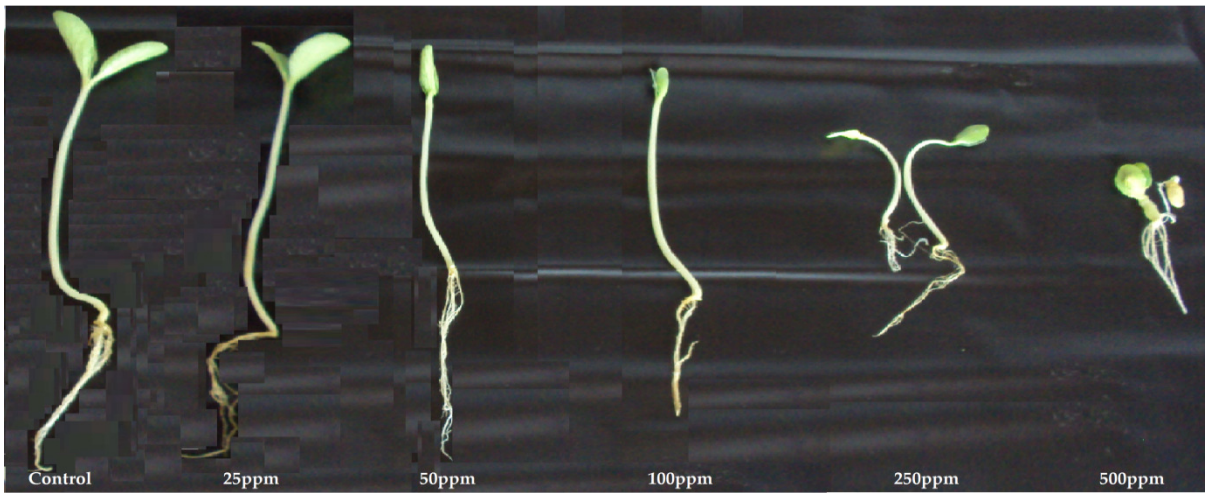

Figure- 1
Effect of different concentrations of Cadmium on the growth and vigor of germinated seedlings of *Cucurbita maxima* **in lab conditions**

Table- 2
Effect of different concentrations of Cadmium on Seedling fresh and dry weight of *Cucurbita maxima*

Treatment Cadmium (Cd) ppm	Seedling fresh weight (mg)	Seedling dry weight (mg)
25	2.167±0.19	0.103±0.01
50	2.04±0.16	0.098±0.01
100	1.827±0.15	0.090±0.01
250	0.783±0.13	0.040±0.01
500	0.350±0.05	0.019±0.003
Control	2.287±0.13	0.109±0.01

Each data presented is an average of four replicates.

Seedling fresh weight and dry weight were highest when seeds were treated and grown in distilled water as control; however Cd^{2+} treatments reduced fresh weight and dry weight of *C. maxima* different concentrations. The reduction was increased with increasing concentration of Cd^{2+}, though to a different extent. The reduction ranged from 5.25% (with 25ppm Cd^{2+}) to 84.7% (with 500ppm Cd^{2+}) with respect to the fresh weight and similarly, the dry weight ranged from 5.50% (with 25ppm Cd^{2+}) to 82.57% (with 500ppm Cd^{2+}), over control. The present results showed that the reduction in fresh and dry weight of *C. maxima* is not much affected at lower level Cd^{2+} concentrations, however it considerably reduced as the level of Cd^{2+} concentration increased further (250ppm to 500ppm) and it may be the result of inhibition of chlorophyll synthesis and photosynthesis[18]. The present observations are in conformity with the reports of Kabir *et al*.[1] on *Thespesia populnea*.

