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Toxic Effects of Lead on Growth and Some Biochemical and Ionic Parameters of Sunflower (*Helianthus annuus* L.) Seedlings

[1]Hamid Noorani Azad, [1]Amir Houshang Shiva and [2]Rokhsareh Malekpour
[1]Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
[2]Department of Biology, Kazeroun Branch, Islamic Azad University, Kazeroun, Iran

Abstract: Lead (Pb) is one of the non essential and toxic heavy metals which can cause oxidative stress in plants. The effects of $Pb(NO_3)_2$ toxicity on growth and some biochemical parameters of record cultivars of *Helianthus annuus* L. were studied under hydroponic condition. Different treatments of $Pb(NO_3)_2$ [control (0), 200, 400, 600 and 800 μM] were used in order to consider changes in dry weight, proline and Pb accumulation in roots and shoots; Total chlorophyll, enzyme activity (catalase and peroxidase) and K^+, Ca^{2+} amounts of leaves. Compared with the control, Pb treatment caused a significant decrease in roots and shoots dry weight, leaves chlorophyll, catalase activity and K^+, Ca^{2+} amounts. In contrast, a significant increase in proline and Pb accumulation of roots and shoots and peroxidase activity of leaves was observed in Pb treatments.

Key words: Catalase, *Helianthus annuus*, lead toxicity, peroxidase, proline, total chlorophyll

INTRODUCTION

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 (Hamid *et al*., 2010). Soil characteristics like low PH, low density of phosphorus (P) and abundance of organic ligands can increase Pb absorption level by plants (Kabata-Pendias and Pendias, 2000). The most significant factors which can distribute lead as a pollutant in the environment are burning of fossil fuels, agricultural manufacturing, mining, pesticides and fertilizers (Ross, 1994; Eick *et al*., 1999). According to Oliver and Naidu (2003) plants show different reactions against Pb toxicity. Some of them are sensitive and the others have more tolerance. In plants, it has been widely reported that accumulation of Pb may cause many physiological, biochemical and structural changes like decline in photosynthetic rate and essential elements absorption (Larbi *et al*., 2002), the roots and shoots growth inhibition, chlorosis and decrease in water potential and plant hormones (Sharma and Dubey, 2005). Also, this metal can generate different types of active oxygen such as Superoxide (O_2^-) and Hydrogen Peroxide (H_2O_2) which can disturb cell membrane activities (Verma and Dubey, 2003). Plant cells have a protective system against oxidative stress including enzyme antioxidant system like catalase and guaiacol peroxidase and non-enzyme antioxidant system (Van Assche and Clijster, 1990; Cho and Park, 2000). Nowadays, the usage of fertilizers including micronutrients and macronutrients has increased. One of the most toxic heavy metals which may be found in some kind of fertilizers is lead that can be absorbed and accumulated in plants. Then enters the food chain to form hazard for human health (Hendrickson and Corey, 1981). Fertilizers play very important role in plant growth like *Helianthus annuus* L. This species has the largest amount of seed oil (40-50%) and it's quality is very high (Scheiner *et al*., 2002). Therefore, it is essential to investigate the effects of lead toxicity on some biochemical characteristics, ion changes and tolerance rate of the seeds in *H. annuus* L.

MATERIALS AND METHODS

Plant materials: The experiment was carried out in a greenhouse of Islamic Azad University- Jahrom Branch in 2009. The record cultivar seeds of *H. annuus* L. were sterilized by Na-Hypochlorite 20% and washed by distilled water. They were transplanted into containers containing sterile sand and irrigated for one week by distilled water. From the second week, the irrigation was carried out by Hoagland solution 0.5% for two weeks. Then the seedlings were transferred to Hoagland solution containers. Plants were grown in a growth chamber under light (day/night: 16/8 h) at 24ºC, 65% moisture and 16000 lux light intensity.

