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TOXIC HEAVY METAL STRESS IN PADDY: METAL ACCUMULATION PROFILE AND DEVELOPMENT OF A NOVEL STRESS PROTEIN IN SEED

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SUMMARY

Impact of toxic heavy metals lead, mercury and cadmium have been studied in paddy with respect to their impact on harvest index, metal accumulation in grain and plant body and seed protein profile. All the three metals brought about significant decline in harvest index. Lead treatment resulted in 8.85 to 14.06% decline in harvest index compared to control. Mercury also showed similar effect, but cadmium treatment caused 14.29 to 25.0% decline in harvest index. For all the three metals accumulation was highest in roots, ranging from 53.0 to 56.0% for lead, 47.0 to 54.0% for mercury and 66.0 to 69.0% for cadmium. Following roots, accumulation was in descending order in stem, leaf sheath, grain and leaf. In grains, accumulation was 7.0 to 8.0% for lead, 10.0 to 11.0% for mercury and 6.0 to 8.0% for cadmium, clearly indicating the possible health hazard. Seed protein profile analysed through SDS-PAGE revealed elimination of a high molecular weight protein (≥ 120) for mercury and cadmium treatment generated a novel 66.5 kDa stress protein.

***Key words*: Heavy metals, paddy, stress protein.**

INTRODUCTION

Environmental deterioration has generated an increase of stress in all forms of life. Of these, stress on agricultural crop is of prime importance since agriculture is lifeline of global society. Abiotic stresses like water stress, salinity stress, high temperature stress are known to adversely affect growth and grain yield of paddy (Pareek *et al*. 1999). Along with these stresses, toxic heavy metal stress is an emerging and more dangerous stress for paddy as well as of other major crops. Metals like lead, mercury, cadmium, arsenic, chromium have no biological function and are toxic to life even at very low concentration (Salt *et al.* 1995). Pollution of soil and water due to toxic heavy metals is mostly of anthropogenic origin and there are many records that agricultural land adjacent to industrial areas are polluted to varied extent by many toxic heavy metals (Klein 1972, Rao 1979). But the most alarming fact is that the water and sediments of Ganga river contain high level of toxic heavy metals like Zn, As and Cr (Saikia *et al.* 1988, Singh *et al.* 1992) particularly in the middle stretch. Paddy fields are generally low-lying areas with bunds erected in such a manner that water accumulate. These facilitate deposition of toxic metals through surface run off water. Moreover, due to inadequate irrigation use of sewerage water is a common practice in India. Different plants absorb toxic and non-toxic heavy metals from soil and water to varied extent and accumulate in different body parts (Chamber and Sidle 1991). This is a matter of serious concern because paddy is the principal food for one third of world population (Anonymous 1991) and staple food for about 65% of India's population.

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Bioaccumulation of toxic heavy metals by various crop plants have been recorded by a number of workers and is a matter of serious health hazard (Mishra and Singh 2000, Ray 1987, Semu *et al.* 1985). Following accumulation in plant, heavy metals enter food chain and the relationship of heavy metal with soil-plant-herbivore in agriculture is well documented (Donovan *et al.* 1969). Crops like paddy are known to respond to abiotic stress like salinity, drought, temperature etc. by synthesizing some stress protein (Pareek *et al.* 1998, 1999). The present study was undertaken to study the response of paddy to three common and widespread heavy metals, viz. lead, mercury and cadmium with particular emphasis on accumulation pattern and synthesis of metal specific stress protein.

MATERIALS AND METHODS

Paddy cv. Mashuri was the plant material used for the present study. Since nitrate forms of heavy metals have high solubility and nitrate ions have no adverse effect on plant, therefore, nitrate salts of lead, mercury and cadmium, viz. cadmium nitrate [$Cd(NO_3)_2 4H_2O$] mercuric nitrate [$Hg(NO_3)_2 H_2O$] and lead nitrate [$Pb(NO_3)_2$] were used .

