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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Phytotoxicity of Pb: II. Changes in Chlorophyll Absorption Spectrum due to Toxic Metal Pb Stress on *Phaseolus mungo* and *Lens culinaris*

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**Abstract:** Lead concentration is increasing rapidly in the environment due to increased use of its sources by human society. Photosynthesis has been found to be one of the most sensitive plant physiological process and the effect of metal is multi facial. This investigation discussed the toxicity of Pb on chlorophyll content and carbohydrate of *Phaseolus mungo* and *Lens culinaris*. Growth and pigments contents of both species were highly decreased and inhibited at 250 ppm of Pb concentration. The absorption spectrum of pigments were obtained by UV/Visible double beam Shimadzo Spectrophotometer. The observed spectral change due to enhancement of Pb dose, led to significant reduction in chlorophyll biosynthesis with values of 1.004 to 0.023 for *Phaseolus mungo* and 1.415 to 0.426 for *Lens culinaris*. This may be related with the accumulation of Pb in root and shoot. The absorption spectrum shows the change in structure of chlorophyll, which indicated that absorption of Pb as compare to essential mineral ion was higher. Pb replaces the magnesium and imparts the color due to which dark green leaves of both species were observed and leaves of plant remain green after experience of death. It may be attributed with less concentration of Mg in chlorophyll. Observation revealed that stomata density in the epidermis was noticeably reduced in presence of Pb due to which quantum efficiency for released of oxygen decreased.

**Key words:** Pb, chlorophyll, visible spectrum, quantum efficiency, Mg

### INTRODUCTION

As many of the Pb pollutant are indispensable for modern human life, soil contamination with Pb is not likely to reduce in near future. It is easily taken up by plants from soil and accumulated in different organs (Baryla *et al.*, 2001; Ewais, 1997). Photosynthetic pigments are essential for plant development. The studies on the effect of heavy metals on the photosynthesis apparatus (Csatorday *et al.*, 1984) have raised the question about possible mechanism of their toxicity towards its integrity and functional aspects (Baker, 1981) whereas leaf chlorophyll content (Doring *et al.*, 1969) provides valuable information about physiological status of plant. Reflectance measurement makes it possible to quickly and nondestructively assess, in situ, the chlorophyll content in leaves. The process of photosynthesis is adversely affected by toxicity. Plant exposed to Pb show a decline in photosynthesis rate which results from distorted chloroplast ultra structure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, inhibited activities of

Calvin cycle enzymes, as well as deficiency of CO<sub>2</sub> as a result of stomata closure. Pb inhibits chlorophyll synthesis by impairing uptake of essential elements of chlorophyll such as Mg and Fe. The mechanism proposed for this inhibition is the replacement of magnesium in the chlorophyll molecule. Consequently cells accumulate protoporphyrin and synthesis of chlorophyll is blocked. In this regard the reduction of chlorophyll content is a common symptom of heavy metals toxicity. This may be attributed to inhibition of reduction steps in the biosynthesis pathways of the pigments (Hartnut *et al.*, 1990). It damages the photosynthetic apparatus due to its affinity for protein N- and S- ligands. The chlorophyll content over a wide range of its changes can be assessed during leaf ontogeny using reflectance near 700 nm (Gitelson *et al.*, 2002, 2003). Reflectance spectra of leaves and fruits undergo remarkable changes under deficiency of mineral nutrition, pollution, different stress conditions, during adaptation to variable solar irradiation and in the course of senescence (Merzlyak *et al.*, 2003; Solvchenko *et al.*, 2001). Reduced accumulation

of chlorophyll in Cd, Cu, Mn and Ni treated plant have been reported by Siedlecka *et al.* (2001). The substitution of heavy metal in place of Mg as a central ion in the porphyrine ring of chlorophyll or bacteriochlorophyll *in vivo*, for algae, lichen, water plants and *in vitro* for isolated pigments were reported by Ewais (1997). Leaf chlorosis was attributed to a marked decrease in the chloroplast density caused by a reduction in number of chloroplast per cell and change in cell size suggesting that Cd interfered with chloroplast (Azmat *et al.*, 2005a) replication and cell division.

