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Phytotoxicity of Pb: I Effect of Pb on Germination, Growth, Morphology and Histomorphology of *Phaseolus mungo* and *Lens culinaris*

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Abstract: This study examined the effect of toxic metal Pb on *Phaseolus mungo* and *Lens culinaris*. Results showed that Pb markedly affect the *Lens culinaris* as compared to *Phaseolus mungo*. A pronounced effect of Pb on plant roots of both species shows the rapid respond to absorbed Pb, through a reduction in rate of seed germination, morphology with change in branching pattern. Histomorphological changes in root and vascular tissues in both species may be related with the conduction of water in the plant followed by inhibitory effect on germination of seeds and retarded the growth of seedlings. Pb also decreases the germination percent, germination index, root/shoot length which also depend upon the nature of plant species.

Key words: Pb, seed germination, vascular tissues

INTRODUCTION

Contamination of soil by heavy metals is of widespread occurrences as results of human and industrial agricultural activities. Among heavy metals lead (Pb) is a potential pollutant that readily accumulates in soils and sediments. The transport and distribution of Pb from stationary or mobile source is mainly via air (Singh *et al.*, 1997). Pb that is discharged into the air over areas of high traffic density falls out mainly within the immediate metropolitan zone, residence time for these small particles is of the order of days and is influenced by rainfall. The Pb in the soil and in vegetation decreases exponentially with the distance from the road. Pb is also found in the sediments of streams in the vicinity of highways (Samardakiewicz and Wozny, 2000). Terrestrial and aquatic plants accumulation lead in industrially contaminated environment (Wierzbicka and Obidzinska, 1998). Plants absorbs Pb and accumulation of the metal have been reported in roots, stems, leaves, root nodules and seeds etc. which increases with the increase in exogenous Pb level (Mesmar and Jabor, 1991). The effect of Pb on wheat and lens shows drastic effect where roots were more effected then shoots (Ye *et al.*, 1997). Toxic metal ions enter in cells by means of the same uptake processes as essential macronutrients metals ions (Seregin *et al.*, 2001). The amount of metal absorbed by a plant depend on the concentration and speculation of the metal in the solution, its movements successively from the bulk soils to the root surface, then into the root and finally in to the shoot (Malkowski *et al.*, 2002). The effect of Pb depends on the concentration, type of salts and plant species involved. Though effects are more pronounced at higher concentration and duration in some cases lower concentration might stimulate metabolic processes (Paivoke, 2002). The major processes affected are seed germination, seedling growth, photosynthesis, plant water status mineral nutrition and enzyme activities. The present study will examine the Pb stress on *Phaseolus mungo* and *Lens culinaris*. The results will discuss in terms of seed germination, seedling growth and photosynthesis in relation with leaf size and structure of roots and vascular tissues of two species.

MATERIALS AND METHODS

Phaseolus mungo and *Lens culinaris* were grown into pots with control laboratory scale. The seed germination was carried out in different concentration of Pb based Hoagland solution in July and Aug 2005. Seed germination of two species were recorded daily and compared with control pot. Transverse Section (T.S.) of root and shoot were taken after 3 weeks. The slide was prepared by staining with safranine T and T.S. of both species were observed in camera Lucida microscope. The diagram was drawn by pencil on white paper. Histomorphology of roots of both species were compared with control plant. External morphology and seed germination were also compared with control plant.

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RESULTS AND DISCUSSION

Phaseolus mungo and *Lens culinaris* were chosen to investigate their response and ability to accumulate and tolerate varying levels of toxic heavy metal Pb in their roots and shoots. Plants in the control treatment were grown in the absence of the heavy metal. The accumulation of Pb^{++} in two species were determined as morphological change, seed germination, seedling growth and histomorphology of vascular bundles (Seregin *et al*., 2004).