Soil were collected from farmer's field by digging a pit of 8 inch deep and collecting soil from the depth profile. Soil and FYM were dried and thoroughly mixed at the ratio of 4: 1 followed by thorough mixing of calculated amount of metal salt. The amended soil was filled in plastic tubs of size 25 cm in height and 23 cm in diameter. Each tub was filled with 7 kg amended soil. The controlled set comprised of unamended soil. The amount of heavy metal in corresponding salt was estimated by dividing molecular weight of the heavy metal salt by atomic weight of the heavy metal.

The concentration of heavy metals was viz 100, 300, 500, 700 and 900 mg kg^{-1} for both lead and mercury while for cadmium the same were 100, 200, 300, 400 and 500 mg kg^{-1}. The plastic tubs filled with amended and unamended soil were allowed to stand for 14 days. Thereafter one month old paddy seedlings were transplanted. Watering was done at regular interval and the tubs were stirred at regular interval for better aeration around the root zone. Stirring was done by inserting a glass rod into the tub and moving it gently along the inner face of the tub in vertical position. Transparent polythene sheet was erected during rainy season to avoid flooding of the tubs. After the plants matured and the seeds were ripe observations were recorded for straw yield and grain yield and harvest index was calculated as percentage of grain yield divided by total biological yield.

At the time of harvest the plants were carefully uprooted, washed clean, sun dried and the plant parts were physically segregated, viz . root, stem, leaf sheath and leaf to analyse the content of heavy metal in different parts. Estimation of lead and cadmium were done by atomic absorption spectrometer (Perkin Elmer 2350). The samples were initially sun dried followed by drying in oven at 70°C till constant weight was obtained. Subsequently the samples were ashed in a muffle furnace at 570°C for 3 hours. From the ash acid extract was prepared as per the method described by Kalita (1997). For estimation of mercury the samples were oven dried at 30°C. From the finely cut dry samples acid extract were prepared as per the method described by Munns and Holland (1977). From the acid extract mercury estimation was done in a mercury analyser. Three replication were made for each treatment and the data were analysed by analysis of variance.

For SDS-PAGE analysis the manually dehusked grains were washed with distilled water, blotted dry and 300 mg dehusked grains were grounded in a pre-chilled morter with ice cold 0.5 M Tris buffer pH 6.5. The samples were homogenised in a vortex shaker and centrifuged in a refrigerated centrifuge at 8000 rpm for 10 minutes at 4°C. Sample weight to extract volume was adjusted to 1:4 ratio. SDS-PAGE analysis was carried out as per the method described by Laemmli (1970) with 15% separating gel and 4% stacking gel. 50 µl extract were properly mixed with sample buffer and loaded. The gel was run at 12 mA for 30 minutes and then at 30 mA for 8 hours. The gel was stained with 0.1% Coomassie Brilliant Blue R-200 to develop the bands. To determine the molecular weight of the individual protein bands standard molecular weight marker (PMW-M from Bangalore Genei) was co-electrophoresed.

RESULTS AND DISCUSSION

The results clearly show that due to treatment with lead, mercury and cadmium there was a decline in harvest index compared to control, which was significant. However, due to differences in the doses of metal there is not much significant differences except in high doses of the metals. The decline in harvest index due to lead (Table 1) and mercury (Table 2) are comparable but cadmium (Table 3) induces comparatively more decline. A number of workers have observed that due to heavy metal treatment there is a decline in biomass yield mostly in a dose dependant manner. Shaikh *et al.* (2001) observed reduction in biomass yield in brinjal following exposure to cobalt and cadmium. Mukherjee and Maitra (1976) observed a reduction in photosynthetic pigments in paddy following exposure to lead. Both groups of authors are of the opinion that reduction in photosynthetic pigments slow down photosynthesis whereby synthesis and accumulation of carbohydrate are affected ultimately causing a decline in biomass yield. Similar observation were recorded by Xiong (1997) for cabbage working with lead; Tomar *et al.* (2000) for *Vigna radiata* due to lead; Sarma and Sarma (1996) for wheat due to chromium. Therefore it appears that decline in biomass yield due to exposure to heavy metal is a general trend. However, the extent of decline in harvest index may be different depending upon the type of metal and in the present study cadmium appears to be most toxic. Deka Baruah (1999) working with Citronella observed that among lead, mercury and cadmium, cadmium was most toxic. This observation was further confirmed by Handique (2000) for lemongrass.

