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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effect of Lead Toxicity on Growth, Chlorophyll and Lead (Pb^+) Contents of Two Varieties of Maize (*Zea mays* L.)

Abdul Ghani, Ameen Ullah Shah and Umer Akhtar
Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

Abstract: Two varieties of maize (*Zea mays* L.) (Neelam and Desi) were exposed to different concentrations of lead [0, 10, 20 and 30 ppm $Pb(NO_3)_2 \cdot 4H_2O$] for 30 days in earthen pots. Exposure of maize varieties to excess Pb resulted in a significant root growth inhibition though shoot growth remained less affected. The results of chlorophyll analysis indicated that the highly toxic Pb level affected photochemical efficiency in Neelam, while no significant effect was observed in the Desi. This result was related to the accumulation of Pb. The results of the present study indicated that, Desi withstands excess Pb with its higher Pb accumulation capacity in roots and better upregulated protective mechanisms compared to Neelam. Therefore, Desi is more tolerant to Pb toxicity compared to Neelam which was found to be susceptible variety.

Key words: Maize (*Zea mays* L.), lead toxicity, Pb accumulation, Pb concentrations

INTRODUCTION

Lead is one of the heavy metals and is considered one of the dangerous environmental pollutants. It is emitted from the industries, motor vehicles, stationary fuel, road dust composition and traffic roads. Lead is not only a toxic element but also can be accumulated in plant organs and agricultural products (Burzynski, 1987; Mahmoud and El-Beltagy, 1998), consequently enter human food chain (Wagner, 1993). As a result of consumption of food, lead accumulates in human body and it may cause renal failure, brain and liver damage and it can attack the nervous system and cause failing of sickness (Lucky and Kenugopal, 1997; Ramade, 1987). Lead is one of the most difficult pollutants to control (Salt *et al.*, 1998). Environmental contamination with lead has accelerated due to its close relationship to industrialization and its wide usage in paints and gasoline.

Lead (Pb) is one of the prominent examples for anthropogenic environmental metal pollution that originates from various activities including mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal planting and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Sharma and Dubey, 2005). Soils contaminated with Pb cause sharp decreases in crop productivity thereby posing a serious problem for agriculture (Johnson and Eaton, 1980). Although Pb is not an essential nutrient for plants, majority of lead is easily taken up by plants from the soil and accumulated in root while only a small fraction was translocated upward to the shoots (Patra *et al.*, 2004). Pb affects several metabolic activities in different cell compartments. The effect of Pb depends on concentration, type of soil, soil properties and plant species. Pb toxicity leads to decrease in germination percentage, length and dry mass of root and shoots (Munzuroglu and Geckil, 2002), disturbed mineral nutrition (Paivoke, 2002), reduction in cell division (Eun *et al.*, 2000).

Pb concentrations in soil and vegetation in many countries has increased with the rapid development of agriculture and industry in recent decades. Our earlier studies demonstrated that some maize varieties were tolerant to several heavy metals such as Cu and Cd (Tanyolaç *et al.*, 2007; Ekmekçi *et al.*, 2008). Additionally, some authors reported that *Zea mays* L. is a Pb tolerant plant (Malkowski *et al.*, 1996; Heidari *et al.*, 2005). In maize crops, Pb metal ions mostly accumulate in their roots and shoots (Patra *et al.*, 2004; Reddy *et al.*, 2005; Kopittke *et al.*, 2007) and these parts are not consumed as food. Therefore, the risk of Pb uptake by crops followed by transfer in the food chain has disappeared. Consequently, there has been considerable need in finding suitable maize varieties that are able to grow on Pb-contaminated soils for land reclamation.

The uptake, transport and accumulation of Pb by plants are strongly depended on soil type and plant species, and they differ significantly with plant species. Maize is the third most important crop following wheat and barley in Pakistan. Maize yields about 3.5 million tons per year and has a sowing area of around 800,000 ha per year (FAO, 2006). So it is very important to understand the differences between two maize varieties having different tolerance capacity in Pb uptake and translocation and distribution of Pb in maize varieties. Also, in literature, there are limited number of publications on the relationship between Pb toxicity and photochemical activity of PSII (Wu *et al.*, 2008). In this study, we aimed to investigate Pb accumulation in maize varieties, to

Corresponding Author: Abdul Ghani, Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

evaluate the effect of Pb toxicity on chlorophyll contents and to determine the effects of lead toxicity on some growth parameters which played an important role in tolerance mechanisms of the two maize varieties.

