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Lead induced root and shoot growth reduction in wheat (*Triticum aestivum* L.) is due to increase in membrane lipid peroxidation

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Abstract
Lead (Pb) is one of the most phytotoxic metals in the agricultural soils and its concentration is continuously increasing mainly through anthropogenic activities. Present study was designed to investigate the phototoxic effects of lead (Pb) on different physiological parameter in *Triticum aestivum* L. Wheat seeds were germinated in petri plate at different concentration (0, 50, 100, 150, 200, 250 and 500µM) of Pb $(NO_3)_2$. The increasing lead concentration gradually and significantly decreased germination (%), fresh and dry weight, shoot and root length, decrease in chlorophyll and carotenoid content with respect to control. The hydrogen peroxide content and lipid peroxidation increased both in shoot and root, but those in root increased drastically over the control values. The peroxidative process in roots in Pb toxicity might be one of the major factors for inhibition of growth and pigment content in wheat seedlings.

Keywords: root and shoot growth, lipid peroxidation

Introduction
Heavy metal pollution has become one of the major environmental problems worldwide. In most of the metal polluted soil lead usually appears associated with other heavy such as zinc (Zn) and cadmium (Cd) (Hernandez-Allica *et al*., 2007). Lead causes major adverse effect on both plants and animals. Naturally Pb is present in soil, sea, lakes, rivers and natural weathering processes apart from the natural processes the main sources of Pb pollution in environmental are mining and smelting activities, Pb containing paints, fertilizers, pesticides, gasoline, Pb-acid batteries, bullets shot, fusible alloys and disposable of municipal sewage sludge containing Pb (Sharma & Dubey, 2005).Significant increases in the Pb content of cultivated soils has been observed near industrial areas. Soils contaminated with Pb cause significant decreases in crop productivity there by caused a serious problem for agriculture.
Among different heavy metals lead is the second most harmful pollutant after arsenic (Pourrut *et al*., 2011). Lead toxicity in plant change morpho-physiological, biochemical and disturbs normal metabolic activities in plant cell components to organ level. Lead toxicity leads to significant reduction in seeds germination percentage, fresh and dry biomass of shoot and root stunted plant growth, chlorosis (Sharma & Dubey, 2005; Ekmekaci *et al*., 2009) reduction in cell division and negative effects on photosynthetic activity (Boucher & Carpentier, 1999). Lead reducing chlorophyll production by obstructs the uptake of essential nutrients such as Mg and Fe by plants (Pourrut *et al*., 2011).At high concentration of lead cause cell death (Seregin & Ivanov, 2001).
The toxic effect of lead on plants varies among the variety, lead concentration as well as soil properties. Lead produces reactive oxygen species (ROS) and increase antioxidant enzyme activity in plants (Mishra *et al*., 2006). Production of excessive ROS includes harmful effects in plant cells, inhibition of photosynthetic activity, ATP production and DNA damage. Reactive oxygen species includes superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are important agents during tissue and membrane injury and also produced after exposure of plants to heavy metal. Generation of excess amount of ROS in heavy metal stress plants may be a consequence of the distribution of the balance between their production and the antioxidative enzyme activity. The effect of Pb stress has been studied in various plant species, including *Triticum aestivum* L. (Ekmekaci *et al*., 2009); Oryza sativa (Verma & Dubey, 2003) and Brassica juncea (Zaier *et al*., 2010). Hydroponic cultures allow an easy observation making quick screening on the basis relative growth and toxicity (Zhivotovsky *et al*., 2011). However soil contamination with Pb effects yield, quality as well as production of wheat.

Materials and Methods

Healthy seeds of wheat (*Triticum aestivum* L.) variety PBW 343 were purchased from KVK Nawada (Bihar). Seeds were surface sterilized with 0.1 % sodium hypochlorite for 10 min and washed under running tap water followed by rinsing 5-6 times with distilled water and germinated in petri plate at different concentration (0, 50, 100, 150, 200, 250 and 500µM) Therefore, 5 days-old seedlings were used throughout the experiments.

Determination of growth parameters: Wheat seeds (20) were germinated on filter paper placed in petri plate, moist with 5 ml of lead nitrate solution and for control moist with 5 ml distilled water. Petri plate was incubated in dark at 25 ^{0}C for proper germination. After 5 days when plumule and redicle length were over 2 mm long, then the number of seed germination and germination percentage was determined. Plant growth was determined by measuring the length of shoot and root. Fresh weight measurement, selected five seedlings randomly. Dry weight was measured after shoot and roots were oven dry at 70 °C for 48 hours to constant weight. The relative water content were measured and calculate using equation (Chen *et al*., 2001) RWC (%) = [(FW– DW) / FW] X 100).

