Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 15 (2020) 49-59

Environment & We An International Journal of Science & Technology

Effect of Cadmium stress on Growth and Development of Cicer arietinum (Fabaceae)

Pooja Baweja*[1], Dharmendra Kumar[2], Vanshika Maheshwari[1], Muskan Singla[1], Ritu[1], Parul[1], Nisha Jariyal[1], Akshita[1]

[1]Department of Botany, Maitreyi College, University of Delhi, Delhi – 110021
[2]Marine Biotechnology Laboratory, Department of Botany, University of Delhi, Delhi – 110007
*Email Id: poojabaweja@gmail.com

Article history:
Received 29 April 2019
Received in revised form
07 January 2020
Accepted 07 January 2020
Available online
10 January 2020

Keywords:
Cadmium;
Morphology;
Growth;
Heavy Metals;
Stress;

Abstract

Heavy metals are major environmental pollutants, which are being accumulated in soil due to various anthropogenic activities, posing a greater risk to plants, animals and humans. When the soil is contaminated with heavy metals, plants take up them through root system. Later, these heavy metals get accumulated in plants affecting physiological and metabolic processes, leading to agricultural loss. In the present study, one of the heavy metal i.e. cadmium is selected to study its effect on *Cicer arietinum* (Chick Pea). Different concentrations of cadmium is used i.e. 25 µg/ml, 75 µg/ml, and 150 µg/ml to study its effect on various morphological & biochemical parameters such as % seed germination, number of leaves, number of branches, root length, shoot length, cotyledons, chlorophyll pigments, total proteins, phenols, lipids, and carbohydrates at 15th day of growth. A significant difference has been observed in most of the cadmium treated plants, which are discussed in the research article

Introduction

Anthropogenic activities such as industrial, domestic, agricultural and technological applications have led to the addition of various heavy metals in the environment. These heavy metals have potential effects on human health and environment. Although, plants require certain heavy metals for their growth and development, the excessive amount of these metals can become toxic and can have adverse effect on growth, development and activities of soil microorganisms and plants. Cadmium, a heavy metal, is distributed in the earth's crust at an average concentration of about 0.1mg/kg and its highest concentrations are found in sedimentary rocks and marine phosphates, which contain about 15mg cadmium per kg. It is a non-essential element that negatively affects plant growth and development. It can alter the uptake of minerals by plant through its effect on the availability of minerals from the soil, or through a

reduction in the population of soil microbes. Stomatal opening, transpiration and photosynthesis have been reported to be affected due to high levels of cadmium in soil. The metal is being taken up by roots more readily from nutrient solutions than from the soil directly and afterwards, it is transported to various tissues to finally get accumulated in roots, shoots and fruits (Wang *et al*., 2009). In plants, chlorosis, leaf role, necrosis, stunted growth are some of the major easily visible symptoms of cadmium toxicity (Liu *et al*., 2014). These negative effects of cadmium on plants may be associated with that cadmium interferes with several physiological processes such as imbalance of mineral nutrients (Gouia *et al*., 2000), variation of many enzyme activities (Hasan, 2009), and modification of gene expressions (Herbette *et al*., 2006; Liu *et al*., 2011).

*Cicer arietinum*_commonly known as chickpea or Bengal gram is a genus of the legume family Fabaceae. The plant grows to 20–50 cm (8–20 in) height and leaves are small feathery growing on either side of the stem, flowers are white, with blue, violet, or pink veins. Pod is with two or three seeds. India is world leader in chickpea (Bengal gram) production and the total production of India is approximately 15 times in comparison to the second-largest producer, Australia. Other significant producers are Burma, Ethiopia, Iran, Pakistan and Turkey. It is economically very important and is being consumed both by humans as well as animals in various forms.

Materials and Methods

Germination of seedlings: For the germination seeds of *Cicer arietinum* were surface sterilized with 70% ethanol for 30s and rinsed several times with distilled water. The test chemical cadmium sulphate ($CdSO_4^{2-}$) was procured from Sigma-Aldrich, UK. Different concentrations of $CdSO_4^{2-}$ solution (25 µg/ml; 75 µg/ml and 150 µg/ml) were prepared in double distilled water. For all the concentrations of cadmium three replicates of petri plates (Borosil) layered with cotton were prepared with 50 seeds each. One set of control was also prepared in which tap water was used. After every 24 hrs the seeds were sprinkled with their respective test solution to avoid dehydration. The seeds were allowed to germinate for 15 days.

Morphological Parameters: After 15 days the seedlings were analysed for various morphological parameters such as % seed germination, number of leaves, number of branches, root length, shoot length, and length of cotyledons. After 15 days seedlings were preserved at -20^0C for further biochemical studies.

