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PHYSIOLOGICAL RESPONSES OF *PHASEOLUS VULGARIS* TO DIFFERENT LEAD CONCENTRATIONS

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Abstract

Studies were carried out on the phytotoxicity of lead on some physio-biochemical parameters that is chlorophyll, protein, carbohydrate, nucleic acid and phenolic content of *Phaseolus vulgaris*. *Phaseolus vulgaris* seedlings were treated with 25, 50 and 100 ppm concentration of lead acetate and a control (without any treatment) and sown in pots. Increasing lead acetate levels lead to several disruptions of *Phaseolus vulgaris* plants, which are reflected by reductions of protein, chlorophyll, carbohydrate DNA and RNA content. However phenolic content of plants were increasing with increasing levels of heavy metal lead. The effect of lead toxicity was more pronounced at 100 ppm as compared to 25 and 50 ppm lead concentration.

Introduction

Heavy metals are defined as that group of elements that have density higher than about 5gm/cm. Many of them like Co, Cu, Fe, Mn, Mo, Ni, Zn are essential micronutrients and are required for normal growth of plants, which take part in redox reactions, electron transfers and other essential metabolic processes in plants (Michalak, 2006). Pb, Cd, Cr, Hg, Sb, Ag and U have no known biological function as nutrients and found to be more or less toxic to plants and micro-organism (Nies, 1999).

Large areas of agricultural soils are contaminated by heavy metals that mainly originate from former or current mining activities, industrial emissions, the application of agricultural amendments and lime product (Geldmacher, 1984). The contamination of soil by heavy metal enhances plant uptake causing their accumulation in different plants organ (Gimmler *et al*., 2002, Chaturvedi, 2004, Mathe-Gaspar *et al*., 2005). Their presence in environment has become a major threat to plant, animals and human life due to their bioaccumulation tendency and toxicity (Toppi & Gabbrielli, 1999). From soils to plants, transfer of heavy metals is dependent on the following three factor: quantity factor (the total amount of potentially available elements), intensity factor (the activity as well as the ionic ratios of elements in the soil solution) and reaction kinetics (the rate of element transfer from solid to liquid phases and to plant roots) (Brummer *et al*., 1986).

Lead (Pb) is one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentrations are rapidly increased in agricultural soil (McGrath *et al*., 1995). Elevated Pb in soils may adversely effect on soil productivity and even a very low concentration can inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption showing toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Patra *et al*., 2004).

The objective of this work was to study the effects of different levels of heavy metal lead using lead acetate on some physio-biochemical parameters i.e., chlorophyll, protein, carbohydrate, nucleic acid and phenolic content of *Phaseolus vulgaris*.

Material and Methods

Pot experiment with *Phaseolus vulgaris* were carried out in net house of the Department of Botany University of Karachi. Soil was collected from experimental field and sieved through 2mm sieve to discard non soil particles. Two Kg soil was taken in plastic pots of 12cm diameter and 15cm height. Soil was mixed with NPK @ 50 mg/Kg soil. Healthy seeds of *Phaseolus vulgaris* were obtained from the local market. The seeds of *Phaseolus vulgaris* were surface sterilized with 0.1% Mercuric chloride solution for 5 minutes followed by rinsing with tap and distilled water. Twelve seeds of *Phaseolus vulgaris* were planted in the each pot and they were grown in optimal conditions, after two weeks seedlings were reported up to 4 seedlings per pot. Metal treatments of lead were prepared using lead acetate (Pb $(CH_3COO)_2 \cdot 3H_2O$) with concentration of 25, 50 and 100ppm and non treated plants serve as control. All treatment was replicated three times and 20ml of respective treatment was added to each set of pot at every 7^{th} day. The control received only distilled water. At interval of one week the leaf samples from both control and treated plants were collected in early hours of the morning and were kept in labeled sample bags. The plants samples were analyzed for following biochemical parameters.

Chlorophyll were extracted from the leaves and estimated by the method of Maclachlam & Zalik (1963)**.** Estimation of carbohydrate was done in plant extracts by Yemm & Willis (1954) method using Anthron reagent. Estimation of Protein was done in plant extracts by the method of Lowry *et al*., (1951). Total phenols were estimated by using the method described by Swain & Hillis (1959). Total RNA and DNA were estimated by the method of Schmidt & Thannhauser (1945).

Results

Chlorophyll content: The result obtained for the effect of different treatment of lead on total chlorophyll content of *Phaseolus vulgaris* are shown in Fig. 1. Significant (**P<0.01) decrease in total chlorophyll content was observed in lead treated samples as compared to control throughout experimental period. Decline in chlorophyll content were observed with increasing concentration of lead acetate.