Determination of photosynthetic pigments contents: Photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids were extracted and estimated according to (Wellburn, 1994). 0.5 g of leaves of control and Pb treated were cut into tiny segments and incubated in 5 ml dimethyl sulphoxide (DMF) covered with aluminium foil and kept in dark at 4 ˚C for 24h. After 24 h extraction in dark, the leaf segments were well-extracted for residual pigments. The absorbance of chl a, chl b and carotenoids contents was measured at 664, 647 and 480 nm on UV visual Perkin-Elmer double beam spectrophotometer against DMF, as a blank. The contents of Chl a, Chl b and carotenoid were determined using experimental equations of (Wellburn, 1994) and pigment content were expressed as µg g^{-1} FW.

Chla = $11.65OD_{664}$ - $2.69OD_{647}$

Chlb = $20.81OD_{647}$ - $4.53OD_{664}$

Car = (1000 x OD_{480} - 0.89 x chla – 52.02 chlb)/245

Determination of Hydrogen peroxide: Hydrogen peroxide (H_2O_2) content was determined according to (Velikova *et al*., 2000) with minor modification. Fresh shoot and root (0.5g) were separately homogenized in 5 ml 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000g for 15 min at 4^{0}C. Total 4ml assay mixture was prepared by taking 1 ml supernatant 1ml 10mM phosphate buffer (pH 7.0) and 2 ml of 1M KI. The absorbance was measured at 390 nm. H_2O_2 content was determined by using the extinction coefficient (ε) of 0.28 µmole^{-1} cm−1 and the content was expressed as nmole g^{-1} f.wt.

Lipid peroxidation analysis: Lipid peroxidation was estimated according to (Heath & Packer, 1968) with minor modification. Shoot and root (0.5g) tissue ware homogenized in 5 ml (0.1%) trichloroacetic acid (TCA) and centrifuged at 10000g for 10 min. The assay mixture containing 1ml supernatant and 4 ml 0.5% TBA in 20% TCA. The mixture was incubated at 95 ^{0}C for 30 min in a water bath. The reaction was transfer quickly in ice bath and centrifugation at 10000 g for 10 min. The absorbance of supernatant was recorded at 532 nm and 600 nm by using spectrophotometer (UV-VIS). The concentration of thiobarbituric acid reactive substance (TBARS) was calculated as malondialdehyde (MDA) equivalents using the molar extinction coefficient of 155 nmole^{-1} cm−1.

Statistical analysis: Each experiment was performed in triplicate and values were expressed as mean ± standard error. Analysis of variance (ANOVA) was done by using graph pad prism, version 5.01(graph pad software, La Jolla, Ca, USA) software and significant differences were indicated by different letters ($p \leq 0.05$).

Results

Effect of Pb on germination and plant growth: Growth inhibition is a common response of plants to heavy metal stress and also most important indices of heavy metal in agriculture. The effect of different concentration of Pb on plant growth, expressed as germination percentage shoot length, root length, shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) root dry weight (RDW), SDW/RDW and relative water content (RWC) of shoot and root are shown in Figure 1.Lead exposure inhibited the growth of *Triticum aestivum* L. significantly compared with control and reduction of germination percentage shoot length, root length, 45.00%, 79.95%, 97.51%, respectively. The results presented in Figure.1 showed that all concentration of Pb (50,100, 150, 200, 250 and 500 µM) caused significant decreased in SFW,RFW,SDW,RDW 0.11,0.02,0.003, 0.008g respectively. Relative water content not significantly different in shoot but root relative water content decreased 70%.

Photosynthetic Parameters: Chlorophyll content is very useful indicator of heavy metal toxicity in plants wheat seedling treated with Pb showed a significant decreased in total chlorophyll, Chl a, Chl b and carotenoid as compared with control (Figure. 2). Total Chl, and carotenoid contents reduced significantly by 220%, 26.08% respectively as compared to control. These results suggested that Pb treatment at 500 µM damage photosynthetic pigments in wheat seedlings. Significant reduction observed in chl a/chl b and chl/car content in wheat seedlings growing at different concentration of lead.

