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Toxic Effect of Lead (Pb^{++}) on Certain Biochemical Parameters in Gram (*Cicer arietinum* L.) Seedlings

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The problem of environmental pollution with heavy metals due to anthropogenic activities is becoming increasingly urgent to living world. Heavy metals such as lead and mercury have been widely reported to inhibit germination, growth and metabolism in various plant species (Mesmar and Jaber 1991; Munzuroglu and Geckil 2002). Lead is strongly sorbed by soils, forms insoluble crystalline compounds due to complex rhizospheric reactions between plant roots, soil inorganic and organic matters, and soil microbes (Schutzendubel and Polle 2002) and hence seldom reaches high level in leaves and roots. Baumhardt and Welch (1972) reported if the P status of a soil is adequate for normal plant growth, plants do not accumulate Pb. Freshly added Pb salts are more readily available to plants, but high levels due to long-term automobile Pb depositions or Pb sprays have little effects on Pb uptake by plants. Malone et al. (1974) observed the formation of Pb-rich deposits in dictyosome vesicles of corn exposed to high levels of Pb. Translocation of Pb reveals a complicated interaction among nutrients and plant metabolism. When bound on the cell surface or within the cells, Pb ions interact with the functional groups of proteins, nucleic acids, polysaccharides etc, and substitute for other metal ions already bound to these functional groups, leading to metabolic disorders. Pb and other heavy metals also cause production of reactive oxygen species, which are potentially toxic and lead to unspecific oxidation of proteins and membrane lipids and DNA injury (Schutzendubel and Polle 2002). Hampp et al. (1973) reported the toxic effects of Pb on enzymes of reductive pentose phosphate pathway. Several other scientists have reported the inhibitory effect of Pb on transpiration, nodulation, gas exchange, photosynthesis and respiration etc. in plants (Dabas et al. 1995; Kastori et al. 1996; Ernst 1999; Seregin and Ivanov 2001). Review of toxicity effects studies of Pb in mungbean, soybean, maize, sunflower and other plants has shown that cereals and crucifers turn relatively tolerant and well-marked physiological alterations are observed with leguminous plants including diminished capacity of the plant to fix nitrogen (Seregin and Ivanov 2001).

In the present study, seedlings of gram (*Cicer arietinum* L., Family-Leguminosae), an internationally important grain legume crop, were treated with different Pb concentrations i.e. 0 (control), 0.5, 1.0, 2.5, 5.0 and 10.0 mM, respectively, for 10 and 20 d in order to investigate effects on biochemical parameters, namely peroxidase

(PO) and polyphenol oxidase (PPO) activity, total soluble proteins (TSP) and total free amino acid (TFA) content.

MATERIALS AND METHODS

Certified seeds of gram (*C. arietinum*) cv. Radhey were soaked for 24 hr in different Lead acetate (LA) solutions i.e. 0 (control), 0.5, 1.0, 2.5, 5.0 and 10.0 mM, respectively, and subsequently sown in plastic pots filled with acid-washed mineral-free quartz sand. The pots were maintained at natural temperature (25 to 30°C) under diffused sunlight (approx. 80 $\mu mol\ m^{-2}\ s^{-1}$) using 10 hr photoperiod. The control seedlings/plants were irrigated initially with 50 mL Hoagland's nutrient solution, whereas in LA treatments, the pots were first irrigated with 50 mL of nutrient solution containing different test LA concentrations. Plants in all treatments were irrigated on every third day with 15 mL dH_2O up to 3 weeks.

For biochemical studies, 5 plants were harvested separately from each treatment at 10 and 20 d and washed with dH_2O. The root and shoot portions were separately crushed with mortar and pestle and homogenized in chilled (4°C) phosphate buffer (pH-7.0). Homogenates were centrifuged at 4°C for 20 min at 17000 rpm. The supernanents collected separately and stored at -4°C were used for estimation of PO and PPO activity, TSP and TFA contents on fresh weight basis.