*Phaseolus mungo* and *Lens culinaris* are widely used by people of south Asia and as well as in Pakistan and these pulses are imported in Pakistan from other countries. Keeping this view, this study has been planned to investigate the pigments and starch contents of both species under Pb stress. Toxic metal is widespread potent pollutant of environment which is present both in air and water of irrigation. Chlorophyll and carbohydrate will be determined by usual method and absorption spectrum will be observed by UV/Visible double beam Shimadzo Spectrophotometer. The results will be discussed in relation with photosynthesis and growth regarding with carbohydrate concentration.

### MATERIALS AND METHODS

The experiments were performed in growth chamber in department of Botany in Jinnah University for Women in July 2005. *Phaseolus mungo* and *Lens culinaris* were selected for cultivation under PbCl<sub>2</sub> treatment in pots of Hoagland solution.

For chlorophyll and other pigments, an extract of plants in 80% acetone were prepared. Spectral data for both spices were collected at close-ranges of UV/Visible Shimadzo spectrophotometer. The system detects research spectral data in the range of 200 to 800 nm. (Bohlar and Elisabeth, 1987).

Carbohydrates were analyzed in protein free filtrate extract of plants in water using anthrone reagent and complex absorbance was observed by spectrophotometer at 650 nm (Tandon, 1993).

After passing 2 days, the following agronomic parameters were determined a) Plant height b) Root Length c) Shoot fresh mass and shoot dry mass d) Leaf area e) Percentage of germination f) determination of pigments concentration (chlorophyll a and b and total chlorophyll) by spectrophotometer. Pigments in the leaves were extracted with 80% acetone and data were used to calculate the concentration of pigments.

### RESULTS AND DISCUSSION

Results showed that the Pb affects the morphology of leaves (Fig 1 and 2). Up to 50 ppm of Pb concentration, leaves showed normal growth and color while on high dose of Pb, leaves size were shortened with dark color. This indicated that process of photosynthesis was adversely affected by Pb toxicity. Plant exposed to Pb ions shows a decline in ultra structure, restrained synthesis of chlorophyll (Azmat *et al.*, 2005a-c) plastoquinone and carotenoids (Truscott, 1990), obstructed electron transport as well as efficiency of CO<sub>2</sub> as a result of reduced stomata density (Tripathy *et al.*, 1983). *Phaseolus mungo* and *Lens culinaris* when grown in aquatic medium (Pb based Hoagland solution) showed distinct changes in wavelength of chlorophyll a and b. Table 1 and 2 shows the change in maximum optical density with the change in concentration of Pb, which can be attributed with accumulation of Pb in leaves due to which decreased in concentration of chlorophyll (Table 3 and 4) were recorded (Azmat *et al.*, 2005a, b).

