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LEAD TOXICITY AND ITS AMELIORATION EFFECT BY FOLIAR APPLICATION OF HORMONE AND MICRONUTRIENTS IN *VIGNA RADIATA* L. WILCZEK

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ABSTRACT

Studies were carried out on the phytotoxicity of Lead (Pb) on some biochemical parameters like chlorophyll, protein, nucleic acid content and some enzymatic activities of *Vigna radiata* L. seedlings were treated with 100μm and 200μm concentration of Lead nitrate ($PbNo_3$) as well as Copper sulphate ($CuSo_4$) and also with 20ppm and 30ppm Indole Acetic Acid (IAA) at 21 days of sowing and carried out up to 50% flowering stage with a gap of 7 days between the foliar spraying. Lead nitrate solutions have been sprayed to study the intensity of damage and $CuSo_4$, IAA solutions have been sprayed to minimize or overcome the heavy metal stress. Results indicated that increasing lead nitrate level (from100 μm to 200 μm) leads to several disruptions of plants which were reflected by reductions of chlorophyll (from 0.341mg to 0.312mg), protein (268.251mg to 266.032mg), DNA(from 9.033mg to 8.837mg) and RNA(from 0.859mg to 0.827mg) content. However, few enzymatic analyses such as chlorophyllase, catalase and peroxidase activities of treated samples were increasing with the increasing level of lead concentrations. Regarding detoxification, foliar spray of $CuSo_4$ (100μm or 200 μm and IAA (20ppm or 30ppm) showed significant result to nullify the damaging effect of Pb to a great extent either by their individual treatment with Pd or by combined treatment of $CuSo_4$ along with IAA, whereas best amelioration was obtained with Pb+ $CuSo_4$ +IAA treatment (100μm or 200μm +30ppm + 200μm). From the overall experimental observation, conclusion may be drawn that heavy metal like Pb has a positive definite harmful effect over green gram seedlings even in low concentration and these damaging effect could be minimize with the help of micronutrients and hormonal treatments.

INTRODUCTION

Lead (Pb) is one of the toxic heavy metals discharged into the water system through the release of effluents from electroplating, metal finishing, batteries and paint industries (Zamzow *et al.*, 1990). It is also one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentrations are rapidly increased in agricultural soil (McGrath *et al.*, 1995). From soil to plants, transfer of this heavy metal is dependent on the following three factor: quantity factor (the total amount of potentially available elements), intensity factor (the activity as well as the ionic ratios of elements in the soil solution) and reaction kinetics (the rate of element transfer from solid to liquid phases and to plant roots) [Mathe-Gaspar *et al.*, 2005]. Lead's toxicity in plants cause a decrease in the percentage germination, seedling growth, and fresh and dry weight (Richa Marwari *et al.*, 2009) and also cause induced membrane changes such as qualitative and quantitative changes in membrane lipids, changes in structural and functional status of membrane depolarization of membrane, changes in membrane fluidity, inhibition of chloroplast electron transport, cause membrane lipid peroxidation and membrane leakiness, changes in enzyme activity of membrane bound enzymes, changes in membrane transport functions etc. Elevated Pb in soils may adversely affect on soil productivity and even a very low concentration can inhibit some vital plant process such as photosynthesis, mitosis and water absorption showing toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Patra *et al.*, 2004). It was found that the effect of foliar treatment by Pb has been found more toxic to plant growth than the root treatment with Pb (Salim *et al.*, 1992). The main objective of this investigation on green gram is to assess the toxicity effect of Pb and its amelioration effect by foliar application of hormone and micronutrients. Measurement of toxicity and its ameliorating treatments were analyzed by some biochemical parameters.

MATERIALS AND METHODS

Determination of chlorophyll content

Chlorophyll content was estimated by following method of Anon (1949). One gm of finely cut leaves was grinded with addition of 20 ml of 80% acetone and then centrifuged for 5 min in 5000 r.p.m. The residue was grinded in the 20 ml of 80% acetone and again centrifuged and transferred the

supernatant to 100mL volumetric flask and the volume was made up to 100 ml with 80% acetone. The absorbance was taken at 645 nm and 630 nm against the solvent in a spectrophotometer and calculated chlorophyll content in mg/g of tissue.