PO and PPO activity were assayed by the method of Kar and Mishra (1976). In case of PO, reaction mixture contained 2 mL of 0.1 M phosphate buffer (pH-7.0), 1 mL of 0.01 M pyrogallol, 1 mL of 0.005 M H_2O_2 and 1 mL well diluted enzyme extract. The reaction mixture of PPO assay contained all ingradients of peroxidase assay except H_2O_2. Reaction was allowed to proceed for 5 min at 25°C and then stopped by adding 1 mL of 2.5 N H_2SO_4. Absorbance of purpurogallin formed was measured at 420 nm and the enzyme activity is expressed in absorbency units. The TSP was estimated by the method of Lowry et al. (1951). The content of TFA was estimated according to method of Lee and Takahashi (1966) using L-tyrosine as standard. Absorbance of reaction mixtures for all biochemical parameters was recorded using a double beam UV-VIS Spectrophotometer model SL 164 (ELICO Ltd., India). Analytical controls were run for calibrating the instrument to zero and these comprised of boiled enzyme extract for PO and PPO activity and buffer at the place of supernanent for TSP and TFA.Each experiment was repeated three times with five replications per treatment. The data (3x5) were statistically analyzed for calculation of LSD (Least squared difference) values at 95% confidence limits (Panse and Sukhatme 1967).

RESULTS AND DISCUSSION

PO and PPO activity, TSP and TFA content of *C. arietinum* plants grown at different LA levels for 10 and 20 d are summarized in Table 1. At 10 d, PO activity in root was maximum at 10.0 mM (3.50) and minimum at 0.5 mM (2.08). At 20 d, the

activity was comparatively less than 10 d and recorded maximum at control (0.10). At 10 d, the seedling tissues have relatively poor cellular differentiation, high water content and high Pb ions in root cells which collectively promote formation of active oxygen forms. In response to oxidative stress, an increase in PO and PPO activity helps in neutralizing free radicals and tolerating the metal stress. At 20 d, the seedling tissues become highly differentiated/mature, contain lesser water and Pb ions get distributed in entire plant body. At this stage, Pb ions displace the essential metal from PO and PPO metalloenzymes and lead to decline in their activity. Activity of PO and PPO also depend the particular phase of plant development (less in mature tissues) and both these factors cause reduction in activity of these enzymes at 20 d. These observations of lead toxicity in gram seedlings conform to similar effects of mercury and cadmium on antioxidant enzymes in *Phaseolus aureus* (Shaw 1995). The activity decreased with increase in LA level up to 2.5 mM and reached to minimum (0.04). Further increase to 5.0 and 10.0 mM stimulated the enzyme activity (0.05). In severity of metal stress, transport of absorbed heavy metals to vacuoles and formation of low-insoluble metal-organic acid complexes is a predominant mechanism of metal detoxification (Wierzbicka and Antosiewicz 1993). A similar mechanism at 5.0 and 10.0 mM Pb levels seems to be responsible for elevation of PO and PPO activity as the cytoplasmic Pb level decreases and metalloenzymes face low metal-Pb ion substitutions. At 10 d in control shoots, the activity was 0.96 which is comparatively less than 10 d root tissue (except at 10.0 mM) and presence of LA showed differential stimulation of PO activity. At 20 d in shoots, maximum activity was at control (0.17) and it decreased with increase in LA level reaching minimum (0.04) at 10.0 mM. The PO enzymes perform multiple functions including IAA Oxidase activity (Hoyle 1972) and are distributed almost in all plant tissues. They play basic role in cellular metabolism and differentiation during early phase through regulation of IAA activity whereas in late phase they regulate cell wall formation. In the present study, at higher LA levels and exposure duration, PO activity was low and same thing was true for root and shoot biomass (Lal and Mishra 2004) which indicates that at 20 d the rate of lignification goes down and the cytoplasm becomes hyperhydric in nature.