Protein content: Lead treatment result in decline in total protein content in *Phaseolus vulgaris* after 1 week treatment and this decrease was observed till the end of experimental period (Fig. 2), all the result obtained was significant (**P<0.01). Lead treatment 100 ppm showed much more toxic effect on total protein content as compared to 25 and 50 ppm concentration.

Carbohydrate content: In *Phaseolus vulgaris* plants growing at 25, 50 and 100 ppm lead treatment, carbohydrate content decrease compare to the control (Fig. 3). All the result obtained was significant (***p<0.001). This decrease in total carbohydrate content was observed in the entire experimental period.

Total phenolic content: Data presented in Fig. 4 indicated that the phenolic content of *Phaseolus vulgaris* were significantly (*p<0.05) affected by 25, 50 and 100 ppm lead acetate treatment. Increasing levels of lead from 25 to100 ppm markedly increased the phenolic content of *Phaseolus vulgaris* in contrast to control. This stimulating influence in phenolic content was observed throughout experimental period.

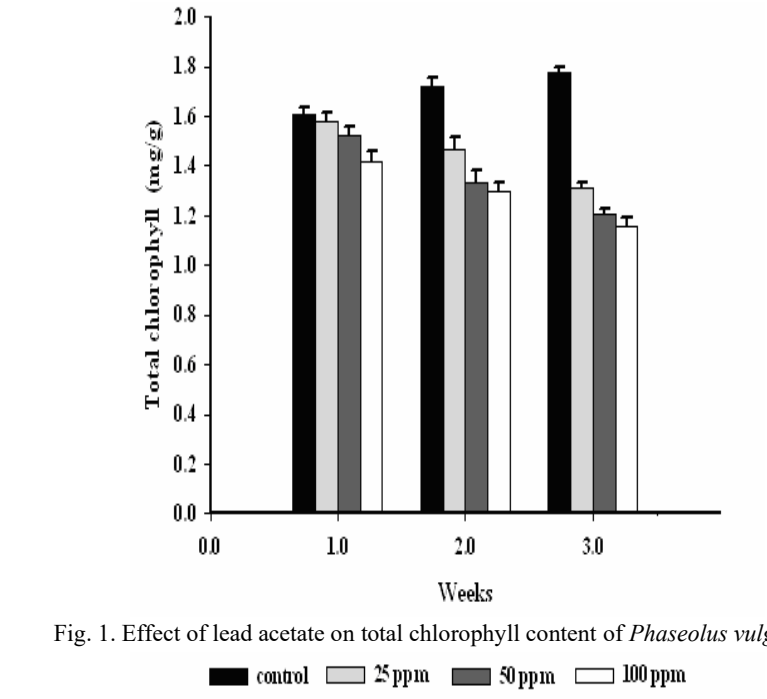

Fig. 1. Effect of lead acetate on total chlorophyll content of *Phaseolus vulgaris*.

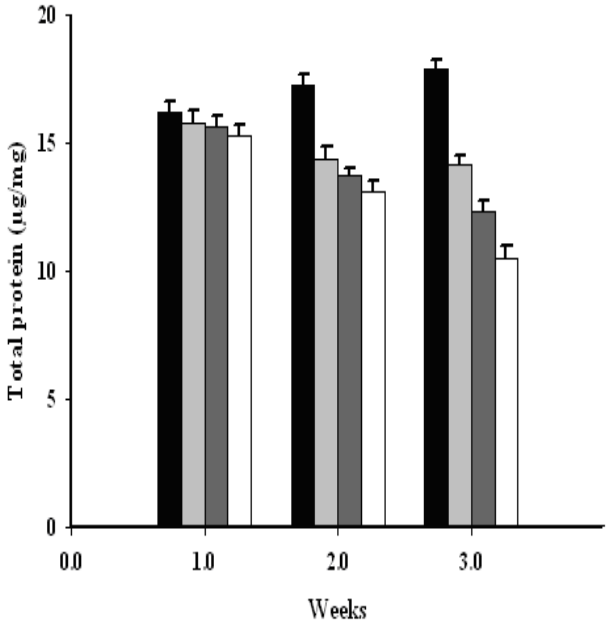

Fig. 2. Effect of lead acetate on total protein content of *Phaseolus vulgaris*.