Biochemical Parameters: Total amount of chl. a, and b were estimated as per Hiscox and Israelstam (1979) method. Carotenoids were calculated using Duxbury and Yentsch (1956) method. Total carbohydrates, soluble and insoluble carbohydrates were determined by Phenol–Sulphuric acid method (Dubois *et al*., 1956). Glucose (Sigma) was used for making standard curve. The optical density was measured at 490nm using Uv – Vis spectrophotometer (Shimadzu). Total proteins were estimated by using Bradford's

(1976) method using Bovine Serum Albumin (BSA) as standard. The optical density was measured at 595nm using Uv –Vis spectrophotometer (Shimadzu). Total lipids were estimated gravimetrically as per Folch *et al*., 1957 method. Total phenols were estimated as per Malik and Singh (1980) using FCR reagent as a blank. Standard curve was prepared by using pyro-catechol 10-100 µg/ml. The optical density was measured at 650nm using Uv –Vis spectrophotometer (Shimadzu). FCR reagent was used as a blank. Standard curve was prepared by using pyro-catechol 10-100 µg/ml.

Statistical Analysis: The relationship between various parameters, different parts of *Cicer* seedlings and concentration of cadmium were determined by Pearson correlation coefficient. To check the difference in the mean value for various parameters against different concentrations were determined by calculating t- value (student's t test). Mean and standard deviation (SD) were obtained by descriptive statistics. All the values were calculated using MS Office (Excel) 2010.

Result and Discussion

Contamination of soil with heavy metals causes degradation of cultivable lands, there by resulting in reduced growth and yield of plants growing in those regions. Increasing evidences, that excessive occurrence of heavy metals is associated with humans and animal diseases have emphasized the need for a better knowledge of the way plants respond to heavy metal stress. The salient findings obtained during present investigations are discussed in the following paragraphs. During present investigation, it has been found that cadmium, being the common heavy metal pollutant, readily absorbed and translocated in *Cicer arietinum* and thus brings about various metabolic effects. Toxic cadmium inhibits plant growth but lower dose may have sometimes stimulatory effect.

Seed germination and morphological parameters: Maximum seed germination of 100% was observed in control followed by seeds treated with 25 µg/ml, 75 µg/ml and 150 µg/ml of cadmium concentration respectively. Number of leaves were found to be maximum (4±0.5) under controlled conditions and cadmium (25 µg/ml) treated seeds (4±0), followed by seeds treated with 75 µg/ml (3±0.89) and 150 µg/ml (3±0.83) of cadmium concentrations. No significant difference is found in the number of leaves in control and the cadmium treated seedlings (p=0.05). Similarly, the number of branches was also found to be maximum in control seedlings (4±1), followed by treated ones. Amongst treated seedlings the maximum branches were present in seedlings treated with cadmium concentration (25 µg/ml) and it decreased as the concentration increased. The significant difference in number of branches was observed in seedlings treated with 75 µg/ml and 150 µg/ml cadmium concentration. Also, the root length was found to be maximum in control plants (23.34$\pm$3.42 cm) and amongst the treated plants, the maximum mean value is at 25 µg/ml of cadmium concentration. Roots in all the treated seedlings were found to be significantly different from control plants. Shoot length is

found to be almost equal in the control (6.66±1.58 cm) and the seedlings treated with 75 µg/ml (6.68±1.07cm) cadmium. At 25 µg/ml and 150 µg/ml concentration the shoot length was found to be 6.18±0.69, 5.6±1.0 cm respectively. Maximum length of cotyledons is found to be in seedlings treated with 25 µg/ml with the value of 0.92 cm. It is found be equal in the control and seedlings treated with 75 µg/ml of cadmium i.e. 7.8 cm (Table 1; Figure 1).

TABLE 1: Comparative account of different morphological parameters of *C. arietinum* at 15 days of treatment with different concentrations of $CdSO_4^{2-}$ (Data:mean ± S.D., *n*= 5; $^{(*)}$ = significant value in relation to control at p = 0.05).

	Control	**25µg/ml**	**75 µg/ml**	**150 µg/ml**
% Seed germination	100%	98%	95%	90%
Number of leaves	4 ± 0.54	4 ± 0	3 ± 0.89	3 ± 0.83
Number of branches	4 ± 1	3 ± 0.44	2.0 ± 0.54$^{(*)}$	2.0 ± 0.44$^{(*)}$
Root length (cm)	23.34 ± 3.42	10.3 ± 1.34$^{(*)}$	6.92 ± 3.38$^{(*)}$	2.58 ± 0.90$^{(*)}$
Shoot length (cm)	6.66 ± 1.58	6.18 ± 0.69$^{(*)}$	6.68 ± 1.07	5.6 ± 0.21
Root:shoot ratio	3.50 ± 2.16	1.66 ± 1.94	1.03 ± 3.15	0.46 ± 4.48
Length of cotyledons (cm)	0.78 ± 0.08	0.92 ± 0.16	0.78 ± 0.08	0.86 ± 0.05