PPO activity was maximum at 1.0 mM (2.18) and minimum at 2.5 mM (0.73). There was sudden enhancement in activity after 2.5 mM till 10.0 mM and it reached closer (2.03) to 1.0 mM. Just like roots, the PPO activity in shoots was also less than 10 d stage. Maximum activity was noticed at control (0.86) which was much less than control and 1.0 mM at 10 d. The activity decreased with increase in LA and reached minimum at 10.0 mM (0.23). With increase in LA level, the magnitude of decrease in enzyme activity narrowed and revealed minimum between 2.5 and 5.0 mM. The polyphenols influence the plant processes by modifying the auxin effect as well as by producing harmful oxidative products that are inhibitory/toxic to cytoplasm. PPO has a direct relation with meristematic activity and this is possibly the reason for higher PPO activity during early phase.

The TSP level in 10 d roots at control was 10.09 mg/g whereas it was minimum at

Table 1. Total soluble protein (TSP), total free amino acid (TFA), peroxidase (PO) and polyphenol oxidase (PPO) activity in *C. arietinum* plants treated for 10 and 20 days with different concentrations of lead acetate.

LA	10 days								20 days							
Treatments (mM)	Root				Shoot				Root				Shoot			
	TSP (mg/g)	TFA (mg/g)	PO	PPO	TSP (mg/g)	TFA (mg/g)	PO	PPO	TSP (mg/g)	TFA (mg/g)	PO	PPO	TSP (mg/g)	TFA (mg/g)	PO	PPO
Control	10.09	6.49	2.92	0.88	20.78	9.98	0.97	1.24	18.16	15.06	0.10	0.47	14.98	12.45	0.17	0.86
0.5	10.85	1.46	2.08	0.63	21.77	6.61	1.60	1.29	18.54	8.03	0.07	0.36	22.58	14.34	0.12	0.61
1.0	9.12	3.15	3.02	1.67	28.27	16.79	2.20	2.18	19.21	8.93	0.05	0.24	21.74	12.00	0.10	0.52
2.5	12.79	2.26	2.50	0.77	13.93	7.82	1.28	0.73	18.51	7.85	0.04	0.20	21.21	10.80	0.06	0.32
5.0	8.47	5.90	2.87	0.58	19.31	13.71	1.78	1.97	18.40	12.66	0.05	0.15	35.61	13.36	0.06	0.31
10.0	17.67	2.54	3.50	2.48	33.77	10.90	3.05	2.03	27.50	5.63	0.05	0.25	21.18	11.32	0.04	0.23
LSD at P=0.05	0.69	0.21	0.17	0.06	1.52	0.72	0.13	0.10	1.32	0.58		0.02	1.73	0.82		0.03

5.0 mM (8.74 mg/g) and maximum at 10.0 mM (14.67 mg/g). At 20 d, the TSP content increased over 10 d stage. It was recorded minimum at control (18.16 mg/g) and maximum at 10.0 mM (27.50 mg/g). At 10 d in shoots, the TSP content was comparatively higher than root tissue. It was minimum at 2.5 mM (13.93 mg/g) and maximum at 10.0 mM (33.77 mg/g). However, at 20 d the TSP content at control was minimum (14.98 mg/g) and maximum (nearly twice to control) at 5.0 mM (35.61 mg/g). There were large differences between minimum values in shoots of 10 and 20 d age.

The TFA content in roots at 10 d at control was 6.49 mg/g (maximum). Addition of 0.5 mM LA decreased TFA to minimum (1.46 mg/g). At other LA levels it showed relatively higher TFA content with fluctuating trend. At 20 d, the TFA content in roots was higher than 10 d stage and was recorded maximum at control (15.06 mg/g) and minimum at 10.0 mM (5.63 mg/g) with almost similar fluctuating trend. In shoots at 10 d, The TFA content was higher than roots. At 0.5 mM LA, TFA decreased to 6.61 mg/g as compared to control (9.98 mg/g) but was maximum at 1.0 mM (16.79 mg/g). At 20 d, the TFA content was greater than 10 d (except at 1.0 and 10.0 mM) and maximum and minimum contents were observed at 0.5 mM (14.34 mg/g) and 2.5 mM (10.80 mg/g), respectively. The increase in exposure duration to different LA levels affected the TFA content in differential manner. In case of control, TFA increased in roots and shoots both but the magnitude was very high in roots (2.4 fold).