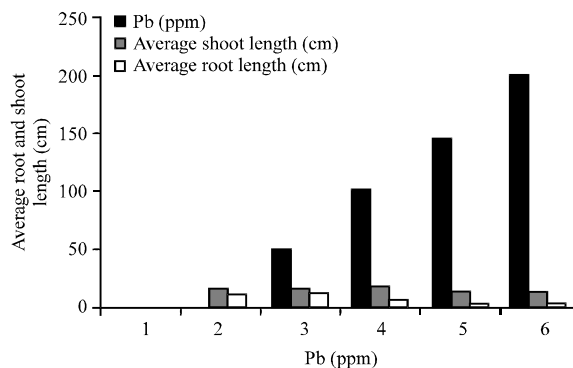


Fig. 1: Effect of Pb on root and shoot length of plant of *Phaseolus mungo*

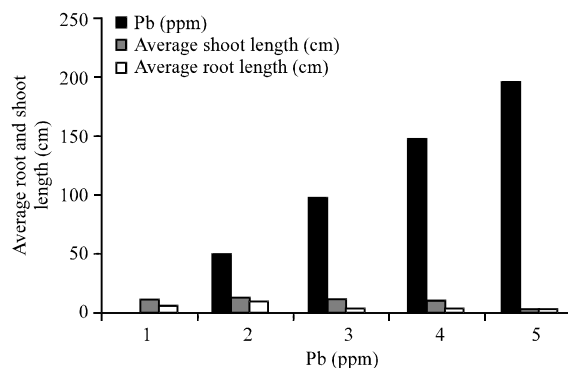


Fig. 2: Effect of Pb on root and shoot length of plant of *Lens culinaris*

Table 1: Effect of Pb (ppm) on pigments absorbance and wavelength in *Phaseolus mungo*

Pb (0) $\lambda_{max}$	OD	Pb (50) $\lambda_{max}$	OD	Pb (100) $\lambda_{max}$	OD	Pb (150) $\lambda_{max}$	OD	Pb (200) $\lambda_{max}$	OD	Pb (250) $\lambda_{max}$	OD
663	0.962	663	0.248	663	0.600	665	0.244	664	0.040	649	0.023
617	0.287	614	0.103	617	0.197	607	0.056	457	0.090	445	0.090
582	0.241	571	0.105	580	0.176	535	0.080	431	0.127	360	0.085
535	0.201	535	0.105	457	0.771	506	0.086	359	0.188	-	0.224
457	1.150	457	0.381	432	1.150	411	0.716	-	-	-	-
432	1.741	432	0.572	372	0.923	373	0.543	-	-	-	-
379	1.148	362	0.512	-	-	-	-	-	-	-	-
345	0.854	-	-	-	-	-	-	-	-	-	-

Table 2: Effect of Pb (ppm) on pigments absorbance and wavelength in *Lens culinaris*

Pb (0) $\lambda_{max}$	OD	Pb (50) $\lambda_{max}$	OD	Pb (100) $\lambda_{max}$	OD	Pb (150) $\lambda_{max}$	OD	Pb (200) $\lambda_{max}$	OD	Pb (250) $\lambda_{max}$	OD
663	1.351	665	0.657	663	0.493	663	0.872	663	0.409	-	-
617	0.349	607	0.152	617	0.144	617	0.238	616	0.124	-	-
584	0.260	535	0.188	535	0.106	584	0.188	-	-	-	-
535	0.183	411	1.834	456	0.625	-	-	-	-	-	-
456	1.555	-	-	432	0.948	-	-	-	-	-	-
432	2.284	-	-	381	0.577	-	-	-	-	-	-

Key:  $\lambda$  = Wavelength, OD = Optical density,  $\lambda = 663$  = Chlorophyll, 617 = Xanthophyll, 583 - 587 = Isocarotene, 530 - 430 = Caroteoids

Table 3: Concentration (mg g<sup>-1</sup>) of pigments of *Phaseolus mungo* under Pb stress

Pb (ppm)	Chlorophyll (a)	Chlorophyll (b)	Cartenoids	Xanthophyll
0	1.004336	0.21322	1.131706	0.285282
50	0.175120	0.10048	0.375052	0.001809
100	0.624798	0.15591	0.758880	0.194998
150	0.257790	0.00637	0.097282	-
200	0.041296	0.01712	0.08831	-
250	0.023012	0.02365	-	-

Table 4: Concentration (mg g<sup>-1</sup>) of pigments of *Lens culinaris* under Pb stress

Pb (ppm)	Chlorophyll (a)	Chlorophyll (b)	Cartenoids	Xanthophyll
0	1.415584	0.18635	1.525482	0.324362
50	0.693966	0.01404	0.61423	-
100	0.515008	0.09737	-	0.144962
150	0.912360	0.14524	-	0.237552
200	0.426726	0.09080	-	0.122998
250	-	-	-	-

Table 5: Effect of concentration of Pb on carbohydrate contents of *Phaseolus mungo* and *Lens culinaris*