Root/Shoot length ratio and Root/Shoot weight ratio: The effects of Cd^{2+} on the root growth of *C. maxima* seedlings were different as compared to the effects on shoot growth. This is clear and evident from the data presented in table 3. Generally R/S length ratio and R/S weight ratio of seedlings are seen to be reducing as the concentration of the Cd^{2+} is increasing when compared to control. The study therefore indicates that the impact of Cd^{2+} is higher on the root growth of *C. maxima* compared to shoot growth. This may be due to the fact that roots are the primary plant organs in direct contact with heavy metal pollutants in the growing media and accumulate metal at a faster and higher rate than shoot or it may be due to the faster

rate of detoxification in shoot than root. The present observations are in agreement with the reports on cadmium induced root growth inhibition in *Brassica juncea* seedlings[19].

Table- 3
Effect of different concentrations of Cadmium on R/S length ratio, R/S weight ratio and Dw/Fw ratio of *Cucurbita maxima*

Treatment Cadmium (Cd) ppm	Root/Shoot length ratio (%)	Root/Shoot weight ratio (%)	Dry wit/Fresh wit ratio (%)
25	77.61±5.59	13.55±1.89	4.77±0.01
50	77.94±7.07	13.21±2.20	4.78±0.02
100	74.62±5.99	12.05±0.40	4.91±0.01
250	72.81±4.59	11.73±1.87	5.10±0.03
500	71.68±6.53	8.19±0.98	5.33±0.05
Control	82.87±7.51	15.51±0.87	4.75±0.01

Each data is an average of four replicates.

Seedling Dry weight/Fresh weight ratio: Data given in table 3 indicates that seedling dry weight/fresh weight ratio of Cd^{2+} treated seedlings tended to increase as the concentration of Cd^{2+} increase compared to control seedlings. The improvement ranges from 0.42% with 25ppm Cd^{2+} treatment to 12.21% with 500ppm Cd^{2+} treatment over control. Therefore the study reveals a positive correlation between concentration of Cd^{2+} treatment and dry weight/fresh weight ratio of germinated seedlings. This correlation may be attributed to the enhanced growth performance of germinated seedling in lower concentrations of Cd^{2+} compared to the higher concentration, at the expense of more reserve food and subsequent decrease in dry weight and similarly decrease of growth in higher concentration due to lesser availability of reserve food and subsequent increase in dry weight[20]. The correlation may also be attributed to the interference and alterations in the cell membrane permeability properties by Cd^{2+} and resultant poor moisture content inside the cells of seedlings with poor growth performance compared to seedlings with better growth performance.

Seedling vigour index (SVI): Seedling vigor index (SVI) is the potential of seed germination and seedling size against the toxicity and tolerance of metals. Results indicate a decreasing trend of SVI in seedlings obtained through different concentrations of Cd^{2+} treatment, when compared with control. The deduction in SVI was more prominent at 250ppm and 500ppm and is 76.81% and 98.04% respectively over control. Reduced seedling vigor index possessed by *C. maxima* with increasing concentration of Cd^{2+} is probably due to less tolerance. Retardation in root growth due to toxic concentrations of cadmium may be a prominent reason for reduction in SVI and this might be due to inhibition of cell division induced by chromosomal aberrations[21].

Tolerance index (TI): The tolerance of germinated seedlings of *C. maxima* was analyzed using different concentrations of heavy metal cadmium. Data given in table 4 shows all concentrations of Cd^{2+} adversely affected the tolerance index. Highest percentage of tolerance was recorded at 25ppm (94.07%) of Cd^{2+} whereas the lowest tolerance index was recorded in 500ppm with an average of 17.21%, when compared with control. Higher rate of metal uptake by roots and its translocation to shoots might be the cause of reduction in seedling growth and biomass production and therefore the potential of root growth have been proven to be an index of metal tolerance in plants[10]. In the present investigation, root component of the *C. maxima* was adversely affected more than the shoot component as the toxicity of cadmium is increased. This may be the reason why the tolerance index of *C. maxima* is decreased drastically at higher Cd^{2+} concentrations of 250ppm and 500ppm. This inference is in agreement with the findings of Shafiq *et al*.[16] in *Leucaena leucocephala* seedlings.