Pb treatments: Pb treatments were arranged with adding different concentrations of $Pb(NO_3)_2$ [control (0), 200,

Corresponding Author: Hamid Noorani Azad, Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

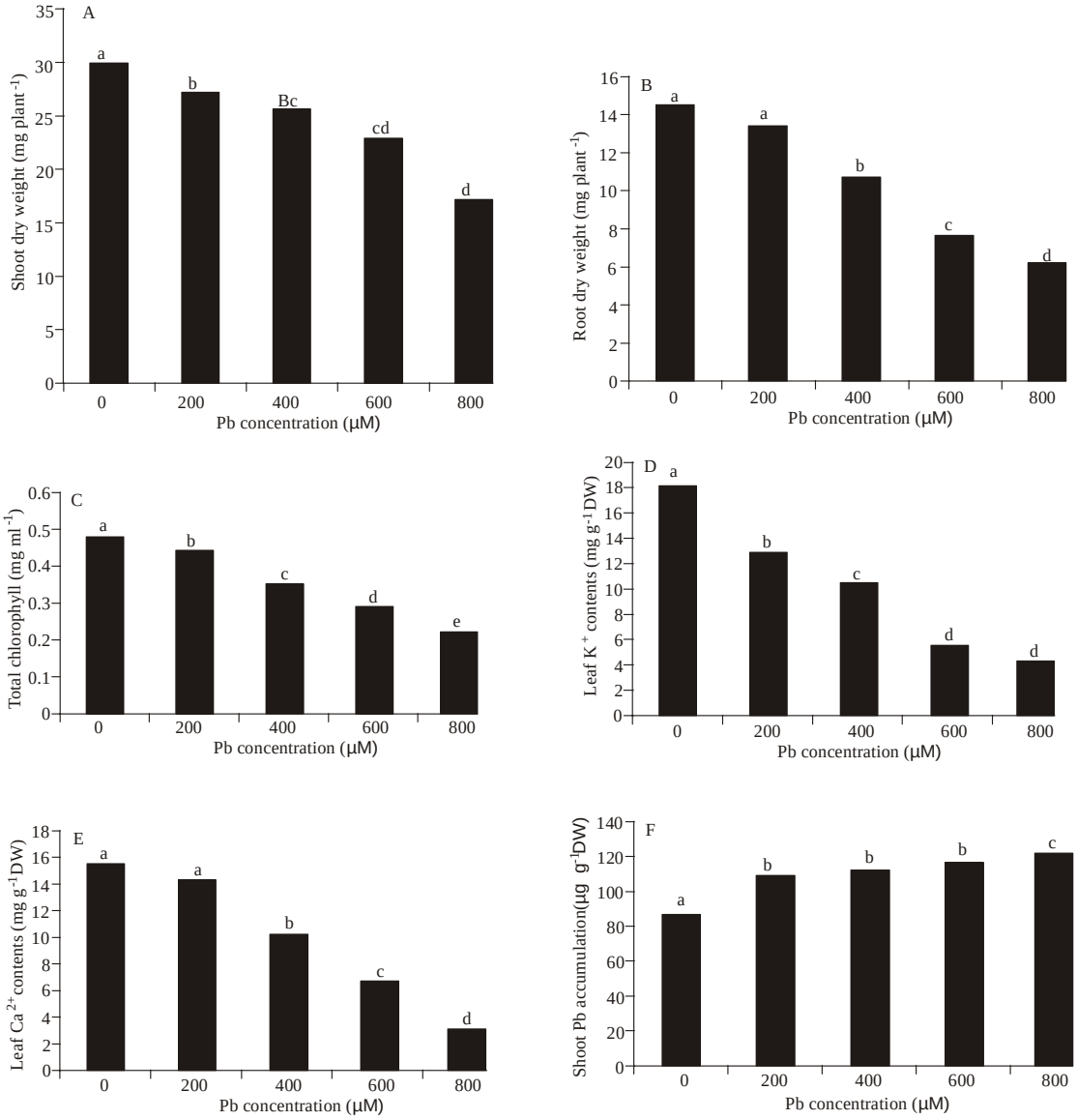

Fig. 1: Effects of $Pb(NO_3)_2$ on shoot dry weight (A), root dry weight (B), leaves total chlorophyll (C), leaf K^+ contents (D), leaf Ca^{2+} contents (E) and shoot Pb accumulation (F) in *H. annuus* L

400, 600 and 800µM] to Hoagland solution (pH = 6-6.5). After two weeks, the plants were harvested, divided into shoots and roots, dried at 70ºC for 48 h and their dry weight was weighted by digital balance (Sartorius BP-315).