In the present study, the heavy metal toxicity can be visualized through seed germination as well in various morphological parameters. It has been observed in present study that there is decrease in percentage of seed germination, root length, shoot length, root:shoot ratio and cotyledon length with increase in cadmium concentration. Less inhibition of seed germination and other parameters shows that the lower concentration (25 µg/ml) is with-in the tolerable range of *C. arietinum*. This decrease can be attributed to the reduction in O_2 uptake and accelerated breakdown of stored food in seed embryo due to physiological disturbances (Heideri and Sarani, 2011). Similarly, Chugh and Sawhney (1999) reported reduced seed germination in pea at 0.5 mM concentration of cadmium. Reduction in plant growth may partially be due to damage to root system and may also be due to denaturation of some hydrolytic enzymes in the cell which play important role in nutrient transport to primary root and shoot. Cadmium is more toxic to roots as it accumulates in roots retarding cell division and elongation. (Mondal *et al*., 2013). The reduced shoot length at higher doses may be due to inhibition of cell division or cell elongation. The retarded roots also transport less nutrients and water to the shoots. Therefore, the reduction in plant growth is also due to decrease in water content due to less water absorption and transport leading to lower water stress tolerance (Barcelo and Poschenrieder, 1990) or it may also be due to decreased carbohydrate formation following an inhibited photosynthesis and CO_2 assimilation. Reduction in shoot and root dimensions under cadmium stress may involve suppression of growth of the component cell. The high concentrations of heavy metals disturb the kinetic and thermodynamic balance in the cell leading to toxic effect and reduced growth. Cadmium causes decrease in Ca concentration in roots and as calcium is needed for growth and thus cadmium may indirectly affects root growth. At various cadmium treatments, the inhibition of root

growth may be due to inhibition of mitosis and reduced synthesis of cell components also (Heideri and Sarani, 2011). At higher concentrations also the browning of cotyledons, shoots and roots is also observed which may be due to damage to cell and cell wall components, changes in metabolic rates and suberin depositions (Punz and Sieghardt, 1993).

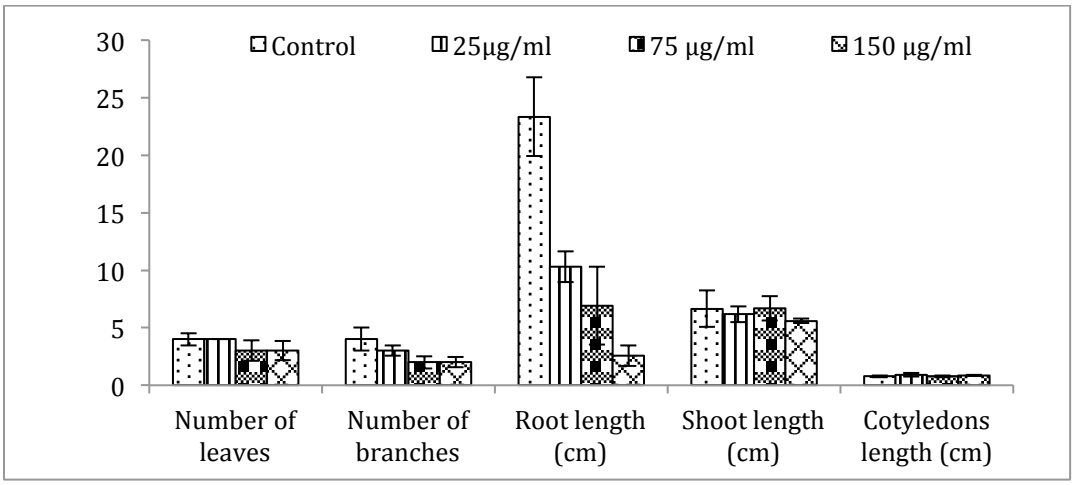

Figure 1: Impact of cadmium ($CdSO_4^{2-}$) on different morphological parameters at 15 days of treatment of *C. arietinum*. Data (mean ± S.E., *n*= 5)

Biochemical parameters:

The photosynthetic activity is suppressed by heavy metals due to disruptive chlorophyll synthesis, photosynthetic activity and enzymes. In the present study, chlorophyll a is maximum in control plants. The treated seedlings show a decline in the chl a content with the variation of 0.51±0.02 mg/g, 0.28±0.02 mg/g, 0.22±0.02 mg/g at the level of 25 µg/ml, 75 µg/ml and 150 µg/ml cadmium respectively. The variation in chlorophyll b is found to be 0.76±0.07 mg/g, 0.73±0.01 mg/g, 0.51±0.01 mg/g, 0.37±0.02 mg/g in the control, 25 µg/ml, 75 µg/ml and 150 µg/ml cadmium concentrations respectively. Similar trend is found in the concentration of carotenoids with maximum values in control seedlings and minimum in seedlings treated with 150 µg/ml cadmium (Table 2; Figure 2). The decline in chlorophyll and carotenoid content in all the seedlings with cadmium treatment is found to be significant at p=0.05 in comparison to control.