Table- 4
Effect of different concentrations of Cadmium on Seedling vigour index and Tolerance index of *Cucurbita maxima*

Treatment Cadmium (Cd) ppm	Seedling vigour index (SVI)	Tolerance index (TI)
25	2557.1±147.41	94.07±8.81
50	2336.0±183.89	89.63±9.47
100	1849.7±109.97	82.41±15.10
250	616.53±27	43.18±6.99
500	52.13±8.02	17.21±3.28
Control	2658.4±178	100.0

Each data is an average of four replicates.

Antioxidant enzyme activity (CAT, POD and SOD): Heavy metals are known to generate toxic reactive oxygen species (ROS) such as H_2O_2, O_2^-, OH^-, OH_2, etc. which degrade important cellular components by inducing oxidative stress[22].The tolerance to damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify reactive oxygen species[23]. But when plants are exposed to environmental stresses, oxidative damages may happen due to the imbalances between the productions of reactive oxygen species (ROS) and their detoxification by antioxidative system[24]. The activity of antioxidant enzymes catalase (CAT), peroxidase (POD) and super oxide dismutase (SOD) was assessed in germinated *C. maxima* seedling under the effect of different concentrations of Cd^{+2} and the results are given in table 5. The results of the present study showed all the tested concentrations of Cd^{2+} caused oxidative stress in *C. maxima*. As Cd^{+2} concentrations increased, SOD, CAT and POD activity enhanced progressively, however in 500ppm Cd^{2+} treatment, SOD and CAT activity slightly decreased whereas decrease was recorded from 100ppm onwards with respect to POD activity. This result was in agreement with the trend observed in *Carthamus tinctorius* L. in respose to cadmium toxicity[25]. The increase in antioxidant enzyme activity in lower concentrations of Cd^{2+} in *C. maxima* may be attributed to the increased expression of the genes encoding antioxidant enzymes in

response to increased production of reactive oxygen species[26]. The decline in antioxidant enzyme activity at higher Cd^{2+} concentration(s) may result from the inactivation of the enzyme by H_2O_2, which is produced in different cellular compartments or from a number of non-enzymatic and enzymatic processes in cells[27].