Germination of seeds: Pb toxicity inhibits germination of seeds and retarded growth of seedlings (Sarvari *et al*., 2002). Pb decreases germination percent, germination index, root/shoot length, tolerance index and dry mass of roots and shoots in both species. High concentration of Pb caused 20 to 70% decreased in the germination of *Lens culinaris* and reduced the growth rate of seedlings by more than to 15 to 60%. Pb reduced the number of seeds germination (Table 1 and 2) and caused elongation of hypocotyl with shortening of roots in *Phaseolus mungo* and *Lens culinaris*. Table 1 and 2 shows the adverse effect of Pb on seed germination of two species where it was found that at higher concentration of Pb, seed germination of both species were highly effected specially of *Lens culinaris*, which may be attributed with toxic effect of Pb which prevents the expansion of embyo and puts obstruction in the imbibition of water.

Pb moves predominantly into root apoplast and there by in a redial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between the root and shoot. This may in part account for the report of higher accumulation of Pb in roots compared to shoots.

Seedling growth: Table 1 and 2 shows the effect of Pb on seedling growth of two species It was observed that seedling growth of *Phaseolus mungo* and *Lens culinaris* were inhibited in presence of Pb and shows that effect of metal on both species were different. The length of root and shoot of *Lens culinaris* were affected more as compared to *Phaseolus mungo* (Fig. 1). It indicated that uptake of an element by plant is primarily dependent upon the plant species, it inherent controls and the soil quality. Table 1 and 2 shows that root growth inhibition was more pronounced than shoot growth inhibition at different concentration of Pb especially in *Lens culinaris*. Pb delayed germination and lowered the ability of seeds to germinate in a dose dependent manner in the species with highly Pb permeable seed coats. It indicates that Pb in *Phaseolus mungo* penetrated into embryos in the final stage of imbibition delayed germination. This shows that seed coats are selectively permeable to Pb ions. It shows that root have an ability to take up significant quantities of Pb while simultaneously greatly restricting its translocation to above ground part, Pb retention in the root is based on binding of Pb to ion exchangeable sites on the cell wall and intracellular precipitation, mainly in the form of Pb carbonate deposited in the cell wall.

Fig. 1: Effect of Pb on growth of *Phaseolus mungo*

Histomorphology of roots: The Transverse section of root of *Phaseolus mungo* (Fig. 2) shows more tolerance towards excess of Pb as compared to *Lens culinaris*. While in both species elongation of root were totally inhibited and at higher dose of Pb which shows the decrease in root hair and at 250 ppm of Pb root hairs were completely abolished and lateral root formation was also effected. Lead toxicity appears as:

a) It prevents the expansion of embryo b) It puts obstruction in the imbibition of water. There are two main functions, which are related with the root hair: (a) A rule in absorption of water and mineral ions from soil, (b) Adhesive property between root and surrounding. Roots hairs are the only cells in root, which have cuticle. The presence of cuticle on the other point of the root would prevent diffusion of any gasses but the root hair may permit since the lack cuticle. Investigation shows that due to the absence of root hair with the increase in concentration of Pb, plant lost its immune system, which may be attributed with accumulation of Pb in roots and damage root hair.

