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ORIGINAL ARTICLE

STUDIES ON THE BIOCHEMICAL ANALYSIS OF MERCURY CHLORIDE TREATED ORNAMENTAL PLANT *Zinnia elegans* (L.)

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ABSTRACT

Contamination of soils by Hg is often due to the addition of this heavy metal as part of fertilizers, lime, sludges, and manures. The dynamics between the amount of Hg that exist in the soil and its uptake by plants. The interaction between Hg and plant systems is of particular importance due to the highly employment in seed disinfectants, fertilizers, and herbicides. The effects of heavy metal stress on biochemical parameter level were studied in the ornamental plant Zinnia *elegans*. *Zinnia elegans* were grown for 90 days and the heavy metal mercuric chloride contaminated soil in pot culture method from the date of seed. Effect of mercuric chloride was observed in treated plants. The selected plant *Zinnia elegans* was grown under mercuric chloride treatment in a specified concentration levels (40, 100, 200, 300, 400 and 800 mg kg^{-1}) from 15th to 90th sampling days. The control plant maintained without the treatment of mercuric chloride. The following biochemical parameters such as total sugar, starch, protein, and amino acid level were affected by the treatment due to mercuric chloride stress.

Keywords: *Zinnia elegans*, Biochemical analysis, Mercury chloride, Ornamental plant.

1.INTRODUCTION

Heavy metal contamination of land and water resources is a growing problem in many countries. Although heavy metals are the natural components of soils in trace level activities such as mining, industry and localised agriculture have contributed to undesirable accumulations of these metals at toxic levels (Alloway, 1995). Lower contents of sugars and starch were observed in the copper stressed wheat leaves (Lanaras *et al*., 1993). Shrotri *et al*. (1979) studied the effect of Zinc on sugars and starch contents in *Zea mays* carbohydrate levels and photoassimilate export from leaves of *Phaseolus vulgaris* exposed to excess zinc was observed by Samarakoon and Rauser (1979). Cadmium uptake and sugar accumulation on young sugar beet under cadmium treatment were assessed by Greger and Lindberg (1986). Similar reduction in quantity of sugar contents due to various other heavy metals such as aluminium in Barley (Guo *et al*., 2008) and chromium in *Brassica juncea* (Singh and Sinha, 2005) and Water lilies (Choo *et al*., 2006) were also reported. Stiborova *et al*. (1986) reported that the protein content was decreased by cadmium and copper treatment in *Hordeum vulgare*. Excessive quantities of mercury and copper became toxic as it interfered with protein synthetic process in *Thalaspi ochroleucum* (Ouzounidou *et al*., 1994). Wheat plants growing in the field of ore bodies containing mercury and copper showed a reduced protein and *Rubisco* activity compared wih control plants grown in garden soil (Lanaras *et al*., 1993). Lidon and Henriques (1993) registered the protein content of rice shoots tend to decrease progressively with increasing copper levels. Effects of copper on RNA and protein synthesis of pea plants was reported by Angelov *et al*. (1993). Total soluble protein content was examined in the leaves of *Zea mays* under various levels of copper treatment (Mocquot *et al*., 1996). Davies *et al*. (2002) studied the effect of zinc on the protein levels of *Festuca rubra*. Zinc toxicity had been found to result in a decrease in amino acid accumulation in *Panax quinquefolium* roots (Ren *et al*., 1993). Demirevska-Kepova *et al*. (2004) reported that high copper level induced the reduction in leaf total soluble protein in barley plants. Choo *et al*. (2006) investigated the reduction of protein and sugar contents in water lilies plants exposed to chromium. Effect of zinc on nitrate reductase activity and soluble protein content in leaves of *Quercus serrate* was assessed by Ghosh and Srivastava (1994). Vijayaragavan *et al*. (2007) investigated the cadmium at all levels (10-50 mg kg^{-1}) produced toxic effect on amino acid and protein contents of radish plant. The changes in soluble protein content and photosynthetic pigments as well as the activity of antioxidant enzymes caused by copper sulfate and cadmium dichloride, respectively in duckweed (*Lemna minor*). Similar

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differential toxicity of heavy metals such as arsenic in maize (Jain and Gadre, 1997), cadmium in *Vigna unguiculata* (Bhattacharjee and Mukherjee, 1994); in *Arachis hypogaea* (Bavaji, 1999), Lead and cadmium in *Vigna* and *Hydrill*a (Bhattacharya and chowdhuri, 1994) and nickel and cadmium in cauliflower (Chatterjee and Chatterjee, 2000) have also been reported.

