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Pb Stress on Phytochemistry of Seedlings of *Phaseolus mungo* and *Lens culinaris*

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Abstract: The influence of Pb treatment was investigated using phytochemistry as stress indicator. Excess of Pb in *Phaseolus mungo* and *Lens culinaris* exhibited higher dry root, stem and leaves with decrease in mineral ions (Na, K, Ca, Mg, Fe, Mn and Zn) uptake in both species. The shoot elongation was negatively correlated with Pb concentration as external morphological symptoms of toxicity were observed. Seed germination of both species grown hydroponically with $PbCl_2$ were highly inhibited. Results obtained by atomic absorption Spectrophotometry from measurements of different essential macronutrient and micronutrient with Pb content both in roots and shoots showed that Pb accumulation was more pronounced in root than shoot. Total protein contents in the seedlings of *Phaseolus mungo* were found to be increased in shoot as compared to roots whereas it is significantly reduced in root and shoots of *Lens culinaris*. Amino acids in the roots of both species were found to be increased and decreased in the shoots of *Lens culinaris* while higher concentration of amino acids was observed in shoots of *Phaseolus mungo*. The higher density and smaller ionic radii of heavy metal plays an integral role to block the asses of essential mineral ions in plants and alter physiological processes of both species.

Key words: Pb, mineral ions nutrient, protein, amino acid, density

INTRODUCTION

Phytotoxicity of Pb depends on the concentration, type of salt and plant species involved. Alarming concentration (Burzynski, 1987; Godzik, 1993) of the metal has been reported in the dust of densely populated urban areas and water and lands of various areas near the industrial waste disposals (Azmat *et al*., 2006). Though effects are more pronounced at higher concentration and duration but in some cases, lower concentration might stimulate metabotic process (Godbold and Kettner, 1991) The major processes affected are seed germination, seeding growth, photosynthesis (Haider *et al*., 2006), plant water status and mineral nutrition (Azmat and Khanum, 2005a, b). Pinero *et al*. (2002) observed that at concentration of 200 ppm in the substrate the cultivated plants increased the biomass production, chlorophyll concentration and total protein contents. However at higher concentration of 600 ppm these variables except the total protein, decreased to levels below the control. Total protein contents in the seedlings of wheat and lens were found to be increase with the increase in the concentration of Pb (Mesmar and Jabor, 1991). Diverse biochemical changes in green plants in response to Pb has been reported by Keresan *et al*. (2001) leads to reduction in protein and nitrogen contents of plants. Pb accumulation in plants increases with the increase in the exogenous Pb level. It appears that the toxic effect of the metal (Jarvis and Leung, 2002) is primarily at physiological level and provision of certain inorganic salts can antagonize the effects to some extent. Pb alters the levels of mineral elements in the root. The level of Ca, Fe and Zn decreases after exposure to Pb in root tips (Eun *et al*., 2002). Pandy and Sharma (2002) reported the accumulation of Co^{2+}, Ni^{2+} and Cd^{2+} in cabbage plant in sand culture, result in inhibition. The effect of calcium and humic acid shows the decrease in concentration of micro and macronutrients of tomato at high level with growth inhibition in seedling (Tûrkmen *et al*., 2004). Reduction in chlorophyll content (Haider *et al*., 2006) due to less concentration of Mg in the three plant species were recorded by Pinero *et al*. (2002).

Geeblen *et al*. (2002) reported significant reduction in contents of Ca, Fe, Mn and Zn uptake by plants due to the elevated concentration of Pb-EDTA, probably due to ion leakage as a observed toxicity. The effect of Pb was more pronounced in planted soil indicating that Pb was taken up by the plants. In Norway spruce (Haussling *et al*., 1998) 40% of Ca taken up was used in root tip growth which decreases with the application of Pb lead in the inhibition of root growth (Rout and Das, 2003), which may be attributed with decrease in cell division or

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cell elongation. It also decreases the level of Ca and Mn in Norway spruce. A decrease in uptake of K, Ca, Mg, Fe and Na were observed in *Picea abies* after treated with Pb (Haussling *et al*., 1998).

Investigations about phytochemistry under Pb stress related with reduction in essential macronutrient, micronutrient, phosphate, amino acids and protein contents in *Phaseolus mungo* and *Lens culinaris* will discuss in this research. The mechanism of replacement of essential mineral ions with Pb due to higher density and small ionic radii will explain the growth inhibitory effect of Pb in both species.