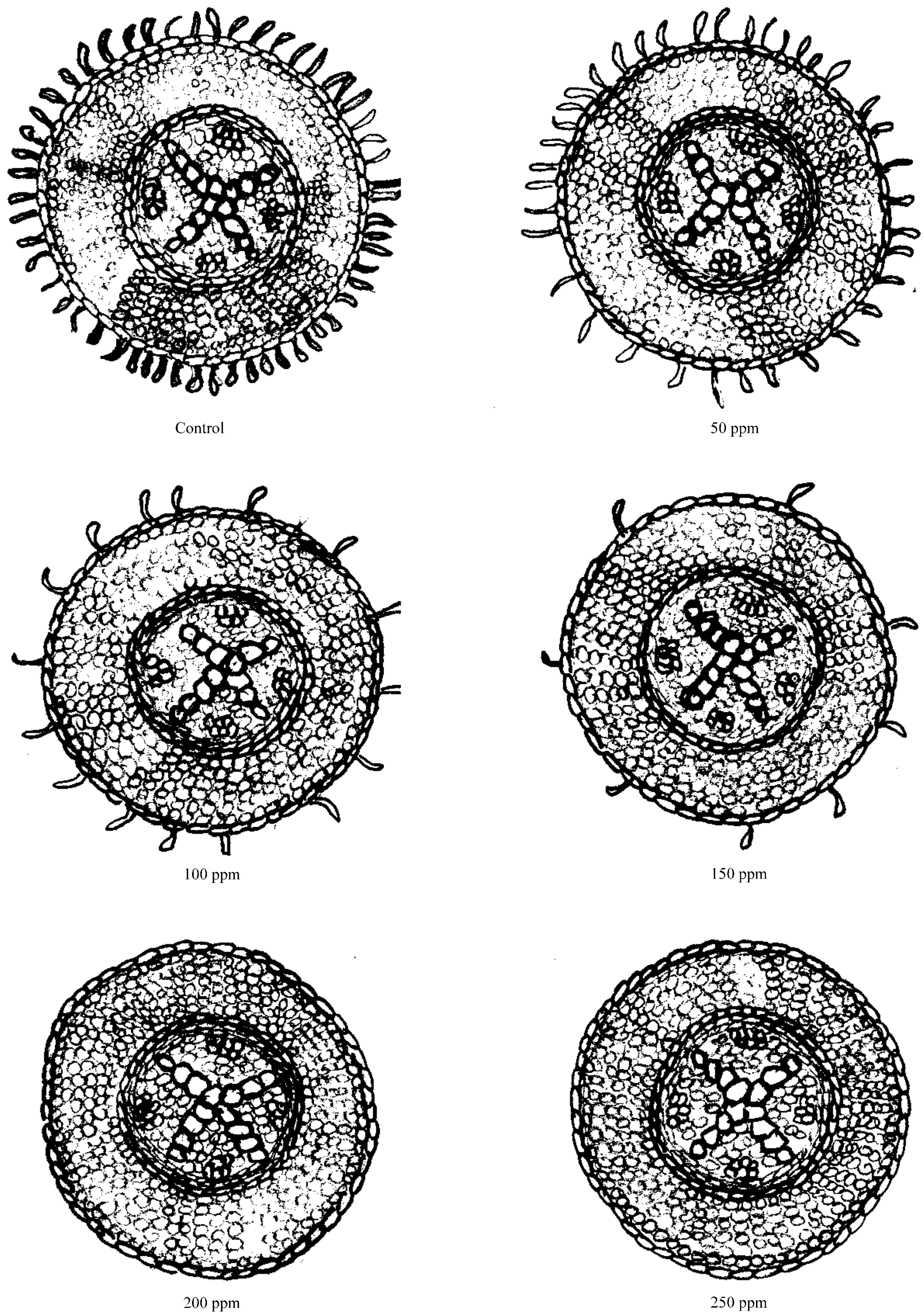

Fig. 2: Effect of Pb on T.S. of root of *Phaseolus mungo*

In *Phaseolus mungo* and *Lens culinaris* species accumulation of Pb in root and shoot appeared in form of inhibition of root hairs and change in tissue structure of vascular bundles. Due to which conduction of water and minerals were effected which ultimately affect the growth of seedlings and morphology of plant. Histomorphology of root of *lens culinaris* shows that change in vascular tissues were observed due to the accumulation of Pb. Figure 3 and 4 shows that at 50 ppm of Pb concentration, T.S. of both species of root shows approximate similar structure as compared to control plant. While change in epidermal region and cortical region with change in structure of vascular tissue were observed with the increase in concentration of Pb. Root hairs were found to be decrease with the increase in concentration of Pb and completely disappeared at 200 ppm where as very little seed germination noted at 250 ppm of Pb (Table 3 and 4). At higher dose I-e 100 ppm of Pb (Fig. 3 and 4), cortical region were increased and 10, 11 and 12 layers were observed at 100, 150 and 200 ppm, respectively as compared to 8 layers of control and 50 ppm of Pb. Xylem and phloem which are responsible for conduction of