The present study has been aimed to find out the biochemical substance total sugar, starch, protein and amino acids level of mercury chloride treated ornamental plant *Zinnia elegans* with six different concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}) level of the soil for the testing periods (15, 30, 45, 60, 75 and 90 days) also record the level of biochemical constituent of the treated *Zinnia elegans* compared to control.

2.MATERIALS AND METHODS

Total sugar analysis

Two grams of fresh leaf materials were plunged in boiling ethanol and allowed to boil for 5 to 10 minutes. Five ml of alcohol was used for every gram of leaf tissue. The extract was cooled and then the tissue was crushed thoroughly in a mortar with pestle. The residue was again re-extracted with 80 per cent alcohol, then filtered, it combined the volume was adjusted (Loomis and Shull, 1937). One ml of ethanol extract was taken in a test tube and evaporated to dryness. To the residue, 1 ml of distilled water and 1 ml of 1 N sulphuric acid were added and incubatated at 49 ˚C for 30 minutes. The solution was neutralized with 1 N NaOH using methyl red indicator. One ml of Nelson's reagent was added to each tube prepared by mixing reagent A and B in the ratio 25:1 (reagent A: 25 g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulhate dissolved in 1000ml; reagent B: 15g of cupric sulphate in 100ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arseno-molybdate reagent (25g ammonium molybdate, 21ml concentrated sulphuric acid, 5g of sodium arsenate dissolved in 475ml of distilled water and incubated at 37 ˚C in a water bath for 48 hr) was added. The solution was thoroughly mixed and diluted to 25ml and read at 495nm in a spectrophotometer. The sugar content of unknown samples was calculated from glucose standard (Nelson, 1944).

Starch analysis

The ethanol insoluble residues taken from ethanol extraction were dried at 60 ˚C for 48 hr in a hot air oven. Three ml of 6N HCl was added to 200mg of the powdered residue and autoclaved at100 ˚C for an hour. The flask was cooled and the volume was raised to 25 ml with distilled water. One ml of aliquot was withdrawn, neutralized with 1N NaOH and sugar was estimated by Nelson's (1944) method. The amout of starch was arrived by multiplying the sugar by the factor 0.9. (Summner and Somers, 1949).

Amino Acids

Ethanol extract of leaf tissues was made as mentioned in the case of sugars. One ml ethanol extract was taken in 25ml test tube and neutralized with 0.1N NaOH using methyl red indicators. One ml of ninihydrin reagent was added (800mg stannous chloride in 500ml citrate buffer, pH – 5.0; 20g ninhydrin in 500ml methyl cellosolve, both solutions were mixed). The contents were boiled in a water bath for 20 minutes, 5ml of dilute solution (distilled water and n-propanol mixed in equal volume) was added, cooled and made up to 25ml with distilled water. The absorbance was measured at 570nm in a spectrophotometer. The standard graph was prepared using leucine (Moore and Stein, 1948).

Protein

Fresh tissue weighing 0.5g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600rpm for 30 minutes and the supernatant was discarded. Five ml of 0.1N NaOH was added to the pellet and it was centrifuged for 30 minutes. The supernatant was saved for the estimation of protein. To 0.5ml of the extract, 5ml of copper reagent 'C' was added (Reagent C: mixture of reagents 'A' and 'B' in the 50:1 ratio. Reagent A:2 per cent Na_2CO_3 in 0.1N NaOH, Reagent B: equal volume of 1 per cent $CuSO_4$ and two per cent sodium potassium tartarate). The tubes were shaken well and allowed to stand in dark for 10 minutes at room temperature, 0.5ml of properly diluted Folin Ciocalteau reagent was added to the solution and mixed thoroughly. The absorbance was read at 550nm in a spectrophotometer against an appropriate blank. Bovine serum albumin was used as the standard (Lowry *et al*., 1951).