Determination of enzyme activities: The enzymes were extracted from fresh plant tissues. The tissues were ground in a chilled mortar at 4ºC with 100 mM potassium phosphate buffer (pH = 7) containing 0.1 mM Na_2EDTA, 0.2 mM Ascorbic acid and polyvinylpyrrolidone 1%. The homogenate was centrifuged for 1 h at 12000×g (Kang *et al*., 2003) and the supernatant was assayed for enzyme activity at 25ºC by spectrophotometer (Bradford, 1976). The measurements of catalase activity were analyzed by adding enzyme extraction to 100mM potassium phosphate buffer (pH = 7.5) and 25mM H_2O_2. The activity of this enzyme was determined by measuring the decrease in H_2O_2 (Pereira *et al*., 2002). The measurements of peroxidase activity were done according to Fielding and Hall (1978). Enzyme extraction was added to reaction medium containing 5 mM potassium

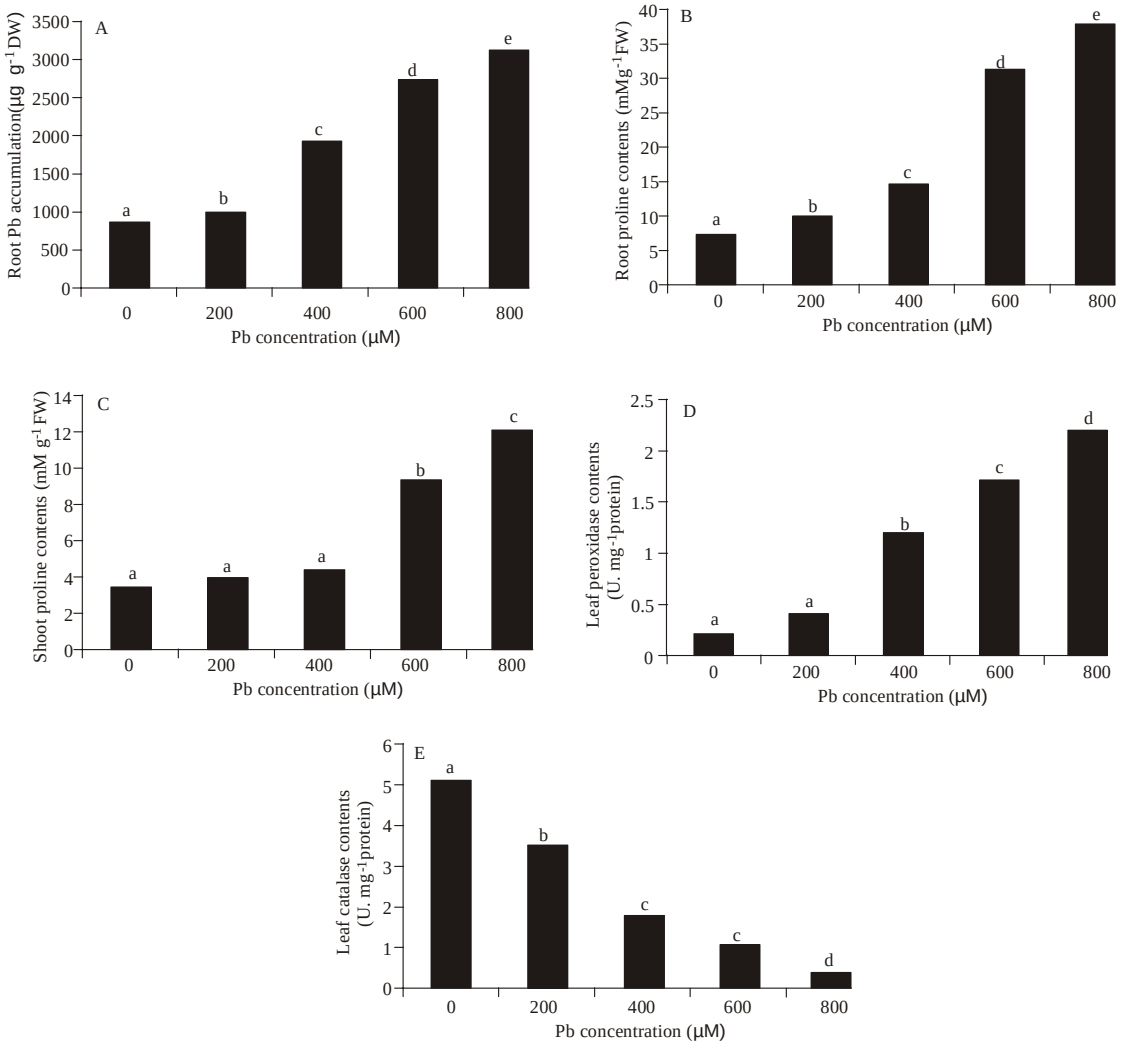

Fig. 2: Effects of $Pb(NO_3)_2$ on root Pb accumulation (A), root proline contents (B), shoot proline contents (C), leaf peroxidase contents (D) and leaf catalase contents (E) in *H. annuus* L

phosphate buffer (pH = 7), 0.1 mM Na_2EDTA, 5 mM H_2O_2 and 30 mM guaiacol. The increase in absorption was recorded at 470 nm.

Determination of roots and shoots proline and leaves Total chlorophyll: Proline determination was analyzed using the method of Bates *et al*. (1973). Proline content was measured by a spectrophotometer (Shimadzu-UV-1601) at 520 nm. For the determination of leaves total chlorophyll, fresh tissues were mixed with 80% acetone and then were determined spectrophotometrically at 634 and 645 nm (Strain and Svec, 1966).