MATERIALS AND METHODS

The growth of the *Phaseolus mungo* and *Lens culinaris* with varying concentration of $PbCl_2$ viz., 50, 100, 150, 200 and 250 ppm were observed in growth chamber in June 2005 in six pots containing half strength Hoagland solution for 2 weeks. Ten to fifteen seeds of *Phaseolus mungo* and *Lens culinaris* soaked in water for 4-6 h were surfaced sterilized with 0.3% calcium hypochlorite for five minutes and rinsed with deionised water. They were introduced into different pots containing 0 to 250 ppm Pb based Hoagland solution. Plants were analyzed after two weeks. Na, K, Mn, Mg, Ca, Fe and Pb were estimated through dry ash method by flame photometer and atomic absorption Spectrophotometer.

Detection of elements: Plants were separated into roots and shoots and dried at 60°C in oven. Dry plant material (1 g) was separately ashed in furnace at 1000°C, were wet ashed with 40 mL (1:1) HNO_3-$HClO_4$ and heated to dryness in platinum dish, few drops of hydrofluoric acid were added and heating was continuous to dryness. The residue was treated with 10 mL concentrated HCl and boiled for 30 min, then 20 mL distill water was added and solution was heated for further 15 min and made up to 50 mL. Solutions then subjected to flame photometry and atomic absorption spectrophotometry for the detection of mineral ions and toxic metals.

Biochemical analysis: For phosphate contents the extracts prepared by dry ashing method, was shaken with ammonium molybdate and stannous chloride. A blue colored complex with phosphate was obtained. Absorbance was measured at 660 nm by spectrophotometer. For protein contents in root and shoot extract in water were treated with Folin cicalteau phenol reagent (half diluted). The extract was left for 30 min at room temperature. A blue coloured complex was developed. Absorbance of the complex was observed at 650 nm on Schimadzo 160 A UV-Visible spectrophotometer. Amino acids were determined by treating the extract of root and shoot with ninhydrin solution in 10% ethanol and heated to 50 to 70°C for few minutes till purple color appeared. Optical density were recorded at 566 nm.

RESULTS AND DISCUSSION

Nutrients are generally absorbed against concentration gradients consequently respiratory energy is required for mineral uptake (Godzik, 1993). In order for respiration continue in the roots, oxygen must be available in the root zone. Roots which become totally submerged or soil contaminated by heavy metals will suffer from lack of oxygen. This will leads to slow growth and inhibitory effect of toxic metal on roots (Fig. 1) of plant (Jones *et al*., 1973). Pb toxicity symptoms were observed three days after the growth began. Excess of Pb caused leaf chlorosis and root blacking. It significantly depressed the leaves size, stem and elongation of roots as compared to control plants (Azmat *et al*., 2006).

Pb alters the levels of mineral elements in the roots by physical blocking of many essential ions from absorption sites of the root. Table 1 and 2 shows that in root tips the level of Na, K and Ca were decrease after the Pb dose. The inhibition of root growth after exposure to Pb may be related with the decreases in Ca in the roots tips in *Phaseolus mungo* and *Lens culinaris* leading to a decrease in cell division or cell elongation. The decrease in concentration of Ca and Mg in the roots in response to higher concentration of Pb was probably a result of osmotic adjustment.

Result showed that high concentration of Pb in the environment causes imbalance of mineral (Na, K, Mg, Ca, Fe, Mn and Zn) nutrients in growing plants (Eun *et al*.,

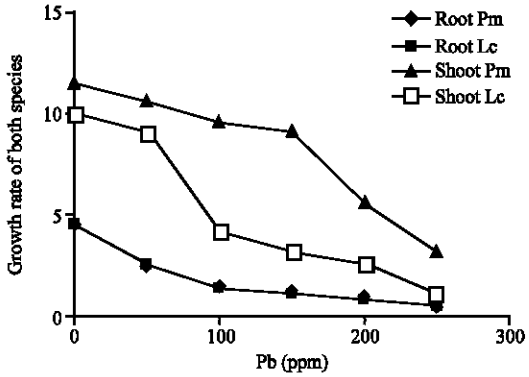

Fig. 1: Pb stress on growth of seedlings of *Phaseolus mungo* and *Lens culinaris*, Pm = *Phaseolus mungo*, Lc = *Lens culinaris*