(Pb) ppm	<i>Phaseolus mungo</i>		<i>Lens culinaris</i>	
	% of Carbohydrate (Root)	% of Carbohydrate (Shoot)	% of Carbohydrate (Root)	% of Carbohydrate (Shoot)
0	0.02±0.0010	0.023±0.03	0.019±0.01	0.027±0.010
50	0.01±0.0010	0.033±0.02	0.016±0.01	0.019±0.010
100	0.011±0.001	0.031±0.01	0.024±0.01	0.024±0.010
150	0.012±0.010	0.02±0.010	0.018±0.01	0.018±0.003
200	0.013±0.010	0.018±0.02	0.017±0.01	0.016±0.010
250	-	0.018±0.01	0.017±0.01	0.016±0.030

Figure 3 and 4 shows decrease in concentration of chloroplast pigments in the absorption spectrum of both *Phaseolus mungo* and *Lens culinaris* with change in wavelength. It may be due to the accumulation of Pb in prophyrin ring of chlorophyll due to which photosynthesis was not effective because light energy absorbed by accessory pigment is transferred to

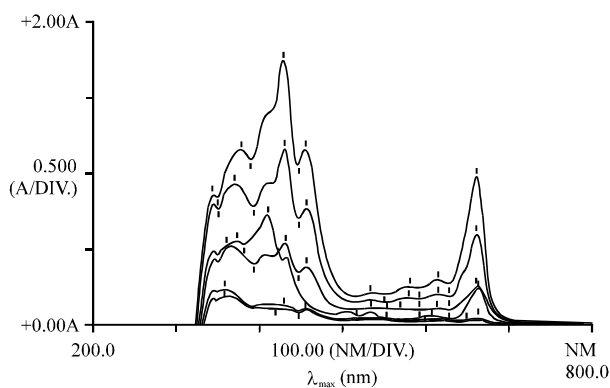


Fig. 3: Spectral change of chlorophyll and other pigments of *Phaseolus mungo* with respect to change in concentration of Pb

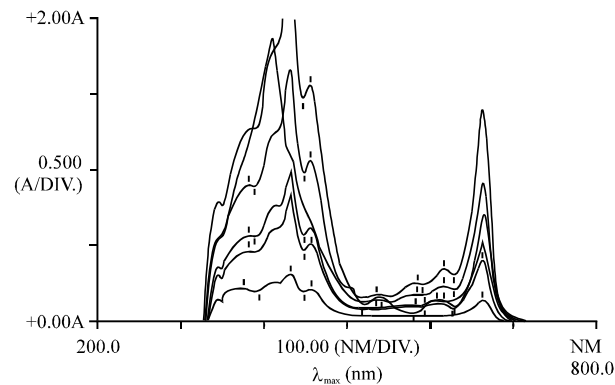


Fig. 4: Spectral change of chlorophyll and other pigments of *Lens culinaris* with respect to change in concentration of Pb

