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Alterations in Growth and Physiology of Wheat to Lead (Pb) Stress Under Soil Culture

Environment

KEYWORDS : Lead toxicity, total chlorophyll content, Chlorophyll a/b ratio, photochemical efficiency of PSII (Fv/Fm)

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ABSTRACT

The present study was conducted to obtain quantitative information about the translocation of lead (Pb) in a soil culture experiment with wheat. The solutions of lead nitrate (500, 1000 and 2500 µM) were applied to achieve stress conditions in comparison to unstressed, water treated control variant. Plants were exposed to Pb solutions for 7 days under experimental dome conditions and various observations were recorded. Growth parameters, such as root length, shoot length and dry weight, exhibited a significant decline with increasing Pb treatment. In addition, the level of photosynthetic pigments decreased upon exposure to Pb in a linear manner. These results suggested manifestation of Pb-induced stress, which was confirmed by reduced Photochemical Efficiency of PSII (Fv/Fm). After 7 days of treatment, malondialdehyde accumulated in the range of Pb ~11-38% with respect to control over 500-2500 µM Pb exposure. Further, superoxide dismutase activity increased with increasing concentrations. On the other hand, a significant decline was observed in guaiacol peroxidase activity in wheat roots in a concentration- dependent manner. The study concludes that short-term Pb treatment alters growth and physiology of wheat grown under soil culture.

Introduction

In the present scenario, heavy metal contamination of environment is threatening agriculture, wild life and humans (Maheshwari & Dubey, 2007). Rapid industrialization has added lead (Pb) beyond permissible limits to the soil. Pb pollution in soil is of priority concern to agriculturalists and environmentalists. Since, Pb is not an essential nutrient for plants, most of it is easily taken up by the plants from the soil (Patra, Bhowmik, Bandopadhyay & Sharma, 2004), thereby affecting crop yield, ecosystems and humans. The ability of a plant to take up significant quantities of Pb depends upon its concentration in soil and its bioavailability. Generally, Pb is present in the forms of Pb-phytochelatins, Pb-nitrate, Pb-acetate, Pb-sulfide and Pb-citrate in the soil, which are readily available and absorbed into the plants (Lopez, Peralta-Videa, Parsons & Gardea-Torresdey, 2009).

The physiology of metal toxicity begins with the increased supply of metal to the root, which results in the failure of well defined "essential to life" plant process. The first visible symptom of metal toxicity includes reduction in growth (Lin, Wang, Luo, Du, Guo & Yin 2007) followed by impaired plant metabolism (Seregin & Ivanov 2001). The toxic amounts of heavy metals can exert deleterious effects on photosynthetic pigments and photosynthetic efficiency of growing plants (Kososbrukhov, Knyazeva & Mudrik, 2004; Maksymiec, Krupa & Wójcik, 2007).

Previously we have examined that Pb interferes with the growth and physiology of wheat grown hydroponically in a time- and concentration-dependent manner (Kaur, Singh, Batish & Kohli, 2012, 2013). To test whether the phenomena of Pb phytotoxicity remains same in field conditions, an experiment was planned out in soil culture. The aim was to examine the effect Pb (as lead nitrate; 0-2500μM) on the growth and physiological responses in young wheat seedlings (*Triticum aestivum* L.) under soil culture. For the present study, wheat has been chosen as target species since; it is a widely grown cereal crop in India. The observations were made with respect to root length, shoot length, dry weight, chlorophyll content and quantum efficiency of PSII.

Material and Methods

Experimental Design-Raising of plants: Plants were raised in propylene pots of dimensions 8.5 × 8.5 × 7.5 cm filled with 0.5 kg garden soil (soil: sand:: 3:1, *w/w*) incorporated with 100 ml of lead nitrate [$Pb(NO_3)_2$; MW=331.21; purity 99%; Merck Ltd., Mumbai, India] solutions at the rate of 0, 500, 1000, 2500 μM, respectively. Soluble fertilizers (100 mg N, 150 mg P_2O_5, and 100 mg K_2O kg^{-1} dry soil) were also applied at the same time. Five replicates were maintained under experimental dome conditions for each treatment including control. Certified seeds of Wheat (*Triticum aestivum* L.) used in the present study were purchased locally from the seed store. These were surface-sterilized with 30% sodium hypochlorite for 10 min, thoroughly washed with distilled water. Seeds were evenly sown in each pot to a depth of 0.5 cm and the pots were watered daily with the respective treatment solutions to maintain sufficient water content in the soil for whole duration of the experiment. Ten seeds were sown per pot and after 7 days root and shoot length were measured with the help of a centimeter ruler. Seedling dry weight was determined by oven drying at 80°C for 24 h. The remnant materials were washed in 10 mM $CaCl_2$ to remove lead accumulated on their surface. Apical portion (~1.5-2.0 cm) of roots was removed, stored at -20°C and used for various biochemical estimations for subsequent analysis. All the chemicals and reagents used in current study for biochemical estimations and enzymatic assay were of reagent grade and procured from the best available sources.