Pb content in roots and shoots: The experiment was carried out using atomic absorption method. 0.5 g plant dry material was digested in 10 mL H_2SO_4-H_2O_2 for 24 hours. The amount of Pb in the solution was measured using an atomic absorption spectrometry (Feng *et al*., 2010).

Determination of Ca^{2+} and K^+ contents in leaves: Ca^{2+} and K^+ determination was carried out using the atomic photometer method (Mansour *et al*., 2005).

The statistics method: Statistical analyses were carried out by analysis of variance (ANOVA) using SAS ver. 9 software. Mean values were analyzed by the Duncan's test.

RESULTS

Pb toxicity symptoms and plant growth: The common symptoms caused by lead nitrate toxicity were yellowing of the leaves and appearance of dark brown pigments,

especially in 800 μM, which might be attributed to nutrient imbalance induced by excess lead. Pb treatment dramatically inhibited the accumulation of both shoot and root biomass and consequently, decreased their dry weight ($p<0.01$) (Fig. 1A, B).

Total chlorophyll: Compared to the control, total chlorophyll slightly declined by increase in lead nitrate ($p<0.01$). Fig. 1C shows the changes of chlorophyll in different $Pb(NO_3)_2$ concentrations. The highest level (0.48 mg/mL) refers to control condition and the lowest level (0.22 mg/mL) refers to the highest concentration of $Pb(NO_3)_2$.

Ca^{2+} and K^+ in leaves: There was a decrease in K^+ and Ca^{2+} by increase in $Pb(NO_3)_2$ ($p<0.01$) (Figs. 1D, E). As Fig. 1E indicates, Ca^{2+} change in 200μM lead nitrate keeps a relatively similar trend with control. However, increase in $Pb(NO_3)_2$ results in a significant fall in Ca^{2+}.