Table 1: Effect of Pb on mineral ions uptake of *Phaseolus mungo*

Pb (ppm)	Na (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Fe (µg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})	Pb (µg g^{-1})
Shoot								
0	89±11	26±03	145±14	70±01	150±12	-	200±23	200±12
50	80±12	20±02	130±12	60±02	141±21	45±02	165±21	50±11
100	71±21	19±03	121±13	56±02	130±23	50±02	145±21	74±15
150	67±11	18±01	109±12	49±02	121±21	59±02	115±22	97±14
200	62±10	15±03	101±14	40±02	110±22	63±02	105±24	108±20
250	50±12	08±02	100±14	35±02	100±18	69±02	100±24	115±24
Root								
0	91±11	14±01	130±13	90±12	158±19	-	200±12	300±20
50	78±13	13±02	120±12	85±13	149±11	25±14	180±23	200±15
100	69±12	11±02	109±14	81±14	138±20	30±12	165±12	179±14
150	60±14	09±02	96±12	75±12	129±14	37±12	140±14	140±15
200	53±10	08±01	85±16	68±12	120±14	43±12	130±14	120±12
250	41±09	05±02	70±11	50±10	120±12	49±14	120±15	110±16

Table 2: Effect of Pb on mineral ions uptake of *Lens culinaris*

Pb (ppm)	Na (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Fe (µg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})	Pb (µg g^{-1})
Shoot								
0	90±11	28±03	143±11	68±11	140±11	-	210±21	208±21
50	88±12	25±04	132±11	61±10	138±11	46±11	170±21	130±20
100	68±12	15±01	115±12	54±03	128±12	55±10	130±21	115±25
150	60±11	12±03	102±12	38±02	120±12	65±10	120±14	101±14
200	61±12	11±03	90±12	30±04	108±13	68±11	108±14	95±11
250	40±21	06±11	70±15	25±01	90±12	73±11	100±25	90±11
Root								
0	98±11	15±03	132±23	82±12	159±24	-	185±12	270±26
50	56±11	10±02	110±11	78±08	140±20	47±09	160±23	240±11
100	50±12	08±03	89±10	60±01	110±16	60±10	145±21	230±12
150	42±08	07±04	80±09	45±06	100±14	65±11	120±21	210±14
200	35±01	03±03	72±08	30±02	90±12	70±12	100±12	195±14
250	30±02	-	5±02	28±12	70±02	75±11	45±11	170±25

Table 3: Pb stress on phytochemical studies of seedlings of *Phaseolus mungo* and *Lens culinaris*

	Phaseolus mungo				*Lens culinaris*			
Pb (ppm)	% protein roots	% protein shoots	% amino acid roots 10^4	% amino acid shoots 10^3	% protein roots	% protein shoots	% amino acid roots 10^5	% amino acid shoots 10^5
0	1.308±0.02	0.496±0.03	1.90±0.01	2.40±0.01	0.820±0.01	1.26±0.01	2.50±0.01	7.30±0.01
50	1.108±0.01	0.617±0.03	2.30±0.02	1.40±0.01	0.610±0.01	1.73±0.01	4.80±0.02	3.60±0.01
100	1.082±0.02	0.647±0.02	2.31±0.03	2.70±0.01	0.600±0.01	0.89±0.01	1.60±0.02	3.60±0.01
150	1.182±0.03	0.690±0.01	2.20±0.02	2.00±0.02	0.470±0.01	0.41±0.01	2.50±0.02	5.50±0.01
200	1.212±0.05	0.726±0.01	3.20±0.02	3.20±0.05	0.350±0.01	0.31±0.02	4.30±0.01	3.50±0.01
250	0.900±0.01	0.736±0.01	3.50±0.02	3.20±0.02	0.150±0.02	0.10±0.02	4.30±0.02	3.10±0.02

2002). Many of observed action of Pb appear to be indirect as results of mineral imbalance with in the tissues of both species. Significant changes in nutrients occur in plants under Pb toxicity The increase in Pb concentration led to decreased quantity of Mg (Table 1 and 2) in both root and shoot which effect on process of photosynthesis (Haider *et al*., 2006).

Reductions in nutrient contents as well as in internal ratios of nutrients occur in seedlings under Pb stress as observed earlier (Pinero *et al*., 2002). It damages the tissues cells of vascular bundles (Azmat *et al*., 2006) which results in the inhibition of conduction of water molecules from root (Eun *et al*., 2002) to aerial parts of seedlings. Table 3 showed significant difference between the two organs of plant (root and shoot) with respect to applied stress (Kornélia and Sarkada, 2002) and accumulation trend of Pb in roots and shoots of both species (Table 1 and 2).