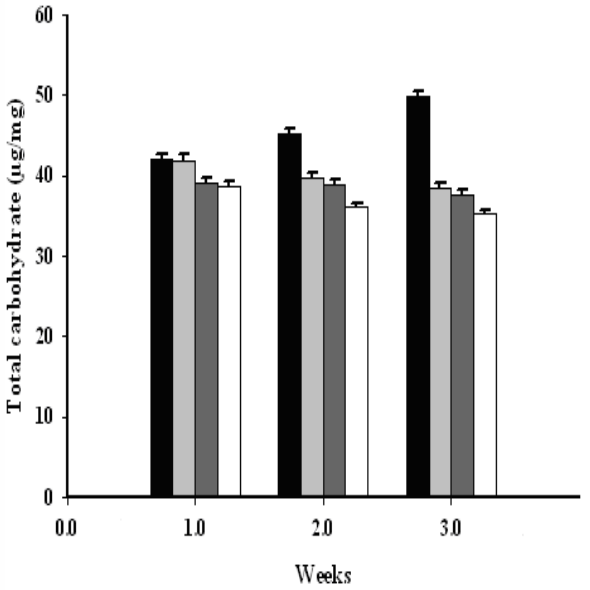

Fig. 3. Effect of lead acetate on total carbohydrate content of *Phaseolus vulgaris*.

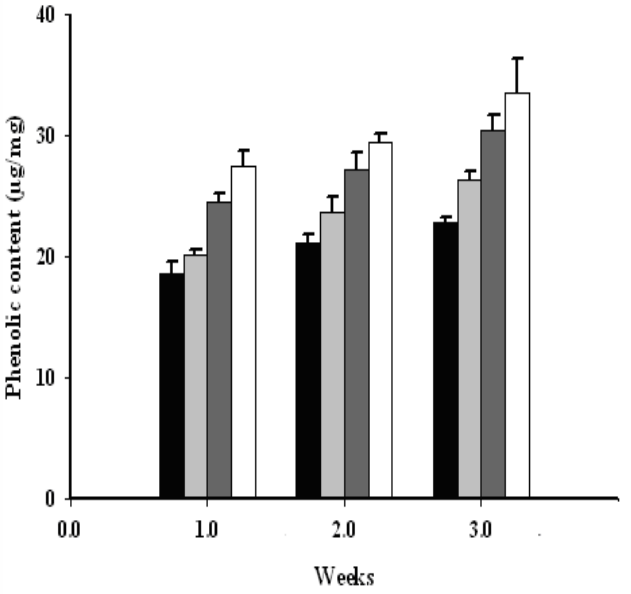

Fig. 4. Effect of lead acetate on phenolic content of *Phaseolus vulgaris*.

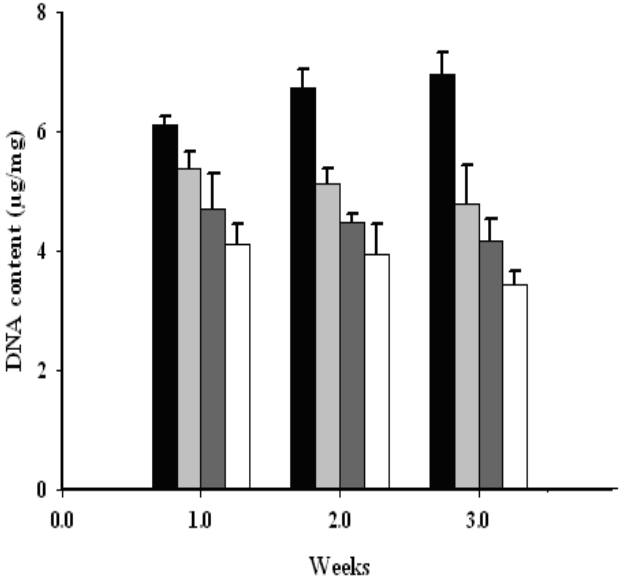

Fig. 5. Effect of lead acetate on DNA content of *Phaseolus vulgaris*.

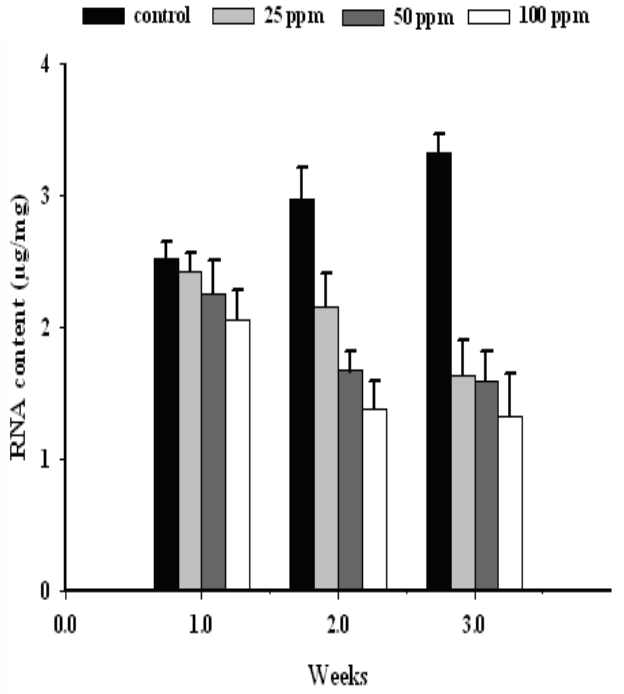

Fig. 6. Effect of lead acetate on RNA content of *Phaseolus vulgaris*.