Effects of Pb on H_2O_2 content and lipid peroxidation: Lead treatment caused a significant increase in $O_2^{\cdot -}$ production in both shoot and root. The results regarding the impacts of Pb on H_2O_2 content in wheat seedlings are depicted in Figure 2.The H_2O_2 content increased 85.01%in shoot and 79.07%in root as compared to control respectively. However, a more pronounced increase was observed in root compared to shoot. MDA is the product of membrane lipid peroxidation and when exposed to ROS its causes cell membrane damage. Exposure of wheat seedling to Pb induced lipid peroxidation in shoot and roots. Figure.2 shows that MDA content increased slightly when the concentration was 100µM but increased dramatically at 500µM Pb concentration. The maximum MDA accumulation 85.17% in root and 46.06% in shoot was observed at 500µM of lead and the difference is highly significant as compared to control.

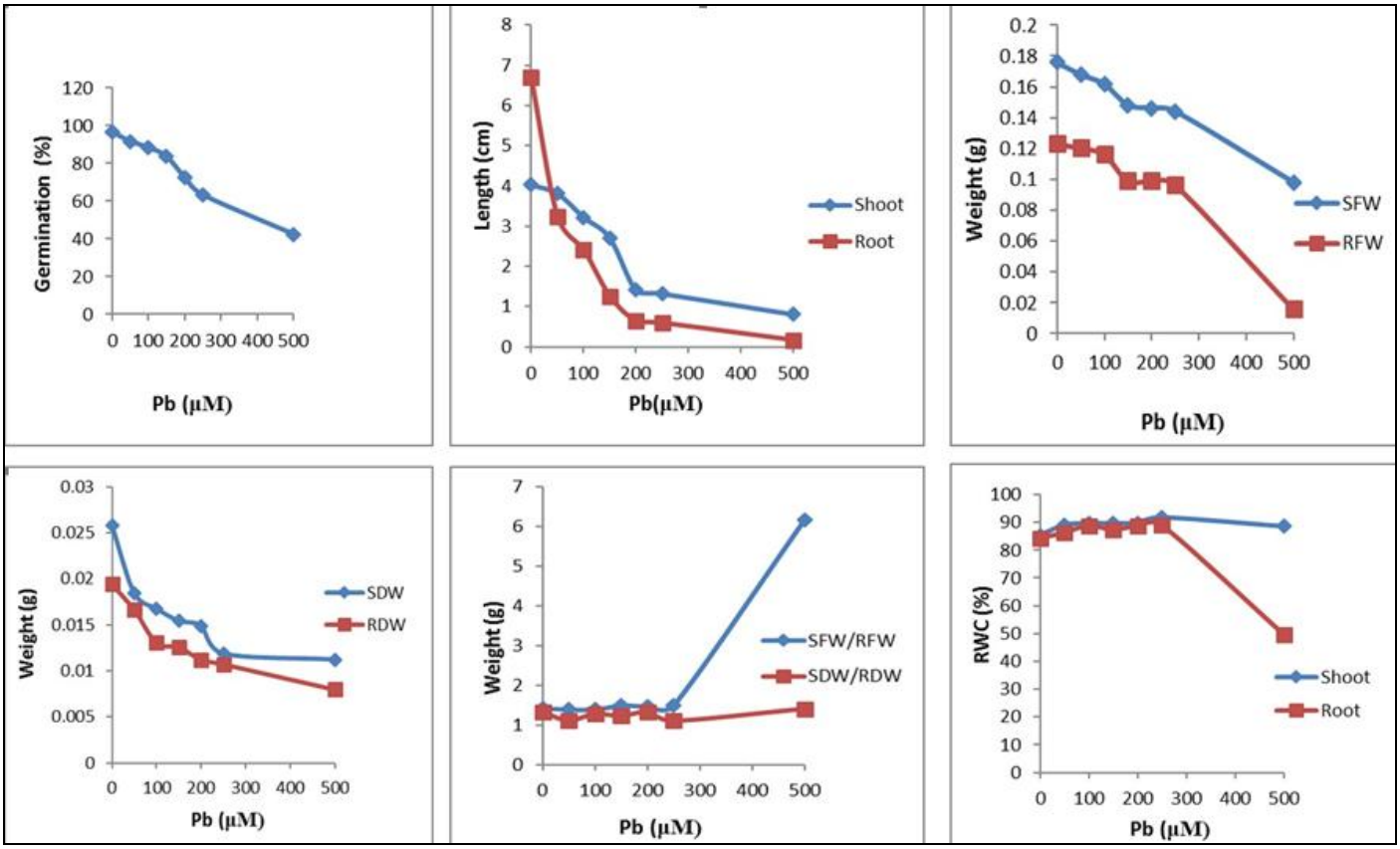

Fig 1: Effect of Pb on germination and shoot or root growth and relative water content (RGW%). SFW, RFW, SDW and RDW represent shoot fresh weight, root fresh weight, shoot dry weight and root dry weight, respectively.