Table 1. Biomass yield of paddy following treatment with lead.

Concentration of lead mg kg^{-1}	Straw yield (g)	Grain yield (g)	Harvest index (%)
00	91.61	40.47	30.60
100	91.05	37.55	27.90
300	84.32	32.37	27.70
500	77.95	29.03	27.10
700	69.44	25.50	26.80
900	62.50	22.30	26.30
C.D.5%	2.75	1.278	0.340
1%	5.83	2.714	0.720

Table 2. Biomass yield of paddy following treatment with mercury.

Concentration of mercury mg kg^{-1}	Straw yield (g)	Grain yield (g)	Harvest index (%)
00	91.61	40.47	30.60
100	90.64	35.70	27.6
300	82.82	31.14	27.3
500	73.43	27.60	27.3
700	65.36	24.35	27.1
900	56.90	21.00	26.9
C.D 5 %	2.241	1.121	0.310
1%	4.750	2.376	0.657

Table 3. Biomass yield of paddy following treatment with cadmium.

Concentration of cadmium mg kg^{-1}	Straw yield (g)	Grain yield (g)	Harvest index (%)
00	91.61	40.47	30.60
100	64.99	23.11	26.23
200	59.67	20.76	25.81
300	54.65	17.17	23.90
400	48.25	15.20	23.95
500	43.80	13.05	22.95
C.D.5%	2.433	1.011	0.464
1%	5.157	2.143	0.983

Accumulation of heavy metals was studied after all field observations were over and the plants attained full maturity and so the values represent final accumulation level. In case of lead, highest accumulation was found in root ranging from 220.80 to 265.50 mg g^{-1} dm representing 52.88% to 56.47% of total accumulated lead. The second highest accumulation was found in case of stem followed by leaf sheath. It is noteworthy that lead accumulations in grains were little higher than the corresponding values for leaf, which was in the range of 30.0 to 36.0µg g^{-1} dm representing. 7.17% to 7.67% of total accumulated lead (Table 4). Irrespective of the accumulation profile the singular factor which influences most the absorption and accumulation is the dose of lead in the soil i.e. there were dose dependant increase in accumulation in all the organs. Mercury accumulation profile was similar and comparable to that of lead – highest accumulation was in root, followed by stem and leaf sheath (Table 5). However, compared to lead, mercury accumulation was considerably higher in grain, which was in the range of 40.0 to 63.0 µg g^{-1} dm representing. 9.83 % to 11.15 % of total accumulation. Cadmium accumulation pattern was similar to that of lead and mercury but on a comparative basis cadmium accumulation was much higher (Table 6). For instance cadmium accumulation in root were in the range of 420.2 to 857.5 µg g^{-1} dm, which was 2 to 3 times higher than the corresponding values for lead and mercury. Moreover, unlike lead and mercury cadmium accumulation was lesser in grain than that of lead. However the values were still higher compared to that of lead and mercury. Due to cadmium treatment leaves

Table 4. Accumulation of lead in different plant parts of paddy at harvesting in pot experiment (µg g^{-1} dm)

Concentration of lead ($mg\ kg^{-1}$)	Lead accumulation ($\mu g\ g^{-1}$ dm)				
	Root ($\mu g\ g^{-1}$ dm)	Stem ($\mu g\ g^{-1}$ dm)	Leaf sheath ($\mu g\ g^{-1}$ dm)	Leaf ($\mu g\ g^{-1}$ dm)	Grain ($\mu g\ g^{-1}$ dm)
00	00	00	00	00	00
100	220.80	60.00	60.20	20.00	30.00
300	240.00	80.30	60.50	28.50	30.80
500	246.50	89.50	63.80	31.50	34.30
700	260.00	91.50	65.50	34.50	35.50
900	265.50	94.50	66.00	40.00	36.00
Treatment S. Ed ±	0.8	0.27	0.24	0.24	0.41
C.D. (0.05)	5.04	1.58	1.08	1.23	1.14
C.D. (0.01)	8.14	2.25	1.54	1.75	1.63