Effect of mineral ions reduction in seedlings: Potassium acts as a coenzyme or activator of many enzyme systems (Kabata-Pendias and Penadas, 1992). High potassium levels are required for protein synthesis and fruit production in most crops (i.e., tomato) as the demand for potassium by the developing fruit is high (Schreinemakers, 1984). A deficiency during fruiting

produces fruit of significant lower quality and size. In some cases Pb toxicity causes leakage of K ions which may depressed the shoot growth.

Potassium deficiency symptoms being as slow growth (Salisbury and Rose, 1969). If the deficiency becomes severe lower leaves develop a marginal chlorosis (Lorenzo *et al*., 2000) giving the appearance of burned edges. Protein synthesis was affected by reduction in uptake of K ions in roots and shoots of *Lens culinaris* (Table 3) while protein contents in shoots of *Phaseolus mungo* were found to be increased which may be related with N-containing metabolites frequently preferentially synthesized under heavy metal stress (Sharma and Dietz, 2006; Lorenzo, 2000) whereas decreased in roots, due to disturbed structure of roots (Azmat *et al*., 2006). Amino acids (Kornélia and Sarkada, 2002) which are building blocks of protein were also affected by Pb toxicity (Table 3) and lower concentrations in roots of both species were recorded (Azmat *et al*., 2006). Figure 3-6 shows the absorption spectrum of protein *Phaseolus mungo* and *Lens culinaris* with different concentration of Pb.

Calcium deficiency is generally a result of an imbalance with potassium and magnesium. It primarily affects leaf size and shape and is the cause of blossom end rot in developing fruit. Magnesium is a constituent of chlorophyll (Haider *et al*., 2006) and is required for activation of many enzymes involved in the energy transfer. A deficiency of magnesium will seriously affect plant growth and development as photosynthesis is directly affected (Haider *et al*., 2006). Deficiency of magnesium frequently occurs due to an imbalance with potassium, appearing as interveinal chlorosis developing first on older leaves.

Mn is essential to activate some enzymes, involved in fatly acid synthesis. DNA and RNA formation and the enzyme isocitrate dehydrogenase in the Krebs cycle. Mn is also involved in the production of oxygen from water in photosynthesis. The decrease in the concentration of Mn causes decrease in the concentration of chlorophyll which may be related with the reduction in quantum yield of oxygen evolved in photosynthesis (Haider *et al*., 2006) Table 1 and 2 shows that concentration of Zn is lowered due to Pb which may results in the stunting of plant and leaf growth and causes the death of plants. Zn is required for the formation of the hormone indoleacetic acid which is an enzyme activator.

Iron is required for the synthesis of chlorophyll and is an essential part of the cytochrome which serves as electron carriers in photosynthesis and respiration. Deficiency of iron in the plant showed the inhibition of process of photosynthesis related with small size of leaves of plant.

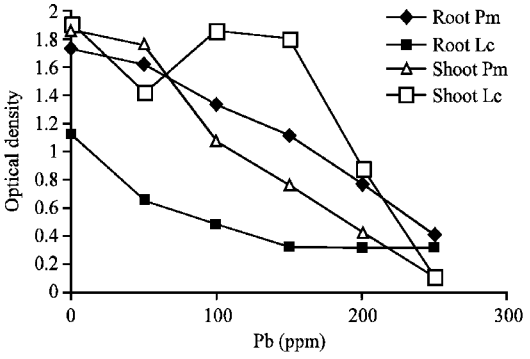

Fig. 2: Pb stress on phosphate contents of seedlings of *Phaseolus mungo* and *Lens culinaris*, Pm = *Phaseolus mungo*, Lc = *Lens culinaris*

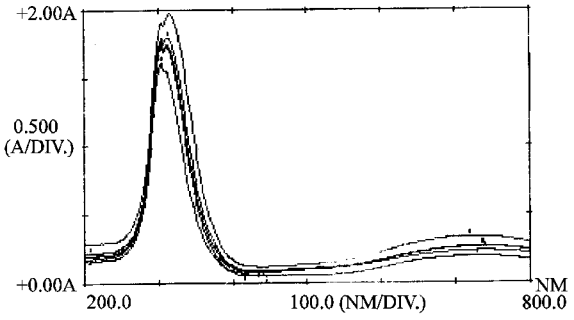

Fig. 3: Absorption spectrum of protein contents of shoots of seedlings of *Phaseolus mungo* under Pb stress