Fig. 3: Effect of Pb on growth of *Lens culinaris*

Table 1: Effect of Pb on morphology and growth of *Phaseolus mungo*

[Pb++]	Germination (%)	Root (cm)	Shoot (cm)	Color of leaves	Size of leave (cm)	Morphology of stems
Control	90	6.5+0.1	11.0+0.1	Light green	3.5±0.1	Erect
50	70	4.5±0.2	12.5±0.2	Light green	2.8±0.2	Erect
100	50	2.5±0.3	16.5±0.3	Dark green	2.6±0.3	Down
150	40	1.5±0.4	10.1±0.1	Dark green	2.5±0.2	Down
200	10	1.0±0.2	5.5±0.3	Dark green	2.0±0.2	Down
250	5	0.5±0.2	3.1±0.1	Dark green	1.8±0.1	Down

Table 2: Effect of Pb on morphology and growth of *Lens culinaris*

[Pb++]	Germination (%)	Root (cm)	Shoot (cm)	Color of leaves	Size of leave (cm)	Morphology of stems
Control	90	4.5±0.01	10.0±0.01	Light green	2.0±0.01	Erect
50	50	2.3±0.01	9.1±0.02	Light green	1.5±0.02	Erect
100	30	1.5±0.01	4.2±0.05	Dark green	1.0±0.01	Down
150	20	1.1±0.01	3.1±0.01	Dark green	0.8±0.01	Down
200	5	0.9±0.01	2.5±0.03	Dark green	0.8±0.01	Down
250	2	0.3±0.01	1.0±0.02	Dark green	0.7±0.01	Down

Table 3: Histomorphlogy of T.S. of root of *Phaseolus mungo* in presence of Pb

Pb (ppm)	Epidermis	Cortex	Endodermis	Phloem	Xylem
0	One layered with 100% of root hair	6 layered	One layered	4 patches	4
50	Do	Do	Do	Do	Do
100	One layered with 50% of root hair	Do	Do	Do	Do
150	One layered with 15% of root hair	Do	Do	Do	Do
200	One layered root hair absent	Do	Do	Do	Do
250	Do	Do	Do	Do	Do

Table 4: Histomorphlogy of T.S. of root of *Lens culinaris* in presence of Pb

Pb (ppm)	Epidermis	Cortex	Endodermis	Phloem	Xylem
Control	One layered with 100% of root hair	8 layered	One layered	4 patches	4 patches
50	Do	Do	Do	Do	
100	One layered with 20% of root hair	10 layered	Do	4 patches but reduce size	Xylem expand
150	One layered with 10% of root hair	11 layered	Do	2 patches with reduce size	Xylem more expand
200	One layered root hair absent	12 layered	Do	No patch	Xylem covered large area
250	-	-	-	-	-