Statistical Analysis

The statistical analysis of the experimental data was carried out as per the procedure given by Gomez and Gomez (1984).

3.RESULTS

Total Sugar

Total sugar content of leaves of *Zinnia elegans* plants under Mercury chloride stress is represented in Table 1. There was a gradual decrease in the total sugar content with increase of Mercury chloride content in the soil. Total sugar content of leaf was maximum at control *Zinnia elegans* plants (viz., 7.546, 9.567, 11.553, 10.457, 8.565 and 7.256) in all the sampling days. With further increase of mercury chloride level (40 – 800 mg k^{-1}), the total sugar content of *Zinnia elegans* was reduced in all the sampling days. Minimum total sugar content of *Zinnia elegans* leaf (viz., 3.465, 5.568, 6.672, 5.475, 4.756 and 3.477) was observed at 800 mg kg^{-1} mercury chloride level in the soil. The total sugar content showed a progressive trend up to the 45^{th} day and it gradually declined on the 60^{th} and 90th day. Significant variations (F value at 1 per cent level) were observed with treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

Starch

Effect of mercury on the starch content of *Zinnia elegans* is represented in Table 2. Starch content of *Zinnia elegans* leaves was high in control (viz., 5.656, 8.365, 9.768, 10.454, 8.543 and 7.876) in various sampling days and decreased further with an increase in the mercury chloride level (40 – 800 mg k^{-1}) of the soil. The lowest starch content was

Table 1. Effect of Mercury chloride on total sugar content (mg g^{-1} fresh weight) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	7.546	9.567	11.553	10.457	8.5654	7.256
40	6.567	9.164	10.676	9.456	8.177	6.556
	(-13.56)	(-11.36)	(-10.56)	(-10.65)	(-13.56)	(-14.45)
100	5.546	8.656	9.566	8.547	7.454	6.246
	(-21.56)	(-15.66)	(-14.46)	(-16.45)	(-21.65)	(-23.56)
200	5.535	8.345	8.646	7.657	6.566	5.564
	(-24.56)	(-20.56)	(-21.56)	(-24.65)	(-25.56)	(-23.65)
300	4.766	7.546	8.356	7.453	6.237	5.145
	(-24.46)	(-19.56)	(-22.66)	(-25.65)	(-26.67)	(-27.64)
400	4.645	6.645	7.646	6.522	5.266	4.656
	(-35.66)	(-28.45)	(-24.45)	(-31.95)	(-32.45)	(-27.76)
800	3.465	5.568	6.672	5.475	4.756	3.477
	(-21.56)	(-28.215)	(-26.88)	(-19.76)	(-25.75)	(-22.57)

Comparison of significant effects	F test
Mercury chloride level	98.64**
Sampling days	327.24**

Figures in parentheses represent per cent reduction (-) over control

Table 2. Effect of Mercury chloride on starch content (mg g^{-1} fresh weight) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	5.656	8.365	9.768	10.454	8.543	7.876
40	4.566	7.766	9.476	10.268	8.165	7.657
	(-8.86)	(-7.67)	(-5.56)	(-6.56)	(-6.66)	(-4.76)
100	4.378	7.545	8.456	9.755	7.579	7.367
	(-18.67)	(-15.76)	(-16.75)	(-19.67)	(-22.67)	(-16.46)
200	3.457	7.076	7.677	8.565	6.567	6.234
	(-25.67)	(-19.755)	(-27.56)	(-26.56)	(-22.65)	(-23.67)
300	3.156	6.656	7.364	8.175	5.458	5.567
	(-26.86)	(-28.45)	(-31.56)	(-23.55)	(-27.46)	(-25.64)
400	3.048	5.565	6.656	7.565	5.166	4.464
	(-33.65)	(-24.57)	(-28.57)	(-25.756)	(-26.64)	(-21.575)
800	2.464	3.655	4.643	5.757	4.645	3.567
	(-48.66)	(-42.75)	(-43.64)	(-31.79)	(-33.56)	(-28.65)