Estimation of photosynthetic pigments: The total chlorophyll content in leaf tissue of treated and control plants were extracted in dimethyl sulphoxide (Hiscox & Israelstam, 1979) after incubation at 60 °C for one hour. The extinction value of recovered chlorophyll was measured at 645 and 663 nm on Shimazdu UV-1800 double beam spectrophotometer against dimethyl sulphoxide, as a blank. Its amount was determined using equation of Arnon (1949) and expressed as μg/mg tissue on dry weight basis (Rani & Kohli, 1991).

$$\text{Chlorophyll a} = (10.63 \times A_{663}) - (2.39 \times A_{645})$$
$$\text{Chlorophyll b} = (20.11 \times A_{645}) - (5.18 \times A_{663})$$

where A_{645} and A_{663} represent extinction values at 645 nm and 663 nm, respectively.

Maximum Potential Quantum Efficiency of PSII: The maximum potential quantum efficiency of PSII of wheat leaves exposed to different concentrations of Pb was measured using the OS-30p Chlorophyll Fluorometer (by Opti Sciences, US). . The observations were recorded after 7 days. A leaf was attached on the leaf holder of the Plant Efficiency Analyser equipment and subjected to dark conditions for about 10 min. Thereafter, its fluorescence characteristics were measured. This was repeated five times for each treatment concentration.

MDA Content and enzyme activity of SOD and GPX: They were calculated as per Singh, Batish, Kohli & Arora (2007).

Statistical Analysis

The experiments were performed in a randomized design with five replicates, each consisting of a single pot with 10 seeds each. All the experiments were repeated and the data presented is of

a single experiment since the differences between two experiments were less than 5%. The data were analyzed by one-way ANOVA and means were separated using post hoc Tukey's test at $P \leq 0.05$.

Results and discussion

Effect of Pb on seedling growth and dry weight: Seedling growth of wheat was found to be adversely affected by Pb treatment (Table 1). The inhibitory effect of Pb on seedling growth was concentration-dependent, where growth declined with increasing concentration. After 7 days, root length decreased by 46 and 51% in response to 1000 and 2500 μM Pb treatment (Table 1). Likewise, a reduction in shoot length was noticed. Shoot length was reduced by 17, 31 and 44% after 7 days upon exposure to 500, 1000 and 2500 μM Pb, respectively. The reduction in shoot length was comparatively lesser, and the inhibitory effect was more pronounced on root length than on shoot length (Fig. 1). This was also evident from a declining root/shoot ratio upon Pb exposure (Table 1). The reduced plant growth further affected biomass of the plant. Parallel to seedling growth, a significant reduction in dry weight of emerged seedlings was also observed in response to metal treatment. The presence of Pb in soil resulted in a striking decrease of root and shoot biomass expressed in terms of dry weight, which decreased in the range of 20-44% after 7 days when exposed to 500-2500 μM of Pb in soil (Table 1).

Pb is one of the toxic metals in the environment and causes drastic morphological and physiological deformities in plants (Kamel, 2008; Kaur, Singh, Batish & Kohli, 2012).Various authors have reported adverse effects of Pb on radical and plumule lengths of plants. The current observations of root and shoot length are in concordance with the results of Ghani (2010), who reported significant root growth inhibition upon Pb exposure in maize (raised in soil), although shoot growth remains less affected. Bashmakov, Lukatkin, Revin, Duchovskis, Brazaityte & Baranauskis (2005) explained that Pb caused suppression of growth process and accumulates in roots. Fernandes and Henriques (1991) suggested that roots hold the heavy metal and prevent its distribution to the aerial parts. The current observations remarked a notable decrease in biomass of treated plants. Earlier, many workers have reported loss of biomass under heavy metal treatment (Lolkema, Donker, Schouten & Ernst, 1984; Verkleij & Prast, 1989). Kosobrukhov (2004) observed a considerable decrease in dry weight of plants under Pb treatment. Previously, it has been reported that increasing Cu supply resulted in decreased root biomass indicating the alterations of physiology and metabolism of test plants (Ouzounidou, Ciamporová, Moustakas & Karataglis, 1995).