Assay of chlorophyllase enzyme activity

Chlorophyllase enzyme activity was measured by the method of Ardao and Vennesland (1960) and Holden (1961). Concentration of the substrate solution was made by 4 mL/g of leaf, 2g fresh leaves were taken, extracted in 50% acetone, centrifuged and the residue was dried at room temperature. Petroleum ether layer was discarded and acetone layer containing chlorophyllides was collected. The volume of this solution was made up to 12 mL with 80% acetone. Absorption was read at 670 nm in spectrophotometer. Enzyme activity was expressed in terms of % substrate split/hour/100 mg enzyme source.

Assay of catalase activity

The activity of this enzyme was assayed by the method of Chance and Machly (1955). Plant tissue was extracted in 0.05 M phosphate buffer (pH 7.4). The homogenate was centrifuged in cold at 10,000 rpm for 20 minutes. The residual H_2O_2 was filtrated against 0.01 (N) $KMnO_4$ and estimating the amount of $KMnO_4$ consumed in terms of H_2O_2 [0.01 (N) $KMnO_4$ = 0.17 mg H_2O_2], total H_2O_2 was calculated. The enzymatic activity was expressed in terms of unit enzyme activity/ min/g fresh weight.

Assay of peroxidase activity

The enzyme activity was assayed by following the method of Chance and Machly (1955). Plant material (leaf) was extracted with 0.05 M phosphate buffer (pH 7.4) and the homogenate was centrifuged in cold at 1000 r.p.m for 20 minutes. The absorbance of the solution was recorded at 0, 30, 60, 90 and 120 seconds after incubation at 420 nm in a spectrophotometer. The enzyme activity was expressed in terms of change in optical density at 420 nm per minute per mg protein.

Extraction and estimation of protein

Total protein was extracted from plant sample using in NaOH and total protein was examined, following the method of Lowry *et al.* (1951).

Extraction of DNA

The DNA extraction and estimation was assessed by the method of Doyle and Doyle (1987). The homogenate was transferred to a 250 mL flask and added 5 mL of 20% SDS and mixed thoroughly using a magnetic stirrer for 15 - 20 min. Potassium acetate (50 mL) solution was mixed and incubated at 0ºC for 30 min in order to precipitate proteins and polysaccharides. Removed the precipitates by centrifuging at 25,000 r.p.m for 15 min, to the supernatant add sixteenth volume of isopropanol and allowed to centrifuge, then stand at 20ºC for at least 20 min to precipitate DNA. Further pellet DNA was centrifuged at 20,000 r.p.m for 15 min and discarded the supernatant, drained off any liquid by inverting the tubes on filter paper for 2-3 min. Now redissolved the DNA pellet in suspension buffer (3 mL) with 1.8 mL

isopropanol and 180 mlit 3 M sodium acetate solution and allowed to stand at 20ºC for 1 hour. Repellent DNA obtained by centrifugation and washed with Ice - cold 80% ethanol and gently dried in vacuum or streaming nitrogen gas. Re-dissolved the DNA pellet in a suitable volume of (0.5 - 5 mL) TB buffer.

Estimation of DNA

Marked tubes were prepared with 1 mL, 2 mL and 3 mL aliquots of the isolated DNA dissolved in standard saline citrate and similar aliquots of a 0.5 mg DNA/ml standard solution was made for all this tubes. Added 6 ml of diphenylamine reagent to each tube and after mixing, the tubes were heated in boiling water bath for 10 min and later tubes were cooled down. The absorbance was recorded at 600nm against blank. A standard graph was prepared using A_{600}nm (ordinate) versus quantity of DNA. The concentration of DNA was calculated by

dissolving in saline citrate solution.

Extraction of RNA

A known weight of fresh plant tissue (100 mg) was homogenized in 4 mL of tris - HCl buffer. Added equal volume of water saturated phenol and centrifuge was done. Phenol layer was removed and added 1 mL of 1.3 M NaCl and 4 mL of phenol - cresol reagents and recentrifuged. The precipitate was washed with 80% ethanol and centrifuged and dissolve in 2 mL of 0.15 M Sodium acetate containing 0.25% SDS. Finally the pure RNA was precipitated by adding 6 ml of ethanol at 20ºC and allowed to stand for 1hour to get the result.