Heavy metal decreases the total chlorophyll, chlorophyll a and chlorophyll b in higher plants. Cadmium interferes with sulphydryl site on the reductase protein during chlorophyll biosynthesis and also destruction of photosynthetic apparatus (Krupa *et al*., 1993). This inhibition is due to the reaction of cadmium with essential groups in both the photo chlorophyll reductase protein and enzymes involved in the light dependent

synthesis of 8- amino laevolinic acid. Cadmium induced decline in chlorophyll content may also be due to disturbance in integration of chlorophyll molecules into stable complexes. In the present experiment, *Cicer arietinum* seedlings, exposed to different concentration of treatments showed reduced level of chlorophyll as compared to control seedlings, such changes may be accounted by decreased Fe content of leaves or by impairment of root transport of Fe (Brown *et al*., 1960). The level of carotenoids also decreases in cadmium treated plants (Mehindiratta *et al*., 1999; Marquez-Garcia *et al*., 2011). Under cadmium stress the degradation of various photosynthetic pigments is due to the damage of PS II reaction center (Deniz *et al*., 2007; Li *et al*., 2008).

TABLE 2: Impact of cadmium ($CdSO_4^{2-}$) on different biochemical parameters of *C. arietinum* (Data:mean ± S.D., *n*= 5; $^{(*)}$ = significant value in relation to control at p = 0.05).

		Control	**25 µg/ml**	**75 µg/ml**	**150 µg/m**
Chlorophyll a (mg/g)	**Leaves**	0.88± 0.02	0.51± 0.02$^{(*)}$	0.28± 0.02$^{(*)}$	0.22± 0.02$^{(*)}$
Chlorophyll b (mg/g)	**Leaves**	0.76± 0.07	0.73± 0.01$^{(*)}$	0.51± 0.01$^{(*)}$	0.31± 0.02$^{(*)}$
Carotenoids (mg/g)	**Leaves**	0.81± 0.02	0.34± 0.02$^{(*)}$	0.42± 0.05$^{(*)}$	0.42± 0.005$^{(*)}$
Soluble carbohydrates (µg/ml)	**Roots**	24.36± 0.08	22.11± 0.03$^{(*)}$	13.56± 0.04$^{(*)}$	13.47± 0.15$^{(*)}$
	Shoots	29.27± 0.24	19.68± 0.32$^{(*)}$	17.35± 0.14$^{(*)}$	16.72± 0.19$^{(*)}$
	Leaves	10.45± 0.02	9.67± 0.05$^{(*)}$	8.35± 0.03$^{(*)}$	7.55±0.35$^{(*)}$
Insoluble carbohydrates (µg/ml)	**Roots**	34.15± 0.04	20.34±0.10$^{(*)}$	13.21±0.02$^{(*)}$	4.623±0.12$^{(*)}$
	Shoots	13.13± 0.02	10.6±0.12$^{(*)}$	10.17±0.03$^{(*)}$	6.64±0.32$^{(*)}$
	Leaves	4.35± 0.07	3.4±0.10$^{(*)}$	3.25±0.16$^{(*)}$	2.8±0.22$^{(*)}$
Total carbohydrates (µg/ml)	**Roots**	58.51± 0.17	42.45±0.22$^{(*)}$	26.76±0.16$^{(*)}$	18.09±0.06$^{(*)}$
	Shoots	42.39± 0.81	30.28±0.52$^{(*)}$	27.51±0.44$^{(*)}$	23.35±0.44$^{(*)}$
	Leaves	14.8± 0.41	13.07±0.34$^{(*)}$	11.6±0.20$^{(*)}$	10.35±0.33$^{(*)}$
Total Proteins (µg/ml)	**Roots**	15.25± 0.07	27.75±0.73$^{(*)}$	37.75±0.53$^{(*)}$	109± 3.0$^{(*)}$
	Shoots	14± 0.23	45.25±0.41$^{(*)}$	137.75±1.6$^{(*)}$	297.75±1.06$^{(*)}$
	Leaves	24± 1.8	25.25±0.8$^{(*)}$	40.25± 1.9$^{(*)}$	89±1.2$^{(*)}$
Total Phenols (µg/gm)	**Roots**	0.006308	0.0049916	0.005636	0.0089348
	Shoots	0.005	0.0045	0.00478	0.006284
	Leaves	0.00309	0.0023186	0.002085	0.0017554
Total Lipids (mg/g)	**Roots**	0.01198	0.0109	0.0097	0.0091
	Shoots	0.0132	0.0115	0.0096	0.0086
	Leaves	0.0135	0.0087	0.0092	0.0107