MATERIALS AND METHODS

Plant materials, growth and treatment conditions: Two maize (*Zea mays* L.) varieties, Desi and Neelam, were used in this study due to their tolerance characteristics at early seedling stage to Pb stress determined by preliminary experiment which was conducted using ten maize varieties from different origin by adding different concentrations of Pb to sand culture media, to investigate the effects of Pb on maize varieties on their germination and growth behavior (Ayhan *et al*., 2007). Seeds of the varieties were surface-sterilized with 30% (v/v) sodium hyphochloride (NaOCl) solution for 20 min. Then, they were washed and imbibed in distilled water for 24 h. The seeds of varieties were germinated under dark conditions at 23±2°C on humidified filter paper with distilled water for 6 days. The seedlings of varieties were planted on plastic pots containing sand culture and watered with Hoagland solution as and when required. Seedlings of the varieties were grown at a constant temperature regime of 23±1°C, 50±5% humidity and at 250 µmol m^{-2} s^{-1} light intensity for 16 photoperiod in a controlled growth cabinet.

At 30th day of the growth, lead stress treatment was initiated by applying Hoagland nutrient solution containing 10, 20 and 30 ppm $Pb(NO_3)_2 \cdot 4H_2O$ to seedlings. Control plants not treated with Pb and Pb stressed plants were grown in the earthen pots under the same conditions for another 8 days. Therefore, the tissues of 38-day-old seedlings were used in the experiment.

Determination of growth parameters: At the end of the experiment, biomass and length of the roots and the shoots (the distance from perlite surface to upper end of the longest leaf; cm per plant) were measured for each Pb concentration. The biomass was measured on dry weight basis after drying at 80°C for 48 h, where DW and FW stand for dry weight and fresh weight, respectively.

Determination of lead (Pb^{+2}) contents: Digested samples were analyzed for Pb of those samples of shoots, roots and leaves which were treated with different concentrations of lead under the following manufacturer's recommended conditions on a perkin Elmer 3100 EDS Atomic Absorption/Emission spectrophotometer, using a fuel which air-acetylene flame, 10 cm burner head, 357.9 nm wavelength, 0.7 nm slit and 20 ma lamp current. Some digests were diluted in order to fall into the linear calibration range of 0-5 mgL^{-1} (Yoshida *et al*., 1976).

Chlorophyll (a, b and total) analysis: Pigment contents of 14 days old plants were extracted by using the formula of Arnon and Hoagland (1949). The leaves were chopped into small pieces that were extracted with 80% acetone. The absorbance was measured at 645 nm and 663 nm for chl a,b and total chl respectively by using spectrophotometer (Hitachi Model-U 2001 Japan). Then chlorophyll a, b and total chlorophyll were calculated according to the Litehtenhaler and Wellburn (1983) formulae:

$$\text{Chl a (mg g}^{-1}\text{ leaf fresh weight)} = [12.7(OD663) - 2.69\,(OD645)] \times V/1000 \times W$$

$$\text{Chl b (mg g}^{-1}\text{ leaf fresh weight)} = [22.9(OD645) - 4.68\,(OD663)] \times V/1000 \times W$$

$$\text{Total Chl(mg g}^{-1}\text{ leaf fresh weight)} = [20.2(OD645) - 8.02(OD663)] \times V/1000 \times W$$

Where: OD = Optical Density, V = Volume of sample
W = Weight of sample

Data analysis: The experiment was performed in a randomized design with three replicates. Differences among the Pb concentrations and between the varieties, were tested using SPSS statistical program. Statistical analysis of the data given as percentage was performed after arcsin transformation in order to stabilize variances. After transformation, the data was acceptably normal with homogenous treatment variance. Statistical variance analysis of the all data was performed using ANOVA and compared with least significant difference (LSD) at the 5% level.