Estimation of RNA

RNA estimation was done by the colorimetric method. Prepared standard RNA (50 mgRNA/mL) solution in ice - chilled 10 mM Tris - acetate. 1 mM EDTA buffer (pH 7.2) or any other. Dissolved the isolated RNA in the above buffer solution to an approximate concentration 50 mg/mL. Prepared series of tubes containing 0.5 mL, 1 mL, 1.5 mL and 3 ml of isolated RNA, 0.5 mL, 1m; 1.5 mL and 3 mL of 50 mg standard. Added 0.4 mL of 6% alcoholic orcinol to each tube and then heat all the tubes in a boiling water bath for 20 min. The tubes were allowed to cool down and reading of the absorbance was taken at 660nm against the blank. Drawn standard curve using 660nm and the concentration of standard RNA and calculated the amount in the isolated RNA solution using the graph.

RESULTS AND DISCUSSION

Foliar treatment of $PbNo_3$ at the concentrations of 100μm and 200μm has been taken as standard solution in respect of intensity of damage. Whereas to ascertain the beneficial effect of $CuSo_4$ and IAA; 100μm, 200μm and 20ppm, 30ppm concentrations were taken respectively as standard after standardization of treatment solutions in several times. It was observed that Chl a, Chl b and total chlorophyll degrades significantly in Pb treated plants compared to control but the degradation of chlorophyll increase with the increase of Pb concentration. Damage was much more sensitive in Chl b with Pb than Chl a (Vodnik *et al.*, 1999) which indicated that chlorophyll synthesis was highly inhibited and this inhibition was reversed by $CuSo_4$ and IAA treatment. Plants treated with Cu and IAA alone showed maximum increase in Chl a, Chl b and total chlorophyll content than that of any other treatments.

Table 1: Effect of lead nitrate toxicity on green gram cv.B_1 and its alleviation by $CuSo_4$ and IAA treatment on chlorophyll a, chlorophyll b and total chlorophyll (mg/g fresh weight)

Treatment		Treatment concentrations	Chl a	Chl b	Chl t
T_0	Control	-	0.329	0.179	0.517
T_1	$PbNo_3$	100μm	0.304	0.069	0.341
T_2	$PbNo_3$	200μm	0.289	0.065	0.312
T_3	IAA	20ppm	0.383	0.16	0.551
T_4	IAA	30ppm	0.401	0.167	0.585
T_5	$CuSo_4$	100μm	0.382	0.199	0.575
T_6	$CuSo_4$	200μm	0.408	0.205	0.592
T_7	$PbNo_3$ + $CuSo_4$	100μm + 100μm	0.335	0.163	0.504
T8	$PbNo_3$ + $CuSo_4$	100μm + 200μm	0.345	0.17	0.513
T_9	$PbNo_3$ + $CuSo_4$	200μm + 100μm	0.314	0.155	0.503
T_{10}	$PbNo_3$ + $CuSo_4$	200μm + 200μm	0.361	0.162	0.509
T_{11}	$PbNo_3$ + IAA	100μm + 20ppm	0.344	0.151	0.493
T_{12}	$PbNo_3$ + IAA	100μm + 30ppm	0.361	0.177	0.511
T_{13}	$PbNo_3$ + IAA	200μm + 20ppm	0.337	0.154	0.503
T_{14}	$PbNo_3$ + IAA	200μm + 30ppm	0.341	0.158	0.51
T_{15}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 100μm	0.364	0.251	0.41
T_{16}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 100μm	0.38	0.077	0.433
T_{17}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 200μm	0.392	0.082	0.44
T_{18}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 200μm	0.403	0.085	0.441
T_{19}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 100μm	0.358	0.061	0.408
T_{20}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 100μm	0.369	0.067	0.411
T_{21}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 200μm	0.446	0.072	0.414
T_{22}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 200μm	0.386	0.078	0.418
	EMS		0.0064	0.00401	0.00005
	SEm		0.0145568	0.03656501	0.004061
	CD (5%)		0.0414892	0.10421613	0.011575

EMS = Error Mean Square, SEM = Standard Error Mean, CD = Critical Differences

Table 2: Effect of lead nitrate toxicity on green gram cv.B_1 and its alleviation by $CuSo_4$ and IAA treatment on chlorophyllase (%substrate splitted/hr/100mg enzyme), catalase (m mol H_2O_2 decomposed/mg protein/min) and peroxidase (unit/mg protein/min) activities