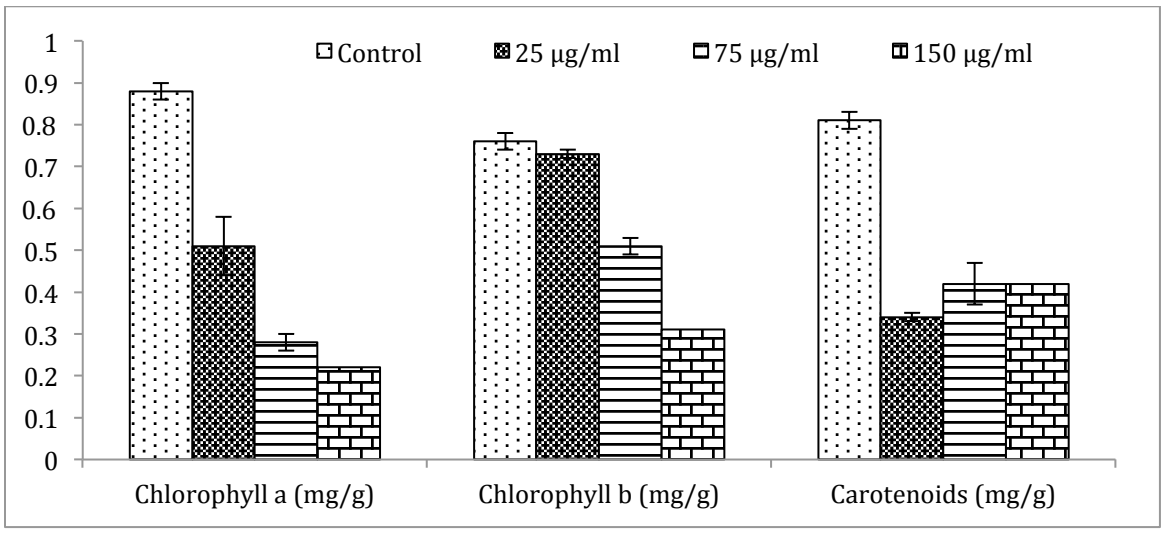

Figure 2: Impact of cadmium ($CdSO_4^{2-}$) on photosynthetic pigments of *C. arietinum* at 15 days of treatment.

Soluble carbohydrates were found to be maximum in the control plants i.e 24.36±0.08 µg/ml (roots), 29.27±0.24 µg/ml (shoots) and 10.45±0.02 µg/ml (leaves) respectively. In all root, shoot and leaves there is significant decline in the soluble carbohydrate content amongst treated plants as shown in table 2. Similar trend has also been found in insoluble carbohydrates content. i.e. maximum concentration is found in control seedlings i.e. 34.15±0.04 µg/ml, 13.12±0.02 µg/ml, 4.35± 0.07µg/ml in roots, shoots and leaves respectively, with a decline in values amongst the treated plants in comparison to the control (Table 2; Figure 3). Very meager information is available on the effect of cadmium on carbohydrates. The addition of cadmium decreases soluble sugars within a concentration dependent manner. In the present experiment the soluble sugar content of root, stem and leaves decline as compare to control plants except in sugars of stem where there is increase at the concentration of 25 µg/ml cadmium. The addition of cadmium decreases the insoluble sugars within a concentration dependent manner. The decrease in sugar level is due to hindrance in the sugar biosynthesis process. Most of the sugars are formed during photosynthesis and in the present experiment as there is decrease in the photosynthetic pigments there is also reduction in the sugar concentration. The correlation analysis has been done between the chlorophyll content and carbohydrate content between control and respective cadmium concentration, and it was observed that as the chlorophyll content is decreasing the carbohydrate content has also been decreased.

The protein concentration is maximum in the seedlings treated with 150 µg/ml of cadmium concentration followed by seedlings treated with cadmium concentration of 75 µg/ml, 25µg/ml and control respectively. Similar trend is observed in roots, shoots and leaves of all seedlings (Table. 2; Figure 4).