Comparison of significant effects	F test
Mercury chloride level	58.25**
Sampling days	172.28**

Figures in parentheses represent per cent reduction (-) over control

Table 3. Effect of Mercury chloride on amino acid content (mg g^{-1} fresh weight) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	3.342	6.656	9.634	8.576	7.765	5.676
40	3.565	6.546	9.667	7.767	7.675	5.764
	(-6.47)	(-4.86)	(-3.67)	(-5.66)	(-2.26)	(-8.84)
100	3.655	6.676	9.328	7.767	6.576(-5.75)	5.768
	(-8.58)	(-7.27)	(-7.84)	(-4.27)		(-12.48)
200	2.898	5.234	8.986	7.678	6.665	5.787
	(-15.53)	(-21.85)	(-15.38)	(-16.85)	(-17.48)	(-21.86)
300	2.675	5.756	8.459	6.656	5.647	4.655
	(-32.37)	(-37.85)	(-28.74)	(-24.48)	(-29.84)	(-31.85)
400	2.565	5.565	7.676	6.565	5.656(-28.88)	3.6765
	(-43.85)	(-28.57)	(-27.47)	(-21.76)		(-36.74)
800	1.675	3.677	5.676	4.765	3.566	2.786
	(-48.64)	(-37.33)	(-31.73)	(-39.58)	(-38.47)	(-36.85)

Comparison of significant effects	F test
Mercury chloride level	58.37**
Sampling days	387.24**

Figures in parentheses represent per cent reduction (-) over control

Table 4. Effect of Mercury chloride on protein content (mg g^{-1} fresh weight) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	18.456	23.743	29.466	25.634	23.634	21.564
40	17.365	22.655	28.767	24.654	22.454	20.442
	(-3.85)	(-3.55)	(-6.56)	(-5.34)	(-8.56)	(-6.56)
100	17.666(-10.87)	21.664 (-5.65)	27.755 (-8.68)	23.446 (-5.47)	22.146 (-6.69)	19.546 (-17.47)
200	16.767(-13.77)	21.366 (-14.37)	27.5465 (-12.95)	22.768 (-19.06)	21.253 (-18.48)	18.768 (-21.06)
300	15.354(-15.75)	20.856 (-13.44)	26.465 (-12.57)	22.164 (-10.06)	20.767 (-13.76)	17.655 (-16.75)
400	14.757(-15.47)	20.267 (-24.47)	25.767 (-19.65)	21.764 (-26.75)	19.767 (-25.67)	16.745 (-27.45)
800	12.654(-26.75)	18.877 (-30.55)	23.5675 (-28.49)	20.767 (-32.65)	17.244 (-30.67)	14.876 (-33.74)

Comparison of significant effects	F test
Mercury chloride level	52.31**
Sampling days	97.57**

Figures in parentheses represent per cent reduction (-) over control

recorded at 800 mg kg-1 mercury chloride level (viz., 2.464, 3.655, 4.643, 5.757, 4.645 and 3.567) in all the sampling days. The starch content of *Zinnia elegans* showed a progressive trend upto the 60th day and it gradually declined on the 75 and 90th day due to the senescence of leaves. F test values for treatment and sampling days were significant at 1 per cent level in Mercury treated *Zinnia elegans* plants.