Photosynthetic pigments and photochemical efficiency: We found that Pb incorporated in the soil caused a significant decline in chlorophyll pigments in wheat seedlings (Table 2). Pb in soil (500-2500 μM) caused 17-66% and 11-24% decline in chlorophyll a and chlorophyll b content, respectively. Due to lead toxicity, the reduction in total chlorophyll content ranging from 14 to 39% was recorded after 7 days. Likewise, a significant decline was observed in chlorophyll a/b ratio in wheat leaves upon exposure to 2500 μM Pb concentration (Table 2).

In order to test if the reduction in chlorophyll content affected photosynthetic efficiency, we measured PSII Photochemical Efficiency (Fv/Fm) in dark-adapted state with fully open PSII. Coinciding with the hypothesis, it was found to be severely affected upon exposure to different Pb concentrations. In control leaves, Fv/Fm values were measured approximately as 0.70 after 7 days (Fig. 2). When Pb concentration was increased from 500 to 2500 µM, the Fv/Fm value started to decline in a linear manner. The decrement was more pronounced at 1000 and 2500 µM, the percent decrease being ~34 and 37%, respectively, after 7 days of exposure. The decrease in photosynthetic efficiency with respect to increasing Pb concentration exhibits a reciprocal relation of Fv/Fm with increasing concentrations of lead nitrate. The present results affirmed that Pb strongly intervenes in photosynthetic electron transport as evidenced by high fluorescence signal, i.e. lower Fv/Fm values (Fig. 2).

In the present study, Pb significantly reduced chlorophyll content, thereby affecting photochemical efficiency of wheat seedlings. In fact, chlorophyll content is suggested as a very useful *in vivo* indicator of heavy metal toxicity for calculating the upper critical tissue concentrations. The current observations are in agreement with Patra, Bhowmik, Bandopadhyay & Sharma (2004), who demonstrated that Pb exposure decreases content of photosynthetic pigments, thereby reducing photochemical efficiency of PSII. Higher concentrations of Pb are known to inhibit chlorophyll synthesis either due to impaired uptake of Mg and Fe by plants (Bruzynski, 1987) or because of increased chlorophyllase activity (Drazkiewicz, 1994). Mallick & Mohn (2003) explained that reduced or partial blockage of electron transport from PSII to PSI limits the reoxidation of Q_A (the primary photosystem electron acceptor), thereby resulting in reduction of Fv/Fm. As a result of reduced photosynthesis, the effect of Pb is apparent as decreased plant growth.

MDA content: Lipid peroxidation was determined in control and treated samples in terms of malondialdehyde content (MDA) and has been used as an index for the status of lipid peroxidation. After 7 days of treatment, MDA accumulated in the range of ~17-38% with respect to control over 500-2500 μM Pb exposure (Table 3). The current observations are in conformity with the reports of Keser and Saygideger (2010). Parallel to our observations, lipid peroxidation is also reported to be enhanced under As toxicity (Singh, Batish, Kohli, Arora, and Kaur 2007) and Pb toxicity (Kaur, Singh, Batish and Kohli 2012, 2013). These results are indicative of the fact that toxicity of heavy metals is probably exerted through free radical generation.

Activities of Superoxide Dismutase (SOD) and Guaiacol Peroxidase (GPX): SOD activity increased linearly with increasing Pb levels. After 7 days, SOD activity was ~33-223% higher as compared to control in response to 500-2500 μM Pb exposure (Table 3). A significant ($P<0.05$) decline was observed in GPX activity in wheat roots after 7 days of exposure in a concentration-dependent manner, compared to the control. Pb exposure led to ~78% decrease in GPX activity upon exposure to 2500 μM Pb (Table 3).

In metal stress studies, SOD activity is of more relevance, since it provides first line of defense against oxidative damage (Gratão, Polle, Lea and Azevedo, 2005). Up-regulated SOD activity catalyzes dismutation of $O_2^{\cdot-}$ to O_2 and H_2O_2 and this increase can be attributed to *de novo* synthesis of enzyme protein. The role of SOD under oxidative stress is evident from studies on *Nasturtium officinale* wherein an increase in SOD activity under Pb stress was observed (Keser & Saygideger, 2010).

GPX, a stress marker enzyme, is located in cytosol, cell wall, vacuole and extra cellular spaces. Broadly, this enzyme is known to catalyze phenolic substrates. It decomposes H_2O_2 to generate phenoxy compounds which in turn polymerize to produce cell wall components such as lignin (Reddy, Kumar, Jyothsnakumari, Himmanaik & Sudhakar, 2005). The decrease in GPX activity could probably result in increased concentrations of H_2O_2 to toxic levels, causing oxidative stress to the plants (Sandalio, Dalurzo, Gomez, Romero-Puertas & del Rio, 2001). Parallel to our observations, a similar decrease was reported in GPX activity in *Ceratophyllum demersum* treated with 10-100 μM Pb for 7 days (Mishra, Srivastava, Tripathi, Kumar, Seth & Gupta, 2006).