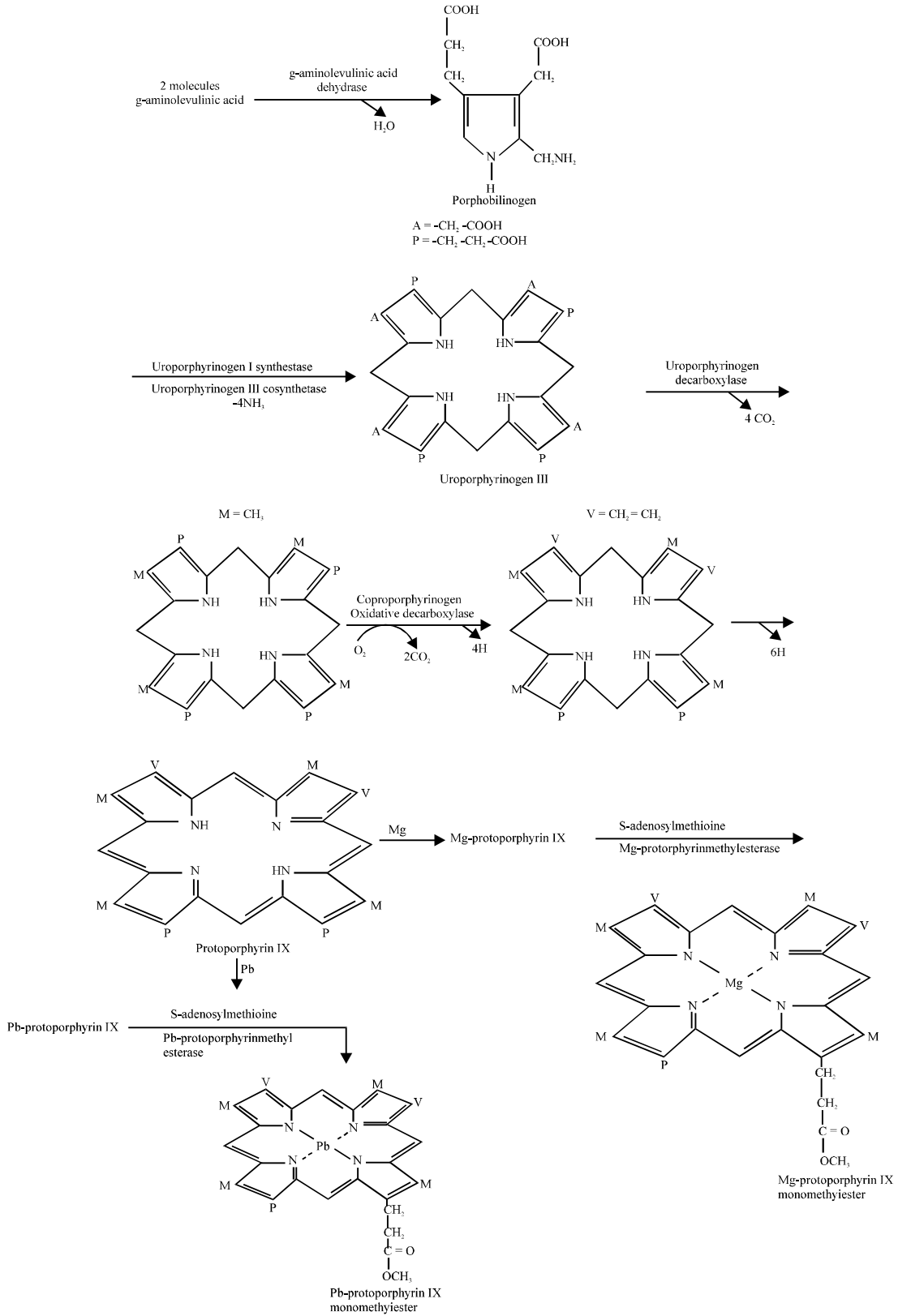


Fig. 5: Continued

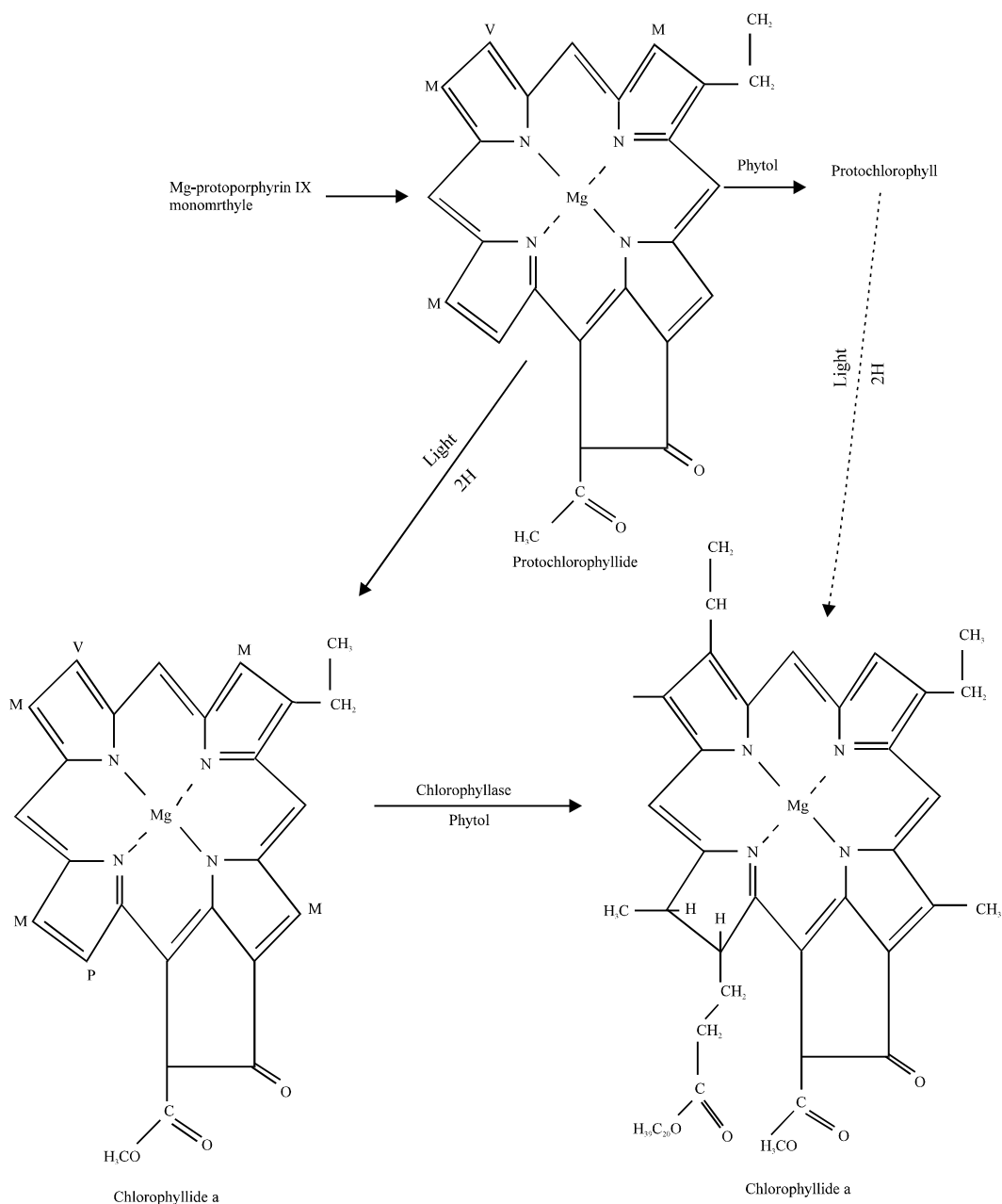


Fig. 5: Biosynthesis of chlorophyll in presence of Pb shows the replacement of Mg ion

chlorophyll then it activates photosynthesis. The above investigation reveals that rate of photosynthesis were decline in red region of spectrum (Ambrosio *et al.*, 1992) that is in the red absorption band of chlorophyll a. It may reduced the quantum yield of oxygen which is released after the quanta of light absorbed and this reduction in quantum yield of oxygen may effect the environment. Decrease in photosynthesis also reduced the absorption of CO<sub>2</sub> and retardation of water molecule which causes the decrease in carbohydrate concentration

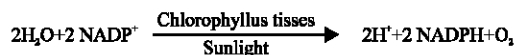
in both species (Table 5). The dark green color of leaves may relate with the increase in the chlorophyll content at 150 ppm of Pb but reduced leaves size at higher concentration with compact root structure indicated that concentration of Mg and Fe in chlorophyll were reduced due to which inhibition of process of photosynthesis and leaf cells of such plants exhibited a reduction in grana stacks together with a reduction in the amount of stomata in relation to the Lamellar system as well as absence of starch grains. Table 5 shows the low carbohydrate

concentration in presence of Pb which also support the above findings. Pb inhibits Chlorophyll b synthesis causing impaired uptake of essential elements such as Mg and Fe (Kowalewska *et al.*, 1987). An enhancement of chlorophyllase activity of chlorophyll b is reported to be more affected than chlorophyll a by dose of Pb.