Amino acids

The results showed in Table 3 indicated that the maximum aminoacids content of *Zinnia elegans* plants occurred in control (viz., 3.342, 6.656, 9.634, 8.576, 7.765 and 5.676) in all the sampling days and minimum amino acid content was observed at 800 mg kg^{-1} of mercury chloride level (viz., 1.675, 3.677, 5.676, 4.765, 3.566 and 2.786) in various sampling days. The amino acid content of mercury chloride treated *Zinnia elegans* leaves progressively decreased with increasing concentrations of applied (40, 100, 200, 300, 400 and 800 mg kg^{-1}) mercury chloride in the soil. The amino acid content is higher at 45th day than the 60, 75 and 90th day. The F test values were significant at 1 per cent level for treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

Protein

The results showed in Table 4 indicated that the maximum protein content of *Zinnia elegans* plants occurred in control (viz., 18.456, 23.743, 29.466, 25.634, 23.634 and 21.564) in all the sampling days and minimum protein content was observed at 800 mg kg^{-1} of mercury chloride level (viz., 12.654, 18.877, 23.567, 20.767, 17.244 and 14.876) in various sampling days. The protein content of mercury chloride treated *Zinnia elegans* leaves progressively decreased with increasing concentrations of applied mercury chloride (40, 100, 200, 300, 400 and 800 mg kg^{-1}) in the soil. The protein content is higher at 45th day than the 60, 75 and 90th day. The F test values were significant at 1 per cent level for treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

4.DISCUSSION

Sugar and starch contents showed a decreasing trend with progressive increase in mercury chloride concentrations in *Zinnia elegans*. However, 40, 100, 200, 300, 400 and 800 mg kg^{-1} levels produced negative effect on the sugar and starch contents, which is in consonance with the findings of Lanaras *et al*. (1993) under copper treatment, Shrotri *et al*. (1979), Samarakoon and Rauser (1979) under zinc treatment and Greger and Lindberg (1986) under cadmium treatment. The reduction of sugar and starch content might be due to the imbalance which might eventually lead to depletion of carbohydrate reserves (Murata *et al*., 1969). Greger and Johansson, (1992) observed that mercury and cadmium caused a diminished carbohydrate concentration in sugar beet (*Beta Vulgaris*), Malik *et al*., (1992) observed that increased accumulation of mercury and cadmium in leaves caused a reduction in carbohydrate reserve in wheat seedlings. Moya *et al*., (1993) reported an inhibition of transport of carbohydrate reserves from the rice seeds from which the rest of the plants were developing when treated with mercury and cadmium. Narwal and Singh, (1993) noted that both mercury and cadmium and other metals decreased reducing, non-reducing and total sugars of maize grown in soil treated with the said metals. According to Satyakala and Jamil, (1997) *Pistia stratiotes* plants when treated with 5-100ppm of mercury and cadmium solutions for 72h., showed decrease in sugar content. In the response of mercury and cadmium there was a steady decrease in the sugar content in *Hydrilla*.

Amino acid and protein content of *Zinnia elegans* were low in all the concentrations of mercury chloride (40, 100, 200, 300, 400 and 800 mg kg^{-1}) level in the soil than in the control plants. These results correlated with the Mocquot *et al*. (1996) under copper treatment and Ghosh and Srivastava (1994). The application of chelators maintained the amino acid content in *C. roseus* better than the Hg treated plants. Amino acid content was increased with the increasing rate of chelators. The amino acid content might be reduced due to the reduction of nitrogen content in plants grown under heavy metal stress. Nitrogen is a precursor for the synthesis of amino acids. Since the nitrogen content of the metal treated plants was reduced, ultimately, amino acids and protein contents of the plants were also reduced as there would be only limited availability of nitrogen for the synthesis of amino acids. Increasing protein with increased chelators addition was coincide with decrease in soluble protein content under high concentration of heavy metals in *Lupinus albus* Costa and Spitz (1997). Ahsan *et al*. (2007) investigated the protein profile alternations during the germination stage following exposure to cadmium; a proteomic approach has been adopted in combination with morphological and physiological parameters.

Costa and Spitz (1997) found decrease of the sucrose level, both in root and shoot, of 15 days old non-nodulated white lupin plants grown with mercury from germination. The both mercury and cadmium cause a decrease in protein content in general provides support to the trend observed by earlier workers in which a decrease was seen in protein content in response to metal application. There was a negative correlation between the increase in metal concentration and the decrease in protein levels both after 5 and 10 days of treatment to *Hydrilla* plant in response to both the metals (Goutam *et al*., 2013). Mohapatra *et al*., (1997) observed that the protein content decreases with corresponding increase in concentration of mercury. Some workers reported an increase in protein content in response to mercury and cadmium (Thompson and Couture 1991; Narwal and Singh, 1993). Decline in protein content in response to application of other metals was also reported by a number of workers (Shukla and Pandev, 1993). (Radha and Srivastava, 2006) reported that protein contents decreased with an increase in mercury and cadmium supply to the varieties of sugarcane.