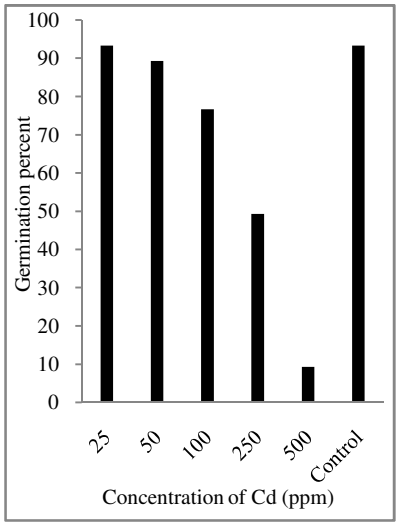

Figure- 2
Effect of Cd^{+2} on germination percent of *C. maxima*

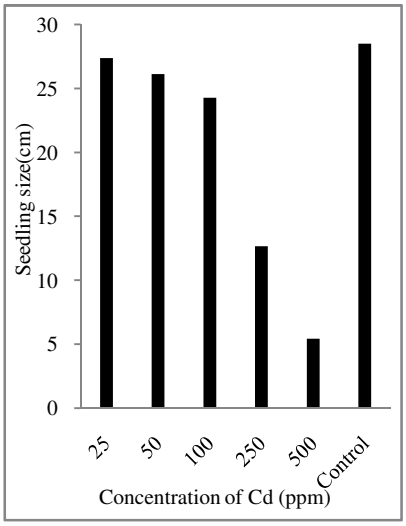

Figure- 3
Effect of Cd^{+2} on seedling size of *C. maxima*

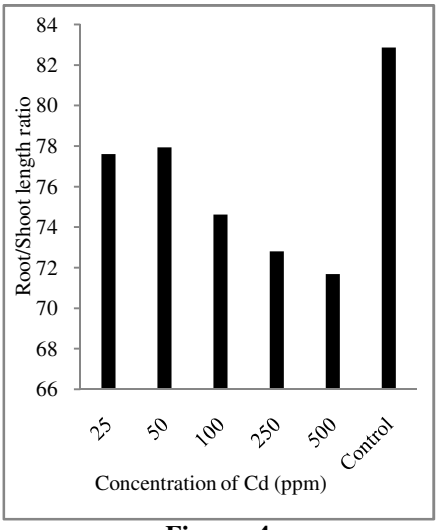

Figure- 4
Effect of Cd^{+2} on root/shoot length ratio of *C. maxima*

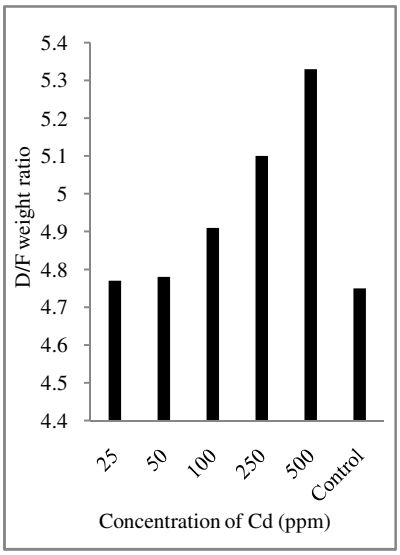

Figure- 5
Effect of Cd^{+2} on dry/fresh weight ratio of *C. maxima*

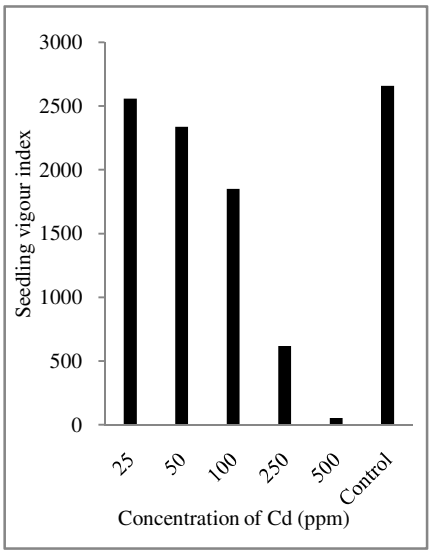

Figure- 6
Effect of Cd^{+2} on seedling vigour index of *C. maxima*

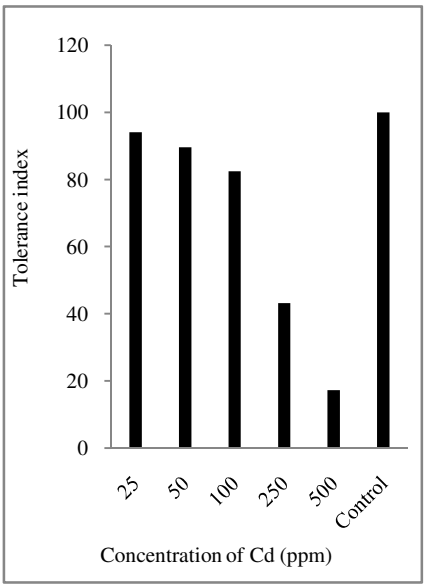

Figure- 7
Effect of Cd^{+2} on tolerance index of *C. maxima*

Table- 5
Effect of different concentrations of cadmium on Antioxidant enzyme activities in *Cucurbita maxima*

Treatment Cadmium (Cd) ppm	SOD activity (% inhibition of NBT)	CAT activity (U)	POD activity (U)
25	38.4±1.21	0.192±0.01	0.46±0.01
50	41.8±2.26	0.223±0.004	0.49±0.03
100	45.6±1.97	0.235±0.004	0.35±0.03
250	49.7±3.1	0.398±0.01	0.25±0.02
500	47.3±1.4	0.366±0.004	0.20±0.01
Control	35.9±0.44	0.166±0.004	0.28±0.01