Pb content in shoots and roots: As can be seen in Fig. 1F and Fig. 2A, Pb accumulation in shoots and roots increased significantly in Pb treatments ($p<0.01$). In general, Pb accumulated in roots more than shoots. The largest amount of Pb in roots was 3116.4 μg/g DW, while in shoots was 122 μg/g DW.

Root and shoot proline: Figs. 2B, C illustrate proline changes in roots and shoots of sunflower seedlings by increase in $Pb(NO_3)_2$. As can be seen, both in roots and shoots, increase in lead nitrate results in a rise in proline. In case of roots it was significant for all treatments ($p<0.01$), but in shoots there was a significant increase only in 600 and 800 μM treatments.

Enzyme activities in leaves: There was a dramatic rise in peroxidase activity and a steep fall in catalase activity in different $Pb(NO_3)_2$ treatments ($p<0.01$) (Fig. 2D, E). Increase in peroxidase activity was significant in all treatments except 200 μM.

DISCUSSION

Lead toxicity can induce complex changes in plants at biochemical and physiological aspects. The most obvious symptoms include leaf chlorosis and reduction of plant growth (Burton *et al*., 1984), Decrease in DNA, RNA and protein synthesis (Verma and Dubey, 2003), cytological changes (Molassiotis *et al*., 2005) as well as imbalance of chlorophyll metabolism (Dalla *et al*., 2005). In the present study, leaf total chlorophyll significantly decreased by increase in lead amount which coincides with Hamid *et al*. (2010). It could be attributed to oxidative stresses and inhibition in chlorophyll synthesis. Lead may prevent enzyme activity like δ-aminolevulinic acid dehydratase (Prasad and Prasad, 1987) or decrease essential elements absorption such as Mg^{2+} and Fe^{2+} (Burzynski, 1987) to inhibit chlorophyll synthesis. Also, some previous investigations have indicated that chlorophyll decrease can be due to its decomposition by increase in chlorophyllase activity (Drazkiewicz, 1994; Hegedus *et al*., 2001).

Regarding decrease in essential elements (Ca^{2+} and K^+) by increase in lead amount, it should be noted that lead can cause nutrient imbalance in plants. It usually prevents cation exchange in roots like K^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} (Kabata-Pendias and Pendias, 1992). Also this metal can limit access to essential elements by roots absorption sites (Sharma and Dubey, 2005).

Based on lead accumulation in roots more than other parts of the plant, it could be concluded that roots play a very significant role in extra lead storage. It has been shown that lead can adhere to roots cell wall, especially in pyrophosphate form (Marschner, 1995). It is inevitable that lead accumulation in roots may be a factor which enhances plant tolerance against Pb toxicity, because of preventing lead transfer from roots to shoots and leaves.

In this experiment, proline increase in roots and shoots was observed which is a result of rise in lead concentration. High level of proline, especially in roots, can eliminate hydroxyl radicals, maintain osmoregulation, prevent enzyme destruction (Kuznetsov and Shevyakova, 1997) and decrease toxicity of heavy metals (Alia and Saradhi, 1991).

It has been shown lead can cause oxidative stress in plants (Verma and Dubey, 2003). Plant cells are equipped with a protective system including antioxidant enzymes like catalase and guaiacol peroxidase which can bridle free radicals (Cho and Park, 2003). In this study, application of lead led to decrease in catalase activity and in contrast increase in peroxidase activity. Decrease in enzyme synthesis or change in subunits arrangement may be a reason for decline in catalase activity (Hertwig *et al*., 1992) and increase in guaiacol peroxidase activity can be due to releasing from cell wall (Gaspar *et al*., 1982). Most of the previous studies indicated that heavy metals like lead are responsible for catalase activity decrease in rice (Verma and Dubey, 2003) and peroxidase activity increase in rice and soybean (Sharma and Dubey, 2005) which coincide our results.

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