Kalita *et al*., (1993) also supports this effect that in presence of cadmium and mercury inhibition of protein synthesis takes place. According to Singh *et al*., (1989) the excess levels of Hg and Cd also decreased the concentrations of soluble protein. Similar trend has been discussed by Garg *et al*. (1997) in *Hydrilla verticillata*. Neelu *et al*. (2000) also supported this view that total soluble protein of leaf, stem and root suffered a pronounced loss with increasing concentration of mercury and cadmium. Narwal and Singh,

(1993) observed an increase in amino acid content in Maize plants in response to mercury and cadmium however the same authors observed a decrease in the α-amino nitrogen content in response to the zinc. Chromium induced biochemical changes with reference to amino acids in the seedling of *phaseolus mungo* (Mahadeswarswamy and Theresa, 1992). Excess cellular concentrations of mercury and cadmium either inhibit the utilization of amino acids or promote protein hydrolysis, thus affecting the normal balance of cellular proteins (Tendon and Srivastava. 2004). In the present investigation Hg and Cd treated seedlings also contained higher amounts of free amino acids as has been observed in different parts of plants under stressful conditions (Dubey and Pessarakli, 1995). As far as effect of mercury and cadmium on amino acid content of *Hydrilla* plant is concerned, it can be said that both of them causing opposite effect in the plant after 5 and 10 days of interval. Addition of kinetin in both metals promotes the amino acid contents in some extent in *Hydrilla* plant after 5 and 10 days of intervals and this was supported by Gupta and Singh. (1972) and suggested that amino acid content showed an increasing trend in the test plant in response to mercury and cadmium with kinetin bearing a positive correlation with the increasing concentrations of the metals.

5.CONCLUSION

The present study clearly indicates that the ornamental plant *Zinnia elegans*, increases the concentration of mercury chloride in treated plants as compared to control due to higher uptake of heavy metals causing accumulation. The maximum accumulation of mercury chloride to reduce the total sugar, starch, protein and amino acids content of the *Zinnia elegans* plant. This reduction progressive trend up to the 45^{th} day and it gradually declined on the 60^{th} and 90th day of all the concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}).

6.REFERENCES

Ahsan, N., D.G. Lee, S.H. Lee, K.Y. Kang, J.J. Lee and P.J. Kim, 2007. Excess copper induced physiological and proteomic changes in germinating rice seeds. *Chemosphere*, 67: 1182-1193 33.

Alloway, B J. 1995. Soil processes and the behaviour of heavy metals. Heavy Metals

Angelov, V.M., T. Tsonev, A. Uzunova, K. Gaielardkiever, 1993. Cu^{2+} effect upon photosynthesis, Chloroplast structure, RNA and protein synthesis of pea plants. Photosynthetica, 28: 341-350.

Bavaji, M., 1999. Cadmium toxicity induced biochemical changes in groundnut leaves *Arachis hypogaea* L. var. JL-24. J. Ecotoxicol. Environ. Monit., 9(3): 213-215.

Bhattacharjee, S. and A.K. Mukherjee, 1994. Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L.) Walp. Seedlings I. Germination behavior, total protein, proline content and protease activity. Pollut. Res., 13(3): 269-277.

Chatterjee, J. and Chatterjee, C. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. Env. Pollut. 109: 69–74.

Choo K, Snoeijs P, Pedersen M .2006. Oxidative stress tolerance in the filamentous green algae *Cladophora glomerata* and *Enteromorpha ahlneriana*. Journal of Experimental Marine Biology and Ecology 298: 111 – 123.