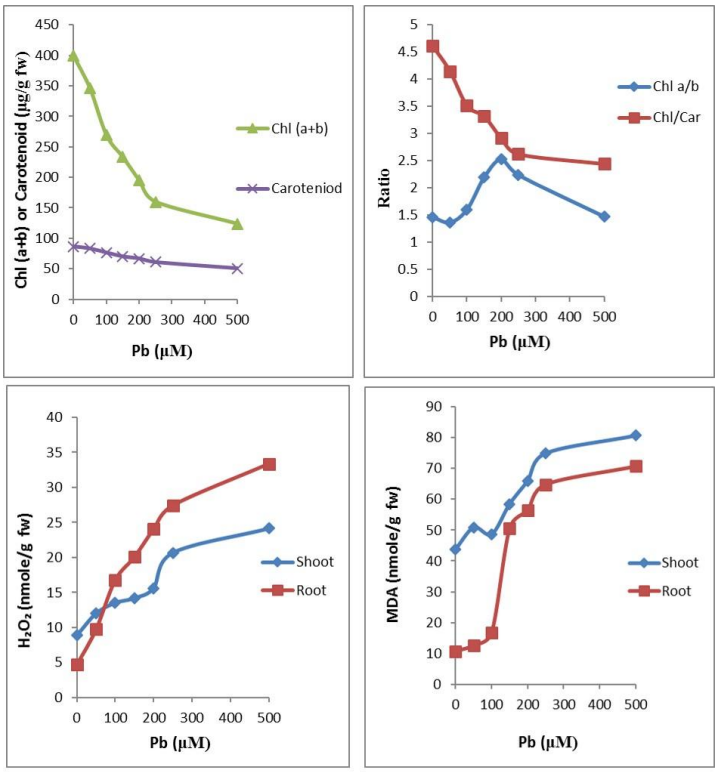

Fig.2. Effect of lead on total chlorophyll (chl a+b), carotenoid, chl a/b, chl/car, H_2O_2 and MDA content in germinated seedlings

DISCUSSION

Lead is one of the most phytotoxic metals in the agricultural soils and its concentration is continuously increasing through anthropogenic activities. The aim of this study was to investigate the effect of lead nitrate $Pb(NO_3)_2$ on wheat seedlings germination. Lead application decreased germination percentage and similar result has been observed by (Mesmar & Jaber, 1991; John *et al*., 2007). These decreases in germination may be due to the interference of Pb with metabolic processes, which loss the viability and

decrease metabolic energy need for generation for embryo. The application of lead nitrate had a significant effect on decrease of shoot and root length l in the studied plant species compared to the control plants. The shoot length decreased 79.95% and root length reduced (97.51%) at 500µM concentration after 5 days of treatment. Similarly (Ghani *et al*., 2010) reported 67% and (Kaur *et al*., 2012) 23 -51 % reduction in root growth of maize and wheat respectively grown in Pb-contaminated soil, whereas shoot length was not more pronounced inhibitory effect then root length (Sharma & Dubey, 2005; Malar *et al*., 2014).In our studies, a significant decrease in fresh weight of shoot and root was observed in plants growing with concentration of Pb ranging from 0.11g and 0.02 g in compared to the control respectively. Similar decrease in fresh weight was also found in stress conditions caused by Pb in *Chlorophytum comosum* (Wang *et al*., 2011) and *Eichhornia crassipes* (Malar *et al*., 2014). Dry weight of shoot and root germinated seedlings was also affected by Pb $(NO_3)_2$, (Lamhamdi *et al*., 2011) also found similar results. Photosynthetic pigments are useful parameters for measurement of lead toxicity in plants such as total chlorophyll and carotenoid. In our study we observed the effect of lead at (500µM) decreased total chlorophyll and carotenoid content as compared to control. Similar result was also found by (Kaur *et al*., 2012) in wheat under Pb toxicity. Hydrogen peroxide content was enhanced in shoot and root after 5 days exposure to 500 µM Pb, over control. Hydrogen peroxide content was observed higher in root than shoot significantly. Such an observation was also reported by (Kaur *et al*., 2012) in wheat during Pb stress. Malondialdehyde (MDA) content increased significantly in shoot and root. These observations are paralleled by earlier studies of (Kaur *et al*., 2015) in wheat seedlings.

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