Treatment		Treatment concentrations	CHLASE	CAT	POX
T_0	Control	-	31.441	5.665	0.492
T_1	$PbNo_3$	100μm	42.803	6.866	0.587
T_2	$PbNo_3$	200μm	43.253	6.944	0.598
T_3	IAA	20ppm	31.066	5.693	0.418
T_4	IAA	30ppm	30.946	5.591	0.407
T_5	$CuSo_4$	100μm	27.416	4.906	0.454
T_6	$CuSo_4$	200μm	26.993	4.849	0.428
T_7	$PbNo_3$ + $CuSo_4$	100μm + 100μm	32.911	4.973	0.525
T8	$PbNo_3$ + $CuSo_4$	100μm + 200μm	31.831	4.821	0.515
T_9	$PbNo_3$ + $CuSo_4$	200μm + 100μm	32.795	4.993	0.501
T_{10}	$PbNo_3$ + $CuSo_4$	200μm + 200μm	31.907	4.805	0.5
T_{11}	$PbNo_3$ + IAA	100μm + 20ppm	41.368	6.69	0.545
T_{12}	$PbNo_3$ + IAA	100μm + 30ppm	41.068	6.488	0.527
T_{13}	$PbNo_3$ + IAA	200μm + 20ppm	41.721	6.64	0.517
T_{14}	$PbNo_3$ + IAA	200μm + 30ppm	40.949	6.106	0.507
T_{15}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 100μm	33.713	5.911	0.477
T_{16}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 100μm	33.111	5.801	0.47
T_{17}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 200μm	32.973	5.613	0.456
T_{18}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 200μm	32.833	5.185	0.448
T_{19}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 100μm	33.665	5.975	0.496
T_{20}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 100μm	33.653	5.695	0.481
T_{21}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 200μm	33.599	5.623	0.473
T_{22}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 200μm	39.537	5.305	0.449
	EMS		5.77700	0.00646	0.00008
	SEm		1.387684	0.046411	0.00503
	CD (5%)		3.955121	0.132279	0.014336

It has also been observed that $CuSo_4$ or IAA or their combination treatments helps to revert back the toxic effect of Pb in respect of chlorophyll retention (Table 1). These results indicate that Cu and IAA had maximum beneficial effect to restore the functional component of photosynthetic apparatus (Abdel and Abdel, 1995).

Table 3: Effect of lead nitrate toxicity on green gram cv.B_1 and its alleviation by $CuSo_4$ and IAA treatment on protein (mg/g fresh weight), DNA and RNA (mg/g fresh weight) content

Treatment		Treatment concentrations	Protein	DNA	RNA
T_0	Control	-	287.667	9.707	0.943
T_1	$PbNo_3$	100μm	268.251	9.033	0.859
T_2	$PbNo_3$	200μm	266.032	8.837	0.827
T_3	IAA	20ppm	292.333	9.85	0.948
T_4	IAA	30ppm	293.331	9.98	0.986
T_5	$CuSo_4$	100μm	290.667	9.887	0.971
T_6	$CuSo_4$	200μm	291.658	10.087	0.987
T_7	$PbNo_3$ + $CuSo_4$	100μm + 100μm	288.564	9.20	0.945
T8	$PbNo_3$ + $CuSo_4$	100μm + 200μm	291.25	9.293	0.951
T_9	$PbNo_3$ + $CuSo_4$	200μm + 100μm	288.12	9.147	0.942
T_{10}	$PbNo_3$ + $CuSo_4$	200μm + 200μm	290.084	9.26	0.95
T_{11}	$PbNo_3$ + IAA	100μm + 20ppm	290.078	9.273	0.948
T_{12}	$PbNo_3$ + IAA	100μm + 30ppm	291.335	9.34	0.954
T_{13}	$PbNo_3$ + IAA	200μm + 20ppm	287.356	9.22	0.943
T_{14}	$PbNo_3$ + IAA	200μm + 30ppm	288.661	9.3	0.951
T_{15}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 100μm	291.339	9.85	0.957
T_{16}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 100μm	292.664	9.917	0.96
T_{17}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 20ppm + 200μm	292.03	10.1	0.964
T_{18}	$PbNo_3$ + IAA + $CuSo_4$	100μm + 30ppm + 200μm	293.688	10.24	0.969
T_{19}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 100μm	290.125	9.78	0.954
T_{20}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 100μm	290.669	9.867	0.958
T_{21}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 20ppm + 200μm	292.335	9.977	0.961
T_{22}	$PbNo_3$ + IAA + $CuSo_4$	200μm + 30ppm + 200μm	293.336	10.08	0.962
	EMs		1.233	0.00302	0.000130
	SEm		0.641092817	0.031728	0.006575
	CD (5%)		0.61661322	0.09043	0.01874