Table 5. Accumulation of mercury in different plant parts of paddy at harvesting in pot experiment (µg g^{-1} dm)

Concentration of mercury ($mg\ kg^{-1}$)	Mercury accumulation ($\mu g\ g^{-1}$ dm)				
	Root ($\mu g\ g^{-1}$ dm)	Stem ($\mu g\ g^{-1}$ dm)	Leaf sheath ($\mu g\ g^{-1}$ dm)	Leaf ($\mu g\ g^{-1}$ dm)	Grain ($\mu g\ g^{-1}$ dm)
00	00	00	00	00	00
100	221.50	65.50	56.30	23.50	40.00
300	250.00	85.00	66.50	30.70	46.10
500	256.00	90.50	80.30	39.40	54.50
700	266.00	94.30	93.50	41.50	57.00
900	269.50	92.20	96.00	44.10	63.00
Treatment S. Ed ±	0.29	0.28	0.22	0.29	0.40
C.D. (0.05)	5.25	1.43	1.73	1.37	1.34
C.D. (0.01)	8.71	2.04	2.47	1.95	1.91

Table 6. Accumulation of cadmium in different plant parts of paddy at harvesting in pot experiment (µg g^{-1} dm)

Concentration of cadmium ($mg\ kg^{-1}$)	Cadmium accumulation ($\mu g\ g^{-1}$ dm)				
	Root ($\mu g\ g^{-1}$ dm)	Stem ($\mu g\ g^{-1}$ dm)	Leaf sheath ($\mu g\ g^{-1}$ dm)	Leaf ($\mu g\ g^{-1}$ dm)	Grain ($\mu g\ g^{-1}$ dm)
00	00	00	00	00	00
100	420.20	80.00	67.50	36.60	43.00
200	424.80	85.50	80.30	52.50	46.10
300	460.00	107.50	91.00	67.80	61.50
400	620.40	129.20	120.30	80.00	65.30
500	857.50	144.50	139.50	81.50	71.50
Treatment S. Ed ±	0.29	0.20	0.06	0.27	0.40
C.D. (0.05)	9.17	2.01	1.88	1.71	1.56
C.D. (0.01)	13.27	2.87	2.68	2.44	2.22

turned yellowish at high concentration; such yellowing was not seen in case of lead and mercury.

The available information is a clear pointer to the fact that heavy metal absorption and accumulation are species specific, metal specific and tissue specific. It appears to be a general trend that maximum accumulation occurs in root followed by stem and other aerial parts as shown by Deka Baruah (1999) in Java Citronella, Handique (2000) in Lemongrass, McGrath (1997) in *Thlaspi caerulescens* etc. Shen *et al.* (1997) working with heavy metals like chromium, copper, manganese, nickel, cadmium and lead found that irrespective of the metal highest accumulation was in root in the test plants *Thlaspi caerulescens* and *T. ochroleucum*. Studies by a number of workers have shown that most plants have a tendency to preferentially absorb and accumulate cadmium in greater quantity than other metals (Wadge and Hutton 1986, Salt *et al.* 1995, Deka Baruah 1999, Handique 2000, Mishra and Singh 2000). In the present study the order of absorption and accumulation was as Cadmium > Mercury > Lead.

It is noteworthy that compared to many other plants paddy has considerable tolerance to cadmium. For some Gramineae family plants like Citronella (Deka Baruah 1999), Lemongrass (Handique 2000) that are hardy perennials, even 200 mg kg^{-1} cadmium is fatal. The present study shows that paddy can withstand even 500 mg kg^{-1} cadmium. But the matter of serious concern is that accumulation of such a high level of lead (minimum 30 $\mu g\ g^{-1}$), mercury (minimum 40 $\mu g\ g^{-1}$) and cadmium (minimum 43 $\mu g\ g^{-1}$) in paddy grain is a possible public health hazard in near future. Equally serious concern is the high accumulation of all the three metals in stem, leaf and leaf sheath. Paddy straw is used as a fodder for domestic herbivores particularly cattle. Therefore it is another route of heavy metal entering the food chain because there is every likelihood that through consumption of such metal contaminated fodder the metals will eventually find its way to milk.