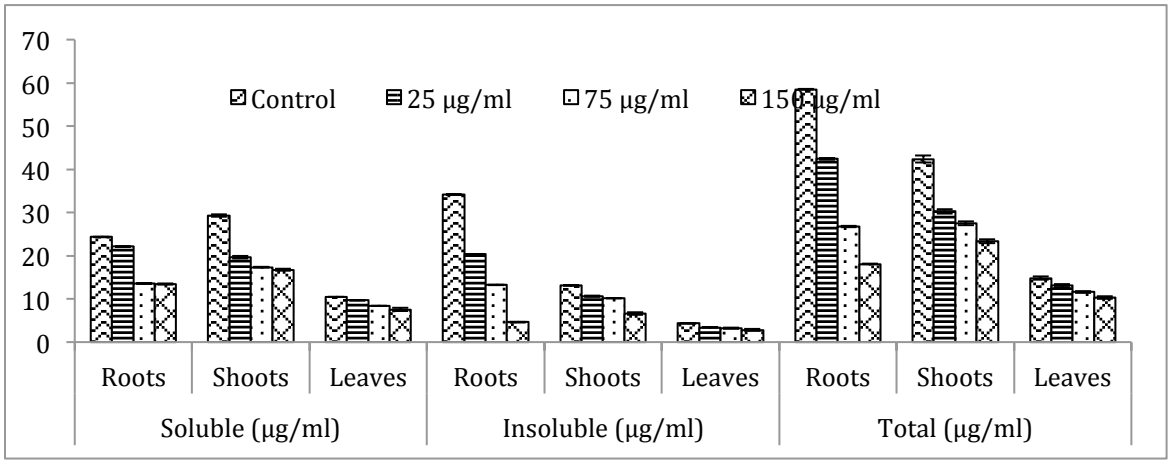

Figure 3: Impact of cadmium ($CdSO_4^{2-}$) on soluble, insoluble and total carbohydrates in roots shoots and leaves of *C. arietinum* at 15 days of treatment.

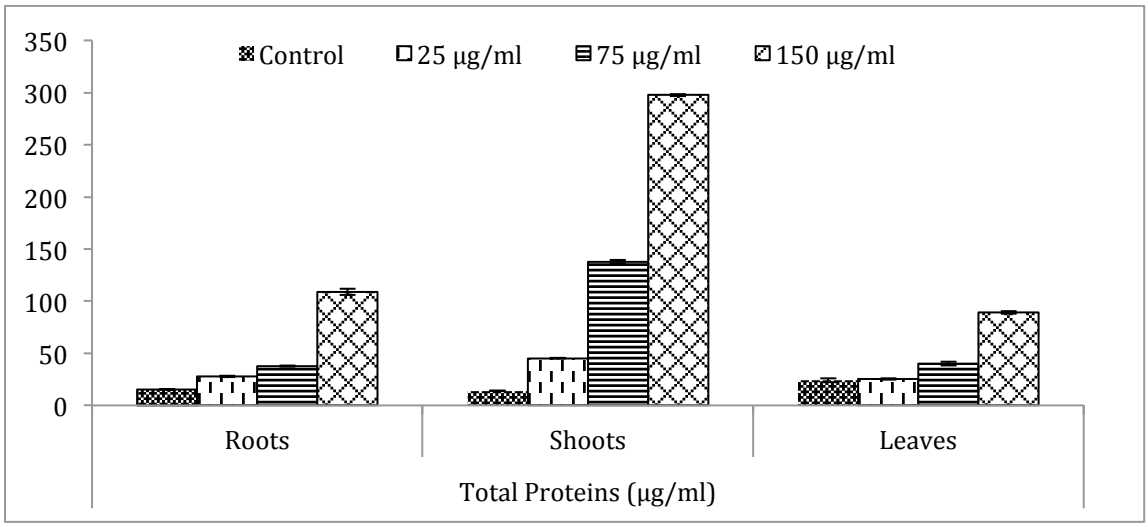

Figure 4: Impact of cadmium ($CdSO_4^{2-}$) on total proteins in roots shoots and leaves of *C. arietinum* at 15 days of treatment.

The protein content of shoots and roots may increase, decrease or remain unchanged in the presence of cadmium. In the present experiment there is increase in protein content of cadmium treated plants as compare to the control plants. At high cadmium concentrations plant synthesizes Cd^{2+} binding stress proteins. The stress proteins such as metallothionines are formed in the plants for the detoxification process, which is important for the survival. These stress proteins regulate the water balance of cells and also protect various enzymes against denaturation and stabilize protein synthesis (Kuznestov *et al*., 1997). Also there is increase in antioxidant enzymes and reactive

oxygen spesies (ROS). The similar increase in soluble protein content has also been found in *Triticum durum* (Alayat *et al*., 2014).

Phenols are protective agents in plants which play a key role in defense mechanism. Any type of stress whether, its pathogenic or environmental, the phenolic concentration always gets affected and generally increases (Chalker – Scott and Fuchigami, 1989). The phenol content was found to be highest in roots of the seedlings treated with 150µg/ml of cadmium concentration followed by seedlings with 75 µg/ml of cadmium, control and 25 µg/ml of cadmium respectively. The similar trend has been observed in shots and leaves (Table 2; Figure 5).