RESULTS

Plant growth: Exposure of maize variety s to Pb stress resulted in a significant root growth inhibition though shoot growth remained less affected (Table 1) Highly toxic Pb level (30 ppm) exhibited substantial growth reduction yielding 76% of the root length of the control in Neelam variety, whereas this value was found in Desi variety as 85% (Table 1). Although Pb content in the roots of Desi was higher than the Neelam for all Pb treatments, Pb-induced root length (or biomass) reduction of Desi was lower than that of variety Neelam (Table 1). However, the growth of maize shoots was rather resistant to Pb stress for both varieties, significant inhibition of root growth was observed in Neelam variety compared to Desi variety (Table 1). Variation of root/shoot ratio was less affected in the Desi compared to its control.

Pb^{+2} accumulation: The lead contents of roots, shoots and leaves of both varieties increased significantly under the Pb stress when compared to control. The increase in Pb content of both varieties was found to be dependent on different Pb concentrations. Pb content of Neelam and Desi in control roots was determined. On the other hand Pb content of both varieties in control leaves was also determined. The absorbed Pb was localized to a greater extent in roots than in leaves and shoots for both varieties. At highly toxic Pb concentration, a ten folds increase in roots and 8 folds increase in

Table 1: The effects of lead concentrations on some growth parameters of two maize varieties grown at different concentrations of lead (Pb^{+2})

Varieties	(Pb^{+2}) concentrations ($mgKg^{-1}$ soil)	Shoot length (cm per plant)	Shoot length (% of control)	Root length (cm per plant)	Root length (% of control)	Dry biomass of root (gDW^{-1})	Root/ shoot ratio
Neelam	Control	34.00±0.70*	100.00	35.31±0.31*	100.00	0.21±0.01**	1.04
	10	33.75±0.58	99.26	26.18±0.48	74.13	0.18±0.01	0.78
	20	30.31±0.86	89.16	24.38±0.42	69.03	0.12±0.01	0.80
	30	27.58±0.89	81.61	23.64±0.36	66.96	0.09±0.01	0.86
Desi	Control	34.98±0.32	100.00	35.74±0.63	100.00	0.23±0.01	1.02
	10	31.08±0.36	88.90	29.94±0.33	83.77	0.18±0.02	0.96
	20	29.20±0.62	83.49	29.01±0.31	81.16	0.17±0.01	0.99
	30	25.67±0.51	73.39	26.66±0.99	74.59	0.11±0.01	1.04
LSD 5%		1.57		1.28		0.03	

*Significant at 5% level of significance

Table 2: Contents of lead (Pb^{+2}) (mg kg^{-1} DW) in different parts of two maize varieties exposed to different concentration of lead (Pb^{+2})

Varieties	Pb concentrations ($mgKg^{-1}$ soil)	Roots	Shoots	Leaves
Neelam	Control	4.1*±1.4	3.4±1.4	2.6±1.8
	10	266±12.4	143.00±6.4	33.93±4.1
	20	330±10.9	165.63±8.7	39.9±5.6
	30	4.4±1.5	177.54±7.5	48.7±6.0
Desi	Control	277±16.4	1.15±1.3	2.8±1.9
	10	384±21.3	149.00±7.6	34.94±3.1
	20	396±17.2	168.33±4.5	42.7±6.4
	30	398±19.2	176.43±7.2	47.6±5

*Significant at 5% level of significance

Table 3: Chlorophyll concentrations (a, b and total) of two maize varieties grown under different concentrations of lead (Pb^{+2})

Varieties	(Pb^{+2}) concentrations ($mgKg^{-1}$ soil)	Chlorophyll (a)	Chlorophyll (b)	Chlorophyll (Total)
Neelam	Control	0.540	0.285	0.8325
	10	0.386	0.366	0.8826
	20	0.334	0.378	0.9228
	30	0.225	0.397	0.9529
Desi	Control	0.640	0.288	0.8535
	10	0.388	0.376	0.9232
	20	0.337	0.380	0.9428
	30	0.227	0.389	0.9848

*Significant at 5% level of significance

leaves and 6 folds increase in shoots of Desi varietiety, whereas an 11 folds increase in roots and 9 folds increase in leaves of Neelam was observed for Pb content when compared with their corresponding controls. The results indicated that the accumulated Pb level in the roots of Desi was higher than that in the roots of Neelam for all Pb treatments but the opposite was observed in the leaves. When compared between plant parts, the Pb in roots was accounted for the majority of the total Pb (90-96%) in both varieties. On the other hand, leaves and shoots usually contained much lower Pb than that of roots.