Gel electrophoresis profile reveals the presence of 14 protein bands in the seed of control set, which are storage protein. The proteins ranged in size from 120.0 to 15.3 kDa. The most prominent among them were the ones with molecular weight 64.5, 54.0, 41.2 and 32.2 kDa. In the present study due to heavy metal treatment both qualitative and quantitative changes were observed for seed protein profile. Following lead treatment (Fig. 1A) there was no change in the protein profile except that the major four bands were thicker and deeper in

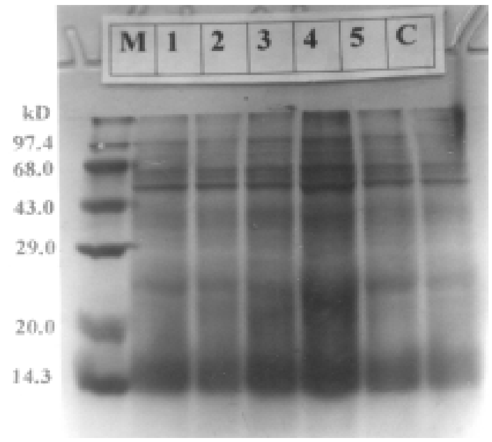

Fig. 1a. Seed protein profile following treatment with lead. Lane M: Marker; Lane 1 to 5 - treatment with 100, 300, 500, 700 and 900 mg kg^{-1} lead respectively C- Control

colour intensity than that of control. Mercury treatment (Fig. 1B) resulted in the elimination of the high molecular weight protein band of 120 kDa size. Except this there was no change in protein profile, particularly the four characteristic bands were unaffected and comparable to those of control in both colour intensity and thickness. Following cadmium treatment (Fig. 1C) a new high molecular weight protein band appeared in all the

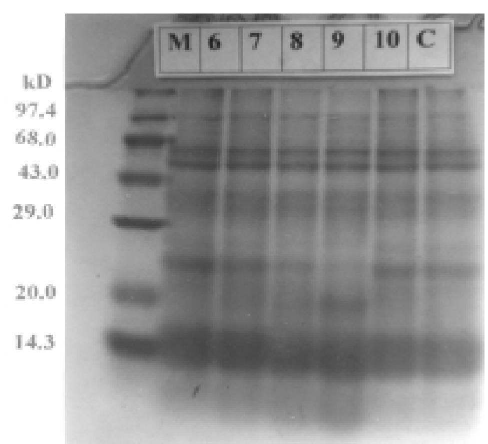

Fig. 1b. Seed protein profile following treatment with mercury. Lane M: Marker; Lane -6 to 10-treatments with 100, 300, 500, 700 and 900 mg kg^{-1} mercury respectively. C- Control.

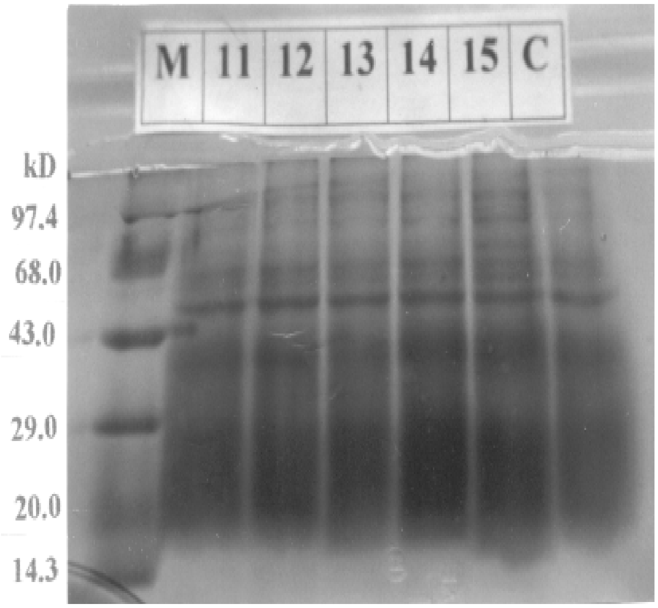

Fig. 1c. Seed protein profile following treatment with cadmium showing the new protein band of 66.5kd.
Lane M: Marker; Lane 11 to 15-treatment with 100, 200, 300, 400 and 500 mgkg^{-1} cadmium. C- Control.

treatments whose size was deduced to be 66.5 kDa. No such band appeared following treatment with either lead or mercury. Except this new band the protein profile remained more or less comparable to that of the control.