Costa, G. and E. Spitz, 1997. Influence of cadmium on soluble carbohydrates, free amino acids, protein content of *in vitro* cultured *Lupinus albus*. *Plant Sci*., 128: 131-140.

Davies, F.T., Puryear, J.D., Newton, R.J., Egilla, J.N. and Grossi, J.A.S. 2002. Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth, and gas exchange. J. Plant. Nutr. 25: 2389–2407.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R. and Feller, U. 2004. Biochemical changes in barley plants after excessive supply of copper and manganese. Env. Exp. Bot. 52: 253–266.

Dubey, R.S. and Pessarakli, M. 1995. Physiological mechaniss of Nitrogen absorption and assimilation in plants under stressful conditions. In Mpessarakli, ed., Hand book of plant and crop physiology. Marcel Dekker Inc., New York, 605 – 625.

Garg, P.; Tripathi, R. D.; Rai, U.N.; Sinha, S. and Chandra, P. 1997. Cadmium Accumulation and toxicity in submerged plant *Hydrilla vertifillata* (I.F.) *Royle, J. of Env. Monitoring and Assessmen*. 47: 167-173.

Ghosh, M.K. and R.C. Srivastava, 1994. Effect of Mg, Zn and Mo salts on nitrate reductase activity and soluble protein content in leaves of *Quercus serrate*. Biol. Plant., 36(4): 599-605.

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research, John Wiley and Sons, New York.

Goutam C. Das, K. Bijay Kumar and B.K. Mohanty, 2013, Effect of mercury and cadimium on the biochemical parameters of *Hydrilla* Plant, International journal of Life science and Pharma Research. Vol 3:2, 58-66.

Greger, M. and Johansson, M. ; Cadmium effects on leaf transpiration of sugar beet *(Beta Vulgaris)*. *Physiol. Plant* ,1992; 86 (3) : 465- 473.

Greger, M. and S. Lindberg, 1986. Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris* L.), Cd^{2+} uptake and sugar accumulation. Physiol. Plant., 66: 69-74.

Guo, J., Dai, X., Xu,W. and Ma, M. 2008. Over expressing GSHI and AsPCSI simultaneously increases the tolerance and accumula- tion of cadmium and arsenic in Arabidopsis thaliana. Chemosphere. 72:1020–1026.

Gupta, G.P. and D. Singh, 1972. Cadmium status of some soils of Indore. J. Ind. Soc. Soil Sci., 20: 49-51. in Soils. London: Chapman & Hill.

Jain, M. and R. Gadre, 1997. Effect of as on chlorophyll and protein contents and enzymatic activities in greening maize tissues. Water Air Soil Pollut., 93: 109-115.

Kalita, M.C.; Devi, P. and Bhattacharya, I. 1993. Effect of cadmium on seed Germination, early seedling growth and chlorophyll content of *Triticum aestivum. Indian J. Plant physiol*. 36(3). 189-190.

Lanaras, T., M. Moustakas, L. Symeonidis, S. Diamantoglou and S. Karataglis, 1993. Plant metal content, growth responses and some photosynthetic measurements on filed-cultivated wheat growing on ore bodies enriched in Hg and Cu. Physiol. Plant., 88: 307-314.

Lidon, F.C. and F.S. Henriques, 1993. Effects of mercury and copper toxicity on growth and the uptake and

tanslocation of metals in rice plants. J. Plant Nutr., 16(8): 1449-1464.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mahadeswarswamy and Theresa, Y.M, 1992. Chromium induced biochemical changes in the seedlings of *Phaselous mungo* L.: GEOBIAS (Jodhpur).19(6): 242-246.

Malik, D.; Sheoran, I.S. and Singh, R. 1992. Carbon metabolism in leaves of cadmium treated wheat seedlings. *Plant. Physiol. Biochem.* (Paris). 30(2): 223-229.

Mocquot, B., J. Vangronsveld, H. Clijsters and M. Mench, 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents and enzyme activities. Plant Soil, 182: 287-300.