Each data is an average of four replicates

The inference in the present study suggest, reactive oxygen species (ROS) could be induced by phytotoxic concentrations of Cd^{+2} leading to increased SOD, CAT and POD activities which play a crucial role in detoxification of elevated concentrations of Cd^{+2} possibly via lignifications and physical barrier formation[28]. The declining activity of antioxidant enzymes at higher concentration(s) might be the result of the acute toxic effect which resulted from lipid peroxidation and cell damage, as reflected by the drastic reduction in vigor when compared with control[29].

Conclusion

One of the major problems affecting the seed germination and productivity of plants is heavy metal stress. Cadmium concentration gets accumulated in agriculturally important crops which are an essential constituent of the food chain, which can significantly impair animal and human health. Threshold values for the inhibition of seed germination and growth of germinated seedlings by heavy metal Cd^{+2} has been reported for survival plants as species dependent. The investigation result clearly reveals Cd^{+2} are inhibitory with respect to seed germination and early seedling growth in *Cucurbita maxima* Duchesne. The intensity of inhibition was directly proportional to the concentration of Cd^{+2} solutions employed. High concentrations of Cd^{+2} treatment is found responsible for decreasing the percentage of tolerance indices in *C. maxima* and that was clearly evident from the inhibition of seedling growth and vigor. Cd^{+2} uptakes by the roots and their translocation to shoots at higher concentration might be the cause of drastic reduction in seedling growth and biomass production. Result of the present study also indicates that cadmium toxicity caused an enhancement in the production and activity of antioxidant enzymes compared to healthy control seedlings, which clearly indicates that seedling growth has been unfavorably affected by the stress due to cadmium toxicity.