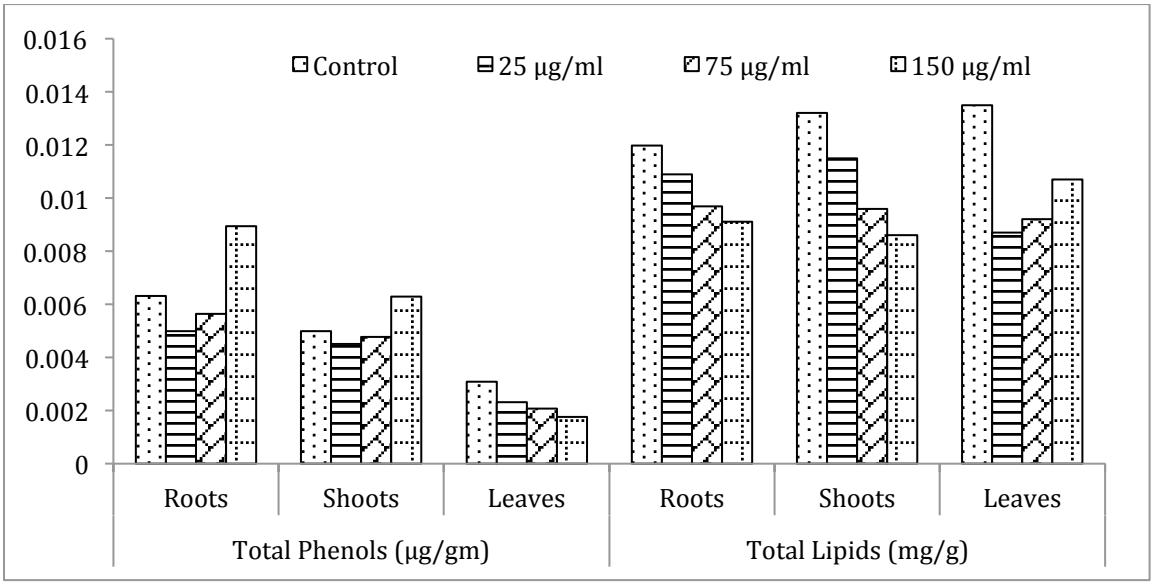

Figure 5: Impact of cadmium ($CdSO_4^{2-}$) on total phenols and total lipidsin roots, shoots and leaves of *C. arietinum* at 15 days of treatment.

The phenolic compounds are electron donators and act as antioxidants, reducing agents or hydrogen donors (Marquez-Garcia *et al*., 2012; Michalak, A. 2006). They can also act as biomarkers for metal exposure. The cadmium exposure can increase the total phenolic compounds and flavonoids in the plants. In the present experiment also, the seedlings grown under higher concentration of cadmium were found to possess high quantity of total phenols suggesting a high level of defense in the seedlings. Lavid *et al*. (2001) similarly observed the increase in phenolic content due to increase in the enzymatic activities for phenol synthesis. Lipids are naturally occurring compounds that are esters of long chain fatty acid and are integral part of membrane. The membranes are the primary structure which are exposed first to ant kind of stress. In the present experiment, it has been observed that the lipid content has been decreased in treated plants as compared to control plants. From cadmium concentration of 25 µg/ml to 150 µg/ml, there is significant decrease in total lipids (Table 2; Figure 5). The decrease in lipid content may be due to reduction lipid synthesis and also decrease in the total

membrane area of cells, due to decrease in number and size of various cellular organelles like chloroplast (Ammar *et al*., 2008; Djebali *et al*., 2005).

Conclusions

Rapid industrialization and urbanization has resulted in pollution beyond the permissible limits. In the present study investigations have been done on the effect of different concentrations of cadmium on growth parameters and different biochemical parameters. The results indicate that Cadmium significantly inhibits the plant growth and development, acting as limiting factor. The increased concentration of cadmium in soil has resulted into oxidative stress in the plants, causing augmentation of phenols and proteins. The present study strongly recommends that a periodical soil study must be done for heavy metals in agricultural lands and irrigation water, so that the concentration of heavy metals do not go beyond the threshold limits as suggested by WHO to avoid health hazards.

Acknowledgements: *The authors are grateful to the Principal, Maitreyi College, University of Delhi, Star College Scheme, DBT, India and Professor Dinabandhu Sahoo, Department of Botany, University of Delhi for providing research facilities.*

Author's Contributions: *Dr. Pooja Baweja (Assistant Professor and project leader) has designed some of the experimental work and also Corresponding Author. Mr. Dharmendra Kumar has worked towards the standardization of various protocols and has also taught various techniques to the students. Students group Ms. Vanshika Maheshwari, Ms. Muskan Singla, Ms. Ritu, Ms. Parul, Ms. Nisha Jariyal and Ms. Akshita have contributed towards the sample preparation, performed various experiments, tabulated the results, done statistical analysis*

References

Alayat, A., Souiki, L., Grara, N., Djebar, M.R., Boumedris, Z.E., Benosmane, S., Amamra, R., Berrebbah, H., 2014. Effects of Cadmium on Water Content, Soluble Protein, Proline Changes and Some Antioxidant Enzymes in Wheat *(Triticum durum desf.)* Leaves. *Annual Research & Review in Biology* 4 (24), 3835-3847.