Studies by a number of workers have established that plants respond to various abiotic stresses at biochemical, physiological level as well as molecular level by synthesizing a class of stress protein or altering the expression of protein resulting in a change in protein profile or a combination of both. Pareek *et al.* (1999) working with paddy (cv. Pusa 169) observed that paddy respond to abiotic stress like salinity, water stress, high temperature by synthesizing a class of stress protein. While the profile of stress protein varies at different growth stages some stress proteins were common to all stages of growth, viz. proteins with 30.0, 22.5 and 22.0 kDa in response to salinity stress. Similar stress proteins due to salinity or water stress have been reported for mungbean by Chakraborty and Mukherjee (2002); *Lathyrus sativus* by Sinha *et al*. (1999). However, stress due to salinity, water or high temperature are quite different from that of heavy metal stress. Many plants respond to heavy metal stress by synthesizing a class of metal binding polypeptide refered to as phytochelatin (PC) and cadmium is a strong inducer of phytochelatin which are low molecular weight protein (Huang *et al*. 1987). Another class of metal stress protein are known as metallothionin (MT), which are known to be aggregates of phytochelatin (Prasad 1995). However the cadmium induced stress protein found in the seed in the present study is unlikely to be phytochelatin or metallothionin, since MTs are low molecular weight protein in the range of 6-7 kDa (Majare and Bulow 2001); while phytochelatins are still smaller with size in the range of 1.5 – 4 kDa (Prasad 1995). While it is difficult to pinpoint the reason for such changes in protein profile it appears that through such changes in protein profile the plant try to adapt to or overcome heavy metal stress.

REFERENCES

Anonymous. (1991). The IRRI Reporter, 3, 4, IARI, Phillipines.

Chakraborty, N. and Mukherjee, S. (2002). Effect of GA_3 on amino acid and protein content and on electrophoretic profile of leaf protein in mung bean under salt stress. Res. *J. Chem.Environ.* **6**: 33-36.

Chambers, J.C. and Sidle, R.C. (1991). Fate of heavy metal in abandoned lead, zinc tailing ponds: I Vegetation. *J. Environ. Qual.* **20**: 745-748.

Deka Baruah, H.P. (1999). Biochemical and physiological assessment of the impact of heavy metal pollutants on Java Citronella (*Cymbopogon winterianus* Jowitt) Ph.D Thesis, Gauhati University, Guwahati, Assam.

Donavan, P.P., Felly, D.T. and Caravan, P.P. (1969). Lead contamination of mining areas in W. Ireland. Survey of animal pastures, food and waters. *J. Sci. Food Agric*. **20**: 43-45.

Handique, G.K. (2000). Bioremediation potentiality of lemongrass (*Cymbopogon flexuosus* Stapf.) against toxic heavy metal pollutants. Ph.D Thesis, Gauhati University, Guwahati, Assam.

Huang, B., Hatch, E. and Goldsbrough, P.B. (1987). Selection and characterization of cadmium tolerant cells in tomato. *Plant Sci.* **52**: 211-221.

Kalita, D. (1997). Identification of organometallic and trace elements in soil sample by atomic absorption spectroscopy. Proc. Forensic Sci. Conf., pp. 21-25. Bhubaneswar.

Klein, D. (1972). Mercury and other metals in urban soils. *Environ. Sci. Technol.* **6**: 560-562.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **227**: 680-685.

McGrath, S.P., Shen, Z.G. and Zhao, F.J. (1997). Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. *Plant & Soil* **188**: 153-159.

Mejare, Malin and Bulow, Leif (2001). Metal binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends in Biotech.* **19**: 67-73.

Mishra, S.N. and Singh, D.B. (2000). Accumulation of lead and cadmium in upper parts of mustard (*Brassica juncea*) seedlings in response to putrescine. *Indian J. Expt. Biol.* **38**: 814-818.