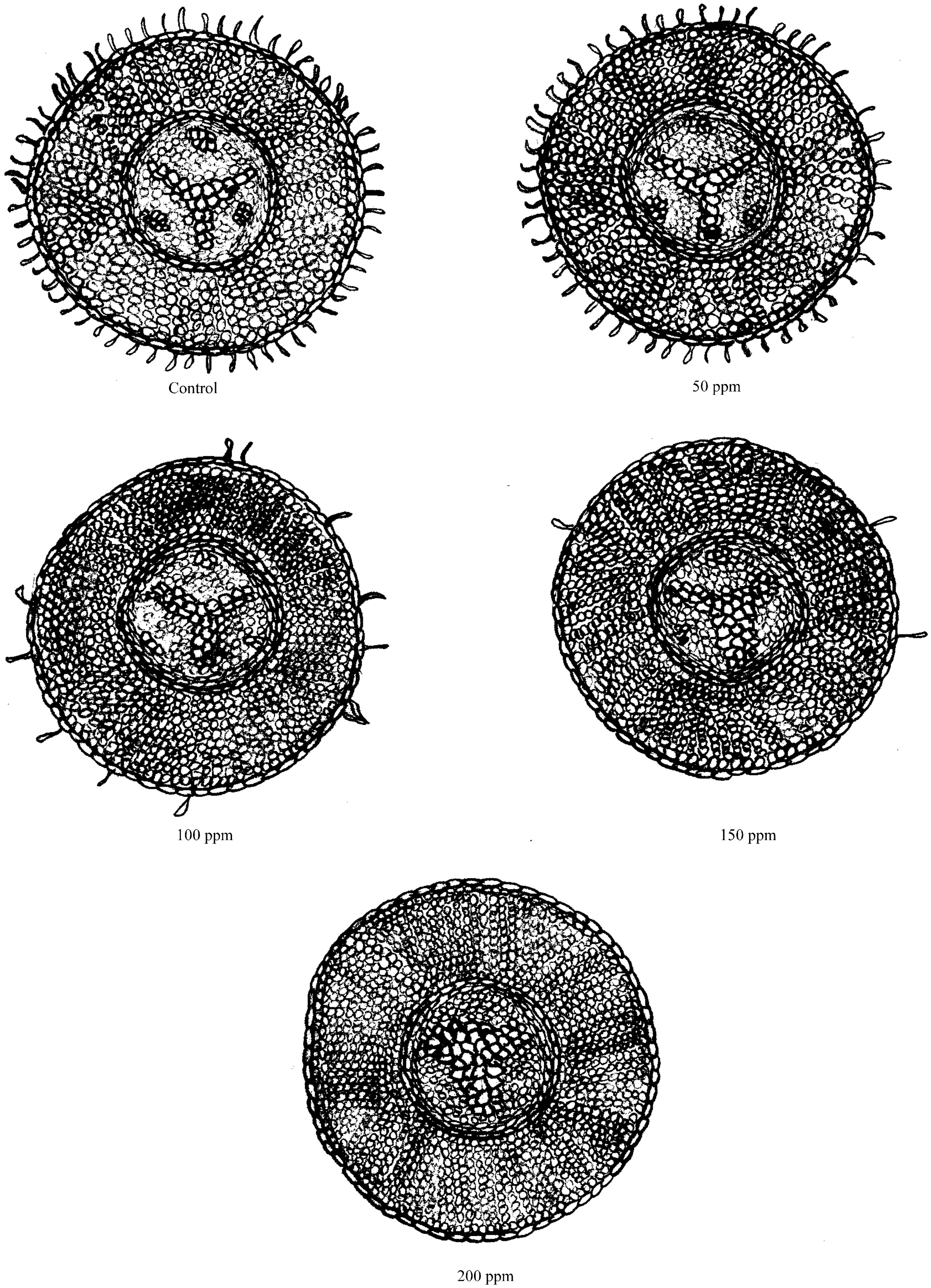

Fig. 4: Effect of Pb on T.S. of root of *Lens culinaris*

water and translocation of food shows adverse effect of Pb on vascular bundle. Figure 3 and 4 reflects the change in structure of xylem at high dose of Pb and it gradually expended at 200 ppm of Pb and occupy more cortical region therefore compact structure of root were observed in which cell division was inhibited. Where as in *Lens culinaris* phloem tissues gradually decreases with the increase in concentration of Pb and finally disappear which may be attributed with inhibitory effect of Pb on tissues of root. It is also related with the size of leaves, which shows the decline processes of photosynthesis. It also proved by Van Helmont conclusion that it was not soil but it was water, which contributes to the growth of the plant.

This study concluded that inside the plant Pb accumulates primarily in the roots but a part of Pb is transferred to the aerial portion. Limited translocation of Pb occurs from root to other organs due to the barrier function of the root endodermis. At lethal (200 and 250 ppm) concentration this barrier is broken and the flux of Pb enters the vascular tissues and significant reduction were observed in the growth of both species.

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