EMS = Error Mean Square, SEM = Standard Error Mean, CD = Critical Differences

Enzymatic analysis of chlorophyllase, catalase and peroxidase were done to ascertain the quantum of damage caused by heavy metal Pb and the activity of said enzyme after amelioration effect. These enzymes are very specific in their action of fluctuates within the plants to the extents of any type of stresses. Table 2 represents the activities of these enzymes under different treatment condition. Result indicated that chlorophyllase activity was significantly increased in Pb treated plants compared to control. The chlorophyllase activity was much less in Pb effective plants treated with $CuSo_4$ and IAA. The enzyme chlorophyllase is a degradative enzyme which breaks chlorophyll molecule of the plants and drastically hampers the photosynthetic processes of the plants. From the results it may concluded that Cu and IAA helps to restore the photosynthetic apparatus as well as decrease of chlorophyllase activity. This conclusion might substantiate by the work of Keshan and Mukherjee (1992) in mung bean where they opined that chlorophyllase enzyme destabilized the structure of the chlorophyll molecule. In case of catalase, activity was much higher compared to peroxidase activity in Pb toxic plants over control. Catalase and peroxidase activities were more or less same like that of control in case of Cu and IAA single treatment, whereas catalase and peroxidase activities decreases significantly in Pb treated plants supplied with Cu and IAA in combination and showed best amelioration result with $Pb + CuSo_4 + IAA$. Catalase is antioxidative enzymes which negate the effect of oxyzen radical produced due to Pb toxicity that might be logical reason to get maximum catalase activity in Pb treatment and minimum in $CuSo_4$ treatment. It showed that there was a synergistic effect of Cu with IAA and Cu was much more prominent than that of IAA. This result suggest that lipid peroxidation is the overall sequence of primary effect of metal toxicity on membrane structure, the beneficial effect of Cu and IAA might be due to inhibition of lipid peroxidation (Cakmak and Horst, 1991). Pb^{2+} increased the activity of catalase and peroxidase and decreased the activity of super-oxide-dismutase (SOD) and the H_2O_2 level. The membrane damage was mediated by reactive oxygen species and hydroperoxides induced by Pb^{2+}. The behaviour of enzymes activities might be due to ROS formation in the tissue induced by Pb toxicity. As an adaptive mechanism antioxidative enzymes like catalase and peroxidase remove free radicals preventing the accumulation of O_2 (Halliwell and Gutterdge *et al.*, 1989). Lead significantly increased H_2O_2 accumulation in cells and decreased ascorbate peroxidase and glutathione reductase activities in green gram. The rate of tissue auto-oxidation was increased in the presence of high levels of heavy metal, indicating that lipid peroxidation of cell membrane was stimulated by endogenous active oxygen radicals (Lixin *et al.*, 1998).

The protein content of seeds collected from treated plants were studied and it was observed that protein content was significantly declined due to toxicity of Pb treatment, whereas protein accumulation much more higher in the amelioration treatments either by Cu or IAA or by combination of them over control (Table 3). This result substantiates the findings by Przymusinski *et al.*, 1991 and Stefanov *et al.*, 1995. Table-III also represents the DNA and RNA content from the seeds of treated plants and found that RNA content decreased with the Pb treated plants but in ameliorating treatments RNA become restored like that that of control. From the results it may be concluded that RNA become much more sensitive than that

DNA in respect of Pb toxicity (Maitra and Mukherjee, 1977). It had already been reported that DNA damage was prominent in presence Pb which causes disturbances in cromatin structure and mutagenesis as well as increase in the activity of DNAase and lowered DNA synthesis and stability (Mullar *et al.,* 2000). It was also reported that RNA synthesis was decreased due to heavy metal toxicity by activating ribonuclease activity (Nagoor and Vyas, 1999).

The experimental data clearly indicate that heavy metal has positive damaging effect on plants even in low concentration; however, the damaging effect can be overcome by hormonal as well as micronutrient treatment to nullify the effect to a great extent.