A strong relationship (Gitelson *et al.*, 2003; Merzlyak *et al.*, 2003) existed between Pb application and a decrease in photosynthesis of the whole plant and is believed to result from stomata closure rather than a direct effect of Pb on the process of photosynthesis. According to Kosobrukhov *et al.* (2004), the photosynthetic activity of plant is governed by many factors including stomatal cell size, number of stomata conductant, leaf area etc. While studying the effects of Pb on the development of thylakoid (Sarvari *et al.*, 2002.) of cucumber and poplar plants. Savari *et al.* (2002) observed increased chlorophyll content either in PS II core or LHC II at low concentrations of Pb treatment, whereas a strong decrease in chlorophyll level of seedlings was seen at the 50 ppm Pb treatment level. The concentration of Pb inside the leaf might have been high enough to directly inhibit chlorophyll synthesis (Sengar and Panday, 1996).

Investigation shows that spectral properties of photosynthetic pigment (Rai *et al.*, 1999) alter in all treated plants where total chlorophyll contents diminished and efficiency of photosynthetic apparatus may be distorted by changes in pigment contents. Table 3 and 4 shows that maximum pigments were observed in control plant, which gets reduced at 250 ppm of Pb concentration. This indicated the visual non-specific symptoms of Pb toxicity as root growth inhibition, reduced leaf size and chlorosis.

**Mechanism:** A mechanism (Nechushtai *et al.*, 1996; Devlin and Witham, 2001) has been proposed based on above findings which show the replacement of Mg from the porphyrin ring of chlorophyll which may be due to the higher entropy of activation of Pb as compared to Mg ion Fig. 5. Pb accumulation (Samardakiewicz and Wozny, 2000) in the roots follow the same pattern of absorption as of essential mineral ion in the roots. Photosynthetic unit is a group of collaborating molecules of pigments, which is essential for photochemical phase. Mechanism showed that when a quantum of light is absorbed and then it migrates through these collaborating molecules to the reaction center or the trapping center, with release of an electron (Nechushtai *et al.*, 1996). This quantum of light may be used for chemical work that is, the generation of assimilatory power, ATP and NADPH but reduced leaves sized due to Pb shows decrease in surface area for photosynthesis and inhibition of chloroplast activity which also reduced the quantum yield of oxygen by following important steps in leaves



Absorption spectrum of treated plants shows the change in spectral properties of pigments as compared to the control plants where maximum absorption was reported (0.962 for *Phaseolus mungo* and 1.352 for *Lens culinaris*) at  $\lambda_{\text{max}}$  663. This change in spectrum revealed the possibility of Pb substitution of Mg in chlorophyll leading to longer shift of wavelength (Gitelson *et al.*, 2003; Merzlyak *et al.*, 2003). Absorption spectrum of chloroplast pigment shows that Pb may interact indirectly with carotenoids pigments (Frank and Cogdell, 1996) affecting carbohydrate environment of photosynthetic antenna. It may relate with the effect of heavy metals on the photosynthetic apparatus with special attention paid to chlorophyll which are much more complex than just simple paid to substitution by other metals for central Mg ion in the porphyrin ring. The probable mechanism shows that if optical density is higher at 150 ppm of Pb, it indicated the maximum accumulation of metal in chlorophyll ring due to which maximum absorption of light took place but further enhancement of Pb shows the decrease in concentration of chlorophyll because leaves size (Williams *et al.*, 1989) were reduced and no growth of seedling were observed.

## CONCLUSION

Pb has considerable attention as a pollutant heavy metal pollutant due to the growing anthropogenic pressure on the environmental. Pb contaminated soils show a sharp decline line in crop productivity mainly through roots.

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