Ammar, W.B., Nouairi, I., Zarrouk, M., Jemal, F., 2008. The effect of cadmium on lipid and fatty acid biosynthesis in tomato leaves. *Biologia* 63/1, 86-93, 2008.

Barcelo, J., Poschenrieder, C., 1990. Plant water relations as affected by heavy metal stress: A review. *Journal Plant Nutrition* 13, 1-37.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248-254.

Brown, J.C., Tiffin, L.O. and Holmes, R.S., 1960. Competition between chelating agents and roots as factor affecting absorption of Iron and other ions by plant species. *Plant Physiology* 35, 878-886.

Chalker-Scott, L., Fuchigami, L.H., 1989. The role of phenolic compounds in plant stress responses. In *Low Temperature Stress Physiology in Crops, ed*. P.H. Li., pp. 68-76. CRC press, Florida, USA.

Chugh, L.K., Sawhney, S.K., 1999. Photosynthetic activities of Pisum sativum seedlings grown in presence of cadmium. *Plant Physiology and Biochemistry* 37, 297-303.

Deniz, T, Yasemin, E, Unalan, S., 2007. Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. *Chemosphere*, 67, 89-98.

Djebali, W., Zarrouk, M., Brouquisse, R., El Kahoui, S., Limam, F., Ghorbel, M.H., Chaïbi, W. 2005. Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biology* 7, 258–268.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356.

Duxbury, A.C., Yentsch, C.S., 1956. Plankton pigment monograph. *Journal of Marine Research* 15, 19-101.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497-509.

Gouia, H., Gorbel, M.H., Meyer, C., 2000. Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiology and Biochemistry* 38, 629-638.

Hasan, S.A., Fariduddin, Q., Ali, B., Hayat, S., Ahmad, A., 2009. Cadmium: Toxicity and tolerance in plants. *Journal of Environmental Biology* 30, 165-174.

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

Herbette, S., Taconnat, L., Hugouvieux, V., Piette, L., Magniette, M. L., Cuine, S., Auroy, P., Richaud, P., Forestier, C., Bourguignon, J., Renou, J. P., Vavasseur, A., Leonhardt, N., 2006. Genome-wide transcriptome profiling of the early cadmium response of Arabidopsis roots and shoots. *Biochimie*, 88, 1751–1765.

Hiscox, J. D., Israelstam, G. F., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* 57(12), 1332-1334.

Krupa, Z., Quist, G., Hurner, N.P.A., 1993. The effect of cadmium on photosynthesis of *Phaseolus vulgaris* – A fluorescence analysis. *Physiologia Plantarum* 88, 626-630.

Kuznestov, V.V., Shevyakova, N.I., 1997. Stress responses of tobacco cells to high temperature and salinity: Proline accumulation and phosphorylation of polypeptides. *Physiologia Plantarum* 100, 320-326.

Li, M., Zhang, L.J., Tao, L., Li, W., 2008. Ecophysiological responses of *Jussiaea rapens* to cadmium exposure. *Aquatic Botany* 88, 347-352.

Liu, L., Sun, H., Chen, J., Zhang, Y., Li, D., Li, C., 2014. Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton (*Gossypium hirsutum* L.). Plants Omic Journal 7(4), 284-290.

Liu, Y., Jiang, H., Zhao, Z., An, L., 2011. An Abscisic Acid Is Involved in Brassinosteroids-Induced Chilling Tolerance in the Suspension Cultured Cells from *Chorispora bungeana*. *Journal of Plant Physiology* 168 (9), 853-862.

Malick, C.P., Singh, M.B., 1980. Plant Enzymology and Hittoenzymology (1st Edn.). Kalyani Publishers, New Delhi.

Márquez-García, B., Fernández-Recamales, M. A., Corbada, F., 2012. Effects of Cadmium on Phenolic Composition and Antioxidant Activities of *Erica andevalensis*. Journal of Botany, 1-6.

Mehindiratta, S., Mahmooduzzafar, T.O., Muhamad, I., 1999. Cadmium induced changes in foliar responses of *Solanum melongena* L. *Phytomorphology* 49 (3), 295-302.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* 15 (4), 523–530.

Mondal, N. K., Das, C., Roy, S., Datta, J. K., Banerjee, A., 2013. Effect of varying cadmium stress on chickpea (*Cicer arietinum* L.) **s**eedlings: an ultrastructural study. *Annals of Environmental Science* 7, *59-70*

Punz, W..F., Sieghardt, H., 1993. The response of roots of herbaceous plant species to heavy metals. *Environmental and Experimental Botany* 33, 85-98.

Wang, C., Zhang, S. H., Wang, P. F., Qian, J., Hou, J., Zhang, W. J., Lu, J., 2009. Excess Zn Alters the Nutrient Uptake and Induces the Antioxidative Responses in Submerged Plant *Hydrilla verticillata* (L.f.) Royle. *Chemosphere* 76 (7), 938-945.