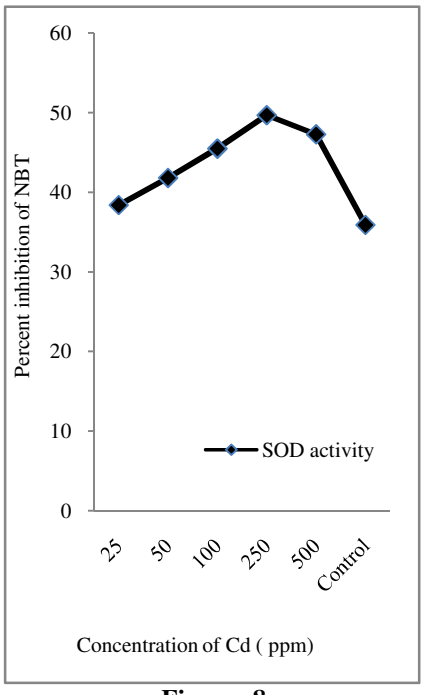

Figure- 8
Effect of Cd^{+2} on SOD activities of *C. maxima*

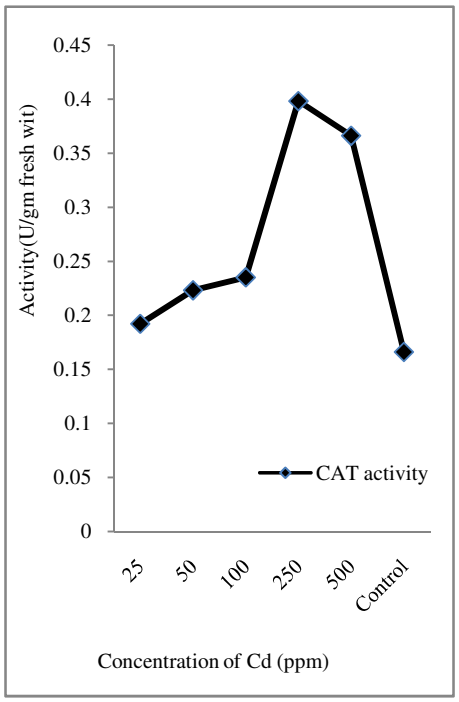

Figure-. 9
Effect of Cd^{+2} on CAT activities of *C. maxima*

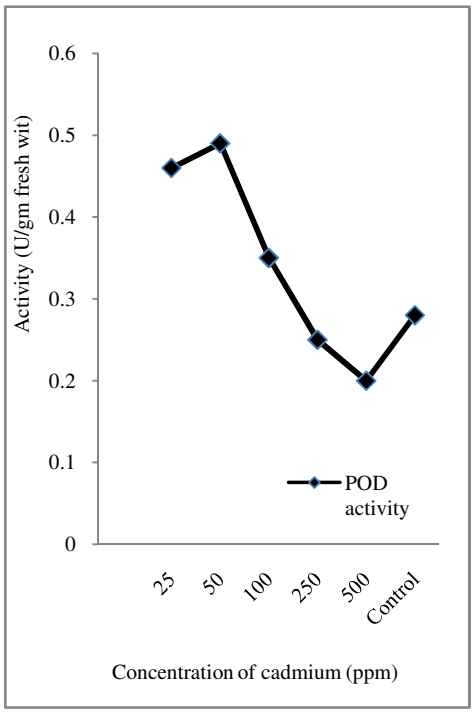

Figure- 10
Effect of Cd^{+2} on POD activities of *C. maxima*

References

1. Kabir M., Iqbal M.Z., Shafiq M. and Farooqi Z.R., Reduction in germination and seedling growth of *Thespesiapopulnea* L., caused by lead and cadmium treatments, *Pak. J. Bot*., **40(6),** 2419-2426 **(2008)**

2. Arun K.S., Cervantes C., Loza-Tavera H. and Avudainayagam S., Chromium toxicity in plants, *Environ. Int*., **31,** 739-753 **(2005)**

3. Gardea-Torresdey J.L., Polette L., Arteaga S., Tiemann K.J., Bibb J. and Gonzalez J.H., Determination of the content of hazardous heavy metals on *Larrea tridentata* grown around a contaminated area. *Proceedings of the Eleventh Annual EPA Conf. On Hazardous Waste Research*, Edited by L.R. Erickson, D.L. Tillison S.C. Grant and J.P. McDonald, Albuquerque, NM, 660 **(1996)**

4. Patra M., Bhowmik N., Bandopadhyay B. and Sharma A., Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance, *Environmental and Experimental Botany,* **52(3),** 199-223 **(2004)**

5. Williams C.H. and David D.J., The effect of superphosphate on the cadmium content of soils and plants, *Aust. J. Soil Res.*, **11,** 43-56 **(1973)**

6. Arduini I., Godbold D.L. and Onnis A., Cadmium and copper uptake and distribution in Mediterranean tree seedlings, *Physiol. Plant*., **97,** 111-117 **(1996)**

7. Wu L. and Lin S.L., Copper tolerance and copper uptake of Lotus purshianus (Benth.) and its symbiotic *Rhizobium loti* from a copper mine waste population, *New Phytol.*, **11,** 531-539 **(1990)**

8. ISTA., International Rules for Seed Testing, Rules 1996, International Seed Testing Association (ISTA), *Seed Science and Technology*, 24 (supplement). Zurich, Switzerland **(1996)**

9. Moradi D., Sharifzadeh P. F. and Janmohammadi M., influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L.), *ARPN Journal of Agricultural and Biological Science*, **3,** 22-25 **(2008)**

10. Wilkins D.A., The measurement of tolerance to edaphic factors by means of root growth, *New Phytol.,* **80,** 623–633 **(1978)**

11. Kakkar P., Das B. and Viswanathan P.W., Modified spectrophotometric assay of superoxide dismutase, *Indian Journal of Biochemical Biophysics,* **2**, 130–132 **(1984)**

12. Luck H., Estimation of catalase. In: Methods in Enzymatic Analysis (Eds), Luck. H. Bergmeyer Academic Press, New York, 885 **(1974)**