REFERENCES

Abdel-AAL, A. E. and Abdel-Nasser, L. E. 1995. Effect of cadmium and lead ions on the growth characteristics, chlorophyll content and some photosynthetic enzymes activity in maize (*Zea mays* L.) seedlings. Alexandria *J. Agricultural Research.* **40(1):** 317-338.

Anon, D. I. 1949. Copper enzymes in isolates chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24:** 1-15.

Ardao, C. and Venneesland, B. 1960. Chlorophyllase activity of spinach chloropiastin. *Plant Physiol.* **35:** 368-371.

Cakmak, I. and Horst, W. J. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max* L.). *Physiol Plant.* **83:** 463-468.

Chance, B. and Maehly, A. C. 1955. Assay of catalase and peroxidase. *Methods Enzymology.* **2:** 764-775.

J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull.* **19**:11-15.

Halliwell, B. and Gutteridge, J. M. C. 1989. Free radicals in Biology and Medicine, Third ed, Oxford Science Publications, New York.

Holden, M. 1961. The breakdown of chlorophyll by chlorophyllase. *Biochem. J.* **78:** 359-364.

Keshan, V. and Mukherji, S. 1992. Effect of Cadmium toxicity on chlorophyll content, Hill activity and chlorophyllase activity in *Vigna radiata* L. leaves. *Indian J. Plant Physiol.* **35(3):** 225-230.

Lixin, L. TieHeng, S. YueHua, J. Luo, L.X. Sun, T.H. and Jin, Y.H. 1998. Effect of cadmium stress on lipid peroxidation in wheat leaves. *China Environmental Science.* **18(1):** 72-75.

Lowry, O. H, Rosebrough, N. J. Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry.* **193:** 265-272.

Marwari, R., Kala, M. and Khan, T. I. 2009. Heavy metals analysis in *Lycopersicum esculentum* L. growing in Sanganer area. *The Ecoscan.* **3(1&2):** 69-74.

Mathe-Gaspar, G., Peter-Mathe, L., Szabo, B., Orgovanyi, N., Uzinger. and Anton, A. 2005. After-effect of heavy metal pollution in a brown forest soil. *Acta Biol Szegediensis.* **49(1-2):** 71-72.

Maitra, P. and Mukherjee, S. 1977. Toxic effects of lead on growth and metabolism of germinating rice (*Oryza sativa* L.) root cells. *Ind. J. Exp. Biol.* **45:** 519-521.

McGrath, S. P. Chaudri, A. M. and Giller, K. E. 1995. Long term effects of metals in sewage sludges on soils microorganisms and plants. *J. Ind. Microbiol.* **14:** 94-104.

Muller, J. Sigel, R.K.O. and Lipperst, B. 2000. Heavy metal mutagenicity: insights from inorganic model chemistry. *J. Inorg. Biochem.* **79:** 261-265.

Nagoor, S. and Vyas, A. V. 1999. Physiological and biochemical responses of cereal seedlings to graded levels of heavy metals. *Annals of Plant Physiology.* **13(2):** 181-186.

Patra, M., Bhowmik, N., Bandopadhyay, B. and Sharma, A. 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Exp. Bot.* **52:** 199-223.

Przymusinski, R. Spychala, M. and Gwozdz, E. A.1991. Inorganic lead changes growth polypeptide pattern of lupin roots. *Biochem. Physiol. Pflan.* **187:** 51-57.

Salim, R. Al. Subu, M. M. Douleh, A. and Khalaf, S. 1992. Effects on growth and oxidative metabolism of pea plants. *J. Experimental Botany.* **52(263):** 2115-2126.

Stefanov, K., Siezova, K., Popova, I., Petkov, V. L., Kimenov, G., Popov, S.1995. Effect of leadions on the phospholipid composition in leaves of *Zea mays* and *Phaseolus vulgaris*. *J. Plant Physiol.* **147:** 243-246.

Vodnik, D. Jentschke, G. Fritz, E. Gogala, N. and Godbold, D. L.1999. Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. *Physiol. Plant.* **106:** 75-81.

Zamzow, M., Eichbaum, B., Sandgren, K. and Shanks, D. 1990. Removal of heavy metals and other cataions from wastewater using *Zeolites. Sep. Sci. Tech.* **25:** 1555-1569.