Mohapatra, D.K. and Mohanty, R.C. 1998. Reduction of mercury toxicity of *Ankistrodesmus convolutes* by addition of nitrogen and phosphorous. Poll. Res. 17(2): 105-109.

Moore, S. and W.H. Stein, 1948. Photometric method for use in the chromatography of aminoacids. J. Biol. Chem., 176: 367-388.

Moya, J.L.,; Ros, R. and Picazo, I. 1993. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate in rice plants. *Photosynth. Res.* 36(2): 75-80.

Murata, T., F.A. Eastein, C. Haskins, Y. Sillivcan and C.H.M. Van Barvel, 1969. Physiological aspects of crop Yield. Amer. Soc. Agro., Crop Science Soc. America, Madison, Wisconsin, USA, 239-259.

Narwal, R.P. and Singh, M. 1993. Effect of cadmium and zinc application on quality of maize. *Indian J. plant physiol.*, 36(3): 170-173.

Neelu, Kumar, M.; Tomar, M. and Bhatnagar, A.K. 2000. Influence of cadmium on growth and development of *Vicia faba* Linn. *Indian J. of Expt. Bio.*, 38, 819-823.

Nelson, N., 1944. A photometric adaptation of the Somogyi's method for the determination of reducing sugar. Anal. Chem., 31: 426-428.

Ouzounidou, G., 1994. Mercury and Copper induced changes on growth, metal content and photosynthetic function of *Alyssum montanum* L. Plants. Environ. Expt. Bot., 34(2): 165-172.

Radha, J. and Srivastava, S. 2006. Effect of cadmium on growth, mineral composition and enzyme activity of sugarcane. *Indian J. Plant Physiol.* 2(3) 306-309.

Ren, F., T. Liu, H. Liu and B. Hu, 1993. Influence of mercury and zinc on the growth, distribution of elements and metabolism of one-year old American ginseng plants. J. Plant Nutr., 16: 393-405.

Samarakoon, A.B. and W.E. Rauser, 1979. Carbohydrate levels and photoassimilate export from leaves of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiol., 63: 1162-1169.

Satyakala, G. and Jamil, K. 1997. Studies on the effect of heavy metal pollution on *Pista stratiotes* L. (water lettuce). J. *Environ. Hlth.* 39(1): 1-7.

Shrotri, C.K., M.N. Tewari and V.S. Rathorem, 1979. Effect f Zinc on chlorophyll, Sugars and starch contents in maize (*Zea mays* L.). Ind. J. Expt. Biol., 17: 58-60.

Shukla, R. and Pandey, G.P. 1993. Effect of zinc toxicity on soybean. *Bionature.* 13(1): 41-48.

Singh S, and Sinha S. 2005. Accumulation of metals and its effects in *Brassica juncea* (L.) Czern. (cv. Rohini) grown on various amendments of tannery waste. *Ecotoxicology and Environment Safety* 62: 118–127.

Singh, S.P.; Thakker, P.N. and Nayyar, V.K. 1989. Effect of cadmium on wheat as influenced by lime and manure and its toxic level in plant and soil. *Int. J. Environ. Stud.* 33 (1/2): 59-66.

Stiborova, M., M. Doubravova, A. Brezinova and A. Friedrich, 1986. Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley. Photosynthetica, 20: 418-425.

Summner, J.B. and G.F. Somers, 1949. Laboratory experiments in Biological chemistry. 2nd ed. Academic Press, New York, pp. 173.

Tendon, P.K. and Srivastava, M. 2004. Effect of cadmium and nickel on metabolism during early stages of growth in gram (*Cicer arietinum L.*) seeds. *Indian J. Agric. Biochem.* 17: 31-34.

Thompson, P.A. and Couture, P. 1991. Short and long term changes in growth and biochemical composition of *Selenastrum capricornutum* populations exposed to cadmium. *Aquat. Toxicol. (Amst)*. 21(314): 135-143.

Vijayaragavan, M., P. Vijayarengan and R. Manivasagaperumal, 2007. Effect of cadmium on germination and seedling growth of radish (*Raphanus sativus* L.). *Adv. Plant Sci.*, 19: 469-471.

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