13. Reddy K.P., Subhani S.M., Khan P.A. and Kumar K.B., Effect of light and benzyl adenine on dark-treated growing rice leaves, II changes in peroxidase activity, *Plant cell Physiol.*, **24,** 987-994 **(1995)**

14. Seyed Amir Moosavi1, Mohammad Hossain Gharineh1, Reza Tavakkol Afshari. and Amin Ebrahimi, Effects of Some Heavy Metals on Seed Germination Characteristics of Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and Safflower (*Carthamus tinctorious*) to Evaluate Phytoremediation Potential of These Crops, *Journal of Agricultural Science*, **4,** 9 **(2012)**

15. Breckle S.W. and Kahle H., Effect of toxic heavy metals (Cd, Ph) on growth and mineral nutrition of beech (*Fagus sylvatica* L.), *Vegetation*, **101,** 43-53 **(1992)**

16. Shafiq M., Zafar I.M. and Athar M., Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*, *J. Appl. Sci. Environ. Manage*, **12,** 61- 66 **(2008)**

17. Mostafa Heidari and Semin Sarani, Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of Mustard (*Sinapis arvensis* L.), *ARPN Journal of Agricultural and Biological Science*, **6(1),** 44-47 **(2011)**

18. Padmaja K., Prasad D.D.K. and Prasad A.R.K., Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* Seedlings by cadmium acetate, *Photosynthetica*, **24,** 399-405 **(1990)**

19. John R., Ahmad P., Gadgila K. and Sharma S., Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L., *International Journal of Plant Production,* 3 (3) (2009)

20. Salisbury F.B. and Ross C.W., Plant Physiology, CBS Publishers and distributors, Delhi, India **(1986)**

21. Liu D.H., Jaing W.S. and Li M.X., Effect of chromium on root growth and cell division of *Allium cepa*, *Israel J. Plant Sci*., **42,** 235-243 **(1993)**

22. Panda S.K., The biology of oxidative stress in green cells: A Review. In: Panda S.K. (ed), *Advances of stress physiology of plants*, 1-13, Scientific Publishers, Jhodpur, India **(2002)**

23. Smirnoff N., The role of active oxygen in the response of plants to water deficit and desiccation, *New Phytol*, **125,** 27-58 **(1993)**

24. Gomez J.M., Hernandez J.A., Jimenez A., Del Rio L.A. and Sevilla R., Differential responses of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants, *Radic. Res*, **31,** 11-18 **(1999)**

25. Shahram Namjooyan, Ramezanali Khavari-Nejad, Franceous Bernard, Shahrokh Namdjoyan and Hamed Piri, The effect of cadmium on growth and antioxidant responses in the saffl ower (*Carthamus tinctorius* L.) callus, *Turk J Agric For.*, **36,** 145-152 **(2012)**

26. Mishra S., Srivastava S., Tripathi R.D., Govindarajan R., Kuriakose S.V. and Prasad M.N.V., Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L., *Plant Physiol Biochem*., **44,** 25-37 **(2006)**

27. Romero-Puertas M.C., Corpas F.J., Rodriguez-Serrano M., Gomez L.A., del Rio L.A. and Sandalio L.M., Diff erential expression and regulation of antioxidative enzymes by cadmium in pea plants, *J Plant Physiol,* **164,** 1346-1357 **(2007)**

28. Saffar A., Bagherieh Najjar M.B. and Mianabadi M., Activity of Antioxidant Enzymes in Response to Cadmium in *Arabidopsis thaliana, Journal of Biological Sciences,* **9,** 44-50 **(2009)**

29. Sai Kachout S., Ben Mansoura A., Leclerc J.C., Jaffel K., Rejeb M.N. and Ouerghi Z., Effects of heavy metals on antioxidant activities of *Atriplex hortensis and Atriplex rosea*, *Journal of Applied Botany and Food Quality*, **83,** 37-43 **(2009)**