Conclusions

From the present study, it is concluded that short-term Pb treat-

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ment alters growth as well as physiology of wheat grown under soil culture. Based on responses generated by wheat seedlings, this study will be helpful to identify toxic critical values of Pb in soils and it will also provide a reference for Pb-caused oxidative stress assessment in soils.

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Table - 1: Effect of Lead (Pb) on seedling growth in wheat.

Lead (µM)	Root Length (cm)	Shoot Length (cm)	Root/ Shoot Ratio	Dry weight (mg seedling^{-1})
0	17.3±0.41a (0)	16.7±1.96a (0)	1.04a	39.1±0.03a (0)
500	16.4±0.33a (-5.2)	13.8±1.20ab (-17.4)	1.19b	31.2±0.02b (-20.1)
1000	9.4±0.49b (-45.7)	11.5±0.76bc (-31.1)	0.82c	28.3±0.02c (-27.6)
2500	8.5±.32b (-50.9)	9.3±0.67c (-44.3)	0.91c	22.1±0.01d (-43.5)

The data were recorded after 7 days' exposure to Pb; means with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test. Figures in parenthesis represent the percent decrease over the control.

Table 2: Effect of Lead (Pb) on photosynthetic pigments in wheat leaves.

Lead (µM)	Chlorophyll a (µg mg^{-1} dry weight)	Chlorophyll b (µg mg^{-1} dry weight)	Chlorophyll a/b Ratio	Total Chlorophyll (µg mg^{-1} dry weight)
0	6.5±0.39a (0)	4.6±0.27a (0)	1.41±0.28a	11.0±1.2a (0)
500	5.4±0.08b (-16.9)	4.1±0.19a (-10.9)	1.32±0.21a	9.5±1.1b (-13.6)
1000	4.0±0.04c (-38.5)	3.7±0.21a (-19.6)	1.08±0.20b	7.7+0.7 c (-30.0)
2500	2.2±0.01d (-66.2)	3.5±0.01a (-23.9)	0.63±0.01c	6.7±0.8c (-39.1)

The data were recorded after 7 days' exposure to Pb; means with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test. Figures in parenthesis represent the percent decrease over the control.

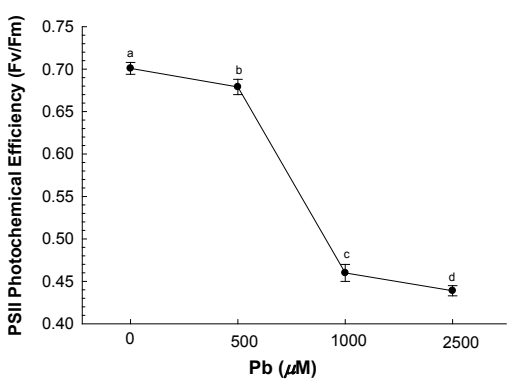
Table 3: Effect of lead (Pb) on lipid peroxidation (MDA content) and alterations in the activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) in the roots of wheat.

Pb (µM)	MDA (nmol g^{-1} FW)	SOD (EU mg^{-1} protein)	GPX (EU mg^{-1} protein)
0	11.2 ± 0.21a (0)	2.2±0.70a (0)	11.6±1.11a (0)
500	13.1 ± 0.22b (+17.0)	3.3±0.12a (+33.3)	7.6±1.01b (-34.5)
1000	14.2 ± 0.37bc (+26.8)	5.1±0.90b (+131.8)	4.20.96c (-63.8)
2500	15.5 ± 0.37c (+38.4)	7.1±0.83c (+222.7)	2.5±0.87d (-78.4)

The data were recorded after 7 days' exposure to Pb; means with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test. Figures in parenthesis represent the percent changes (+, increase; -, decrease) increase over the control.

Fig. 1. Photograph showing seedling growth of wheat after 7 days in soil culture. (Seedlings are arranged as control, 500 µM, 1000 µM, 2500 µM, from left to right)

Fig. 2. Effect of Lead (Pb) on PSII Photochemical Efficiency (F*v*/F*m*) in wheat leaves recorded after 7 days. Data presented as mean±SE. Different alphabets represent significant difference among different concentrations of Pb at $P \leq 0.05$ applying post hoc Tukey's test.



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