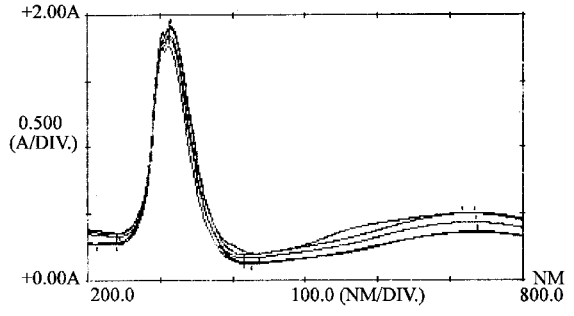

Fig. 4: Absorption spectrum of protein contents of roots of seedlings of *Phaseolus mungo* under Pb stress

Phosphorus is a constituent of ATP, phospholipds and certain coenzymes. It is very important in the plant energy transfer system and deficiency can slow growth considerably. Phosphorus deficiency reduces growth and other older leaves develop a purplish color as anthocyanin pigments accumulate. Phosphorus in the form of mono-or all hydrogen phosphate anions (HPO_4, H_2PO_4).

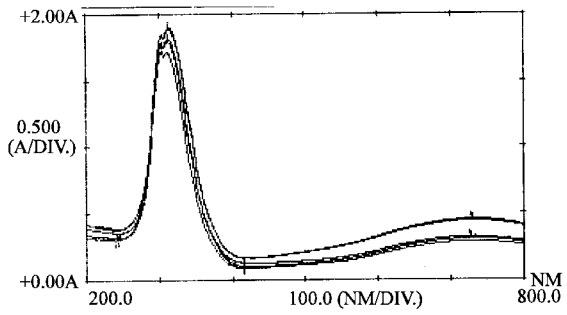

Fig. 5: Absorption spectrum of protein contents of shoots of seedlings of *Lens culinaris* under Pb stress

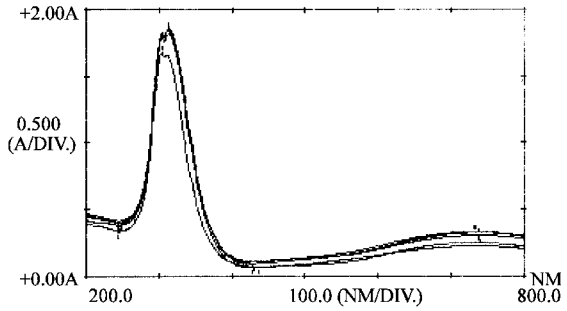

Fig. 6: Absorption spectrum of protein contents of roots of seedlings of *Lens culinaris* under Pb stress

Figure 2 shows decline in phosphorus contents in roots of both species where as in shoot at 50 ppm decrease in phosphorus contents were observed but as the concentration of Pb is increase it shows gradual increased in phosphate concentration but at 200 ppm reduction in phosphate concentration may be due to smaller size of leaves and inhibitory effect of Pb on phosphate ions uptake in root and shoot of both species.

Calcium is required to maintain membrane integrity and is found in cell wall as calcium pectate which cements together adjacent cell walls. Calcium deficiency is generally a result of an imbalance with potassium and magnesium. It primarily affects leaf size and shape (Azmat *et al*., 2006).

Mechanism: Mechanism for decreases (Zlatimira and Doncheva, 2002) in uptake of micro and macronutrients under Pb stress corresponds to, 1) (I) small size of ionic radii of metals, ii) the size of ionic radii of essential minerals ions, (larger as compared to size of heavy toxic metal), iii) higher density of toxic metal and larger entropy of activation due to which Pb will accumulate more rapidly as compared to essential mineral; 2) when it accumulate it damages the tissue structure (Azmat *et al*., 2006; Pahlsson, 1989) and alter membrane enzymes activities. The efflux of K^+ from roots, apparently due to extreme sensitivity of K^+-ATP ase and-SH group of cell membrane protein to Pb, is type of proposed mechanism. Two mechanisms for decreased uptake of micro and macronutrients under Pb toxicity have been suggested. The first mechanism, termed physical, relies on the size of metal ion radii, whereas second mechanism, which is a chemical one, relies on the metal-induced disorder in the cell metabolism leading to change in membrane enzymes activities and membrane structure.

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