Nucleic acid content: Lead treatment 25, 50 and 100 ppm adversely affected the nucleic acid (DNA and RNA) content of *Phaseolus vulgaris*. Different levels of lead had a significant (*$p<0.05$) decrease in nucleic acid content (Figs. 5 and 6). Increasing treatment of lead resulted in decreasing nucleic acid content in every experimental week.

Discussion

It was observed that increasing treatment of lead resulted in decrease in total chlorophyll content of *Phaseolus vulgaris*. Various abiotic stresses decrease the chlorophyll content in plants (Ahmad *et al*., 2007). Several reports show chlorophyll biosynthesis inhibition by metals in higher plants (Prasad & Prasad 1987). The decline in chlorophyll content in plants exposed to lead stress is believed to be due to inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase associated with chlorophyll biosynthesis (Van Assche & Clijsters 1990). Burzynski(1987) suggested that impairment in the supply of Mg^{2+} and Fe^{2+} (required for the synthesis of chlorophylls) resulted in decline in chlorophyll content when expose to lead stress. An enhancement of chlorophyll degradation occurs in lead treated plants due to increased chlorophylase activity (Drazkiewicz, 1994). Our results of decrease in chlorophyll content corroborated with the findings of Siedlecka & Krupa (1996) who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

The results related to protein content illustrate decrease in *Phaseolus vulgaris* plants treated with different levels of lead. Abiotic stress may inhibit a synthesis of some proteins and promote others (Ericson & Alfinito, 1984) with a general trend of decline in the overall content. Our studies coincide with Costa & Spitz (1997) who also reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus.* Mohan & Hosetti (1997) found more pronounced decrease in the protein content with Cd as compared to Pb treatment in *L. minor.* The decrease in protein content in *L. polyrrhiza* may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et al*., 2002) that is found to increase under stress conditions. Increased proteolytic activities in response to heavy metals were also found by Lee *et al*., (1976) who observed increased activities of hydrolytic enzyme of soybean leaves in response to heavy metal stress. It is also likely that these heavy metals may have induced lipid peroxidation in *L. polyrrhiza* and fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content (Davies *et al*., 1987).

In the present investigation lead treatment result in decline in total carbohydrate content of *Phaseolus vulgaris*. Our result corroborated with finding of Ahmed (1978), who found that treatment of plant with lead increased respiration rates of its organ and reduced the photosynthetic rates. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase (Stiborova *et al*., 1987).

Result indicated that increasing levels of lead treatment markedly increased the phenolic content of *Phaseolus vulgaris*. An enhancement of the amount of phenolic compounds can be observed under different environmental factors and stress conditions (Sakihama & Yamasaki, 2002). An increase of phenolics correlated to the increase in activity of enzymes involved in phenolic compounds metabolism was reported (Michalak, 2006), suggesting synthesis of phenolics under heavy metal stress. The

phenolics are generally thought to prevent oxidative damage by scavenging active oxygen species and by breaking the radical chain reactions during lipid per oxidation, these antioxidative effects require the reduced form of phenolics, in the oxidized form act as prooxidants (Sakihama & Yamasaki, 2002). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Sakihama *et al*., 2002).

In the present investigation, different treatments of lead result in decline in total protein content in *Phaseolus vulgaris.* Our results of decline in DNA and RNA content corroborated with the findings of Jana & Choudhuri (1984) who also found a decrease in DNA and RNA content with heavy metal stress. In plants, reduced efficiency of DNA synthesis, weaker DNA protection from damaged chromatin protein (histone) and increased deoxribonuclease (DNase) activity have been reported for Cd, Cu, Cr, Ni, Pb, Hg, Pt and Zn (Prasad & Strzalka, 2002). Elements such as Cu, Ni, Cd and Pb have been reported to decrease RNA synthesis and to activate ribonuclease (RNase) activity, leading to further decrease in RNA content (Schmidt, 1996).

Conclusion

The effect of heavy metals on plants resulted in growth inhibition, structure damage, a decline of physiological and biochemical activities, as well as function of plants.

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