Simultaneous comparison of TSP and TFA at 10 d shows that in control both the constituents are quite high. The presence of LA showed increase in TSP content at certain levels with concomitant decrease in TFA content. It indicates that presence of LA inhibits amino acid biosynthesis and protein degradation, and stimulates synthesis of proteins (with protective role) so that most of the amino acids are converted into peptides and TFA content goes down. Just opposite mechanism seems to explain the low TSP and high TFA content at certain levels. These observations were not earlier reported with lead, however, similar changes have been observed by Taghvi and Vora (1994) in guar seedlings exposed to different industrial effluents.

REFERENCES

Baumhardt GR, Welch LF (1972) Lead uptake and corn growth with soil-applied lead. J Environ Qual 1:92-94

Dabas S, Singh RP, Sawhney V (1995) Nitrogen fixation and ammonia assimilation in mungbean nodules during lead contamination. Physiol Mol Biol Plants 1:135-140

Ernst, WHO (1999) Effects of heavy metals in plants at cellular and organismic level. In: Schuurmann G and Markert B (Eds) Ecotoxicology: Ecological Fundamentals, Chemical Exposure and Biological Effects. Wiley and Sons, Heidelberg, p 587

Hampp R, Ziegler H, Ziegler I (1973) Influence of Lead ions on the activity of

enzymes of the reductive pentose phosphate pathway. Biochem Physiol Pflanzen 164:588-595

Hoyle MC (1972) Indole acetic acid oxidase: A dual catalytic enzyme. Plant Physiol 50:15-18

Kar M, Mishra D (1976) Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol 57:315-319

Kastori R, Petrovic H, Petrovic M (1996) Effects of Pb on water relations, proline concentration and nitrate reductase activity in sunflower. Acta Agron Hungarica 44:21-28

Lal N, Mishra R (2004) Phytotoxic effects of lead on biomass and Photosynthetic pigments in gram (*Cicer arietinum* L.) seedlings. J Eco-Physiol 5:119-223

Lee YP, Takahashi T (1966) An improved colorimetric determination of amino acids with use of ninhydrin. Anal Biochem 14:71-77

Lowry OH, Rosenbrough NJ, Farr AL, Randall RL (1951) Protein measurements with Folin-phenol reagent. J Biol Chem 193:265-275

Malone C, Koeppe DE, Miller RJ (1974) Localization of Lead accumulated by corn plants. Plant Physiol 53:388-394

Mesmar MN, Jaber K (1991) The toxic effect of lead on seed germination, growth, chlorophyll and protein contents of wheat and lens. Acta Biol Hungarica 42:331-344

Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. Arch Environ Contam Toxicol 43:203-213

Panse VG, Sukhatme PT (1967) Statistical Methods for Agricultural Works. ICAR, New Delhi

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351-1365

Seregin IV, Ivanov VB (2001) Physiological aspects of Cadmium and Lead toxic effects on higher plants. Russian J Plant Physiol 48:523-544

Shaw, BP (1995) Effects of Mercury and Cadmium on the activities of antioxidant enzymes in the seedlings of *Phaseolus vulgaris*. Biol Plant 37:587-596

Taghvi SM, Vora AB (1994) Effect of industrial effluents on germination and growth development of guar seed (var. PNB). J Environ Biol 15:209-212

Wierzbicka M, Antosiewicz D (1993) How Lead can easily enter the food chain - a study of plant roots. Sci Total Environ (Suppl) :423-429