Mukherjee, S. and Maitra, P. (1976). Toxic effect of aluminium on growth and nitrogen fixation in vegetable soybean germplasm. *Plant & Soil* **136**: 1976; 25-29.

Munns, Robert K. and Holland, David C. (1977). Rapid digestion and flameless atomic absorption spectroscopy of mercury in fish. Collaborative study. J.A.O.A.C. **60:** 833-837.

Pareek, Ashwani, Singla, Sneh Lata and Grover, A. (1998). Proteins alteration associated with salinity, dessication, high and low temperature stresses and abscisic acid application in seedlings of Pusa 169 – A high yielding rice (*Oryza sativa* L.) cultivar. *Curr. Sci.* **75**: 1023 – 1035.

Pareek, Ashwani, Singla, Sneh Lata and Grover, A. (1999). Analysis of stress proteins at four different developmental stages in field grown rice, *Oryza sativa* L. (cv. Pusa 169) plants. *Curr. Sci.* **76**: 81-86.

Prasad, M.N.V. (1995). Cadmium toxicity and tolerance in vascular plants. *Environ. Exp. Bot.* **35**: 525-545.

Rao Ramani, I.V. (1979). Measurement and characterisation of some heavy metals Hg, Pb, Cd, and Cu in the aquatic environment of the Kalu river. M.Sc Thesis of the University of Bombay.

Ray, M. (1987). Plants as indicator of environmental pollution. In: XIV International Botanical Congress, Abst. No. 6; 147-155. Berlin, Germany.

Saikia, D.K., Mathur, R.P. and Srivastava, R.K. (1988). Heavy metals in water and sediments of upper *Ganga. Indian J. Environ. Hlth.* **31**: 11-17.

Salt, D.E., Blaylock, M., Kumar, N.P.A., Sushenkov,V., Ensley, B.D., Chet, I. and Raskin, I. (1995). Phytoremediation – A novel strategy for removal of toxic metals from the environment with plants. *Biotechnology* **13**: 468- 474.

Semu, E., Singh, B.R., Selmer-Olsen, A.R. and Steenberg, K. (1985). Uptake of Hg from ^{203}Hg labelled mercury compounds by wheat and beans grown on an oxisol. *Plant Soil* **87**: 347-355.

Shaikh T., Dube, B.K., Sinha, P. and Chatterjee, C. (2001). Studies on the growth, yield and metabolism of brinjal (*Solanum melongena* L.) coupled with the uptake of cobalt and cadmium. *J. Environ. Poll* **8**: 159-164.

Sharma, D.C. and Sharma, P.C. (1996). Chromium uptake and toxicity effects on growth and metabolic activities in wheat, *Triticum aestivum* L. UP 2003. *Indian J. Expt. Biol.* **34**: 689-691.

Shen, Z.G., Zhao, F.J. and McGrath, S.P. (1997). Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-accumulator *Thlaspi ochroleucum. Plant Cell and Environ.* **20**: 898-906.

Singh, H.P., Mahavir, L.R. and Mishra, J.P. (1992). Heavy metals in sediments and water in the river Ganga. *J. Assam Sci. Soc.* **34**: 52-56.

Sinha, K.M., Sachdeva, Archana, Johri, R.P. and Mehta, S.L. (1999). Stress induced polypeptides in *Lathyrus sativus. J. Plant Biochem. Biotech.* **8**: 47-51.

Tomar, Manju Kaur, Inderdeep, Neelu and Bhatnagar, A.K. (2000). Effect of enhanced lead in soil on growth and development of *Vigna radiata* (linn.) Wilczek. *Indian J. Plant Physiol.* **5**: 13-18.

Wadge, A. and Hutton, M. (1986). The uptake of cadmium, lead and selenium by barley and cabbage grown on soils amended with refuse incinerator fly ash. *Plant & Soil* **96**: 407-412.

Xiong, G. (1997). Bioaccumulation and physiological effects of excess lead in polluted soils in their uptake by cabbage plants. *Plant & Soil* **113**: 257-264.