Chlorophyll determination parameters: Leaf chlorophylls (chl) content revealed significant differences between the maize varieties and Pb-levels at seedling stage. Total chl contents indicated a sharp decrease in Neelam while Desi maintained a steady level. Higher levels of Pb were much more damaging to Neelam as for as this attribute was concerned.

DISCUSSION

In this study, both maize varieties have the ability to accumulate Pb primarily in their roots and transport it to their leaves in much lesser concentrations. Similar results were reported by (Patra *et al.*, 2004; Sinha *et al.*, 2006). The results also indicated that the accumulated Pb level in roots of Desi was higher than the roots of Neelam in all Pb treatments, but a significantly higher percent of Pb was translocated to the leaves of Neelam compared with Desi. Most Pb remained in the roots Pb transported to the leaves of the varieties. Fritioff and Greger (2006) reported that Pb remained immobile in plants. In this study, 4% of the Pb was translocated to the leaves of Neelam while for Desi it was around 2% at the highly toxic Pb level. The limited transport of Pb from roots to the other organs might be due to the barrier of the root endodermis. Histochemical observations in barley and maize seedlings also showed that, lead can not penetrate endodermis that acts as a barrier to lead uptake to shoots and the stele (Sharma and Dubey, 2005). Tolerance to metals can either be achieved by avoiding the metal stress or by tolerating it or both (Levitt, 1980). Since the tolerant variety took up more lead than susceptible variety, it appears that tolerance depends more on detoxification than on selective absorption. An electron microscopic study of root tips from tolerant plants reveals the presence of Pb in the cell wall as well as the cytoplasm (Sharma and Dubey, 2005). It has been shown that Pb compounds bind less strongly to Phytochelatins (PCS) due to larger ion radius (Pb, octahedral) and high coordination number (Pb, 6-8) (Sharma and Dubey, 2005). Brown and Slingsby (1972) showed that the high tolerance of lead in plants results from Pb accumulation only in the cell wall without penetrating into the protoplast. These results indicate that both varieties might avoid the lead stress by binding it to their cell walls. Also, less migration of lead to the leaves of Desi meant that Desi was more tolerant than Neelam to the lead stress. On the other hand, increased concentrations of Pb induced significant growth inhibition in both varieties as observed in the reduction of the root length (Table 1). Highly toxic Pb concentration inhibited root elongation in Neelam variety whereas this value determined in Desi variety as compare to their controls. Consequently, Pb stored predominantly in roots and while shoots were less affected in both varieties. Similar results were reported by Bashmakov *et al.* (2005). They indicated that 30 ppm of Pb concentrations caused suppression of growth processes and accumulation of Pb especially in roots. It was reported that plants have developed various tolerance and resistance mechanisms in order to diminish the heavy metal stress. One of these mechanisms is to hold the heavy metal in the root and prevent the distribution to the leaves (Fernandes and Henriques, 1991). Yang *et al.* (2000) reported that when exposed to a solution containing Pb, the root biomass of the tolerant variety was higher than that of the sensitive rice variety because of the ability of the tolerant variety to develop adventitious roots. Root development in tolerant variety was associated with a mechanism that altered the Pb in the solution into a form that could no longer be taken up by a newly growing tissue. Similar response in dry biomass of roots of Desi variety was observed in this study (Table 1).

Conclusion: The results of this study showed that higher Pb accumulation in roots in comparison with leaves and better defense systems are the most important characteristics of maize varieties in order to tolerate excess lead. It is clear that Desi is more tolerant to Pb toxicity compared to Neelam which was found to be a particularly less tolerant variety. Desi showed lower Pb accumulation in leaves. Maize varieties were found to be tolerant to Pb treatment without damage for concentrations up to 20 ppm. We consider that *"Maize"* is an excellent crop-model to study heavy metal stress tolerances at the biochemical and growth level due to its ability to withstand certain heavy metal stress conditions. Consclusively, *Zea mays* L. varieties are Pb-tolerant plants so that they can be selected as suitable species growing on the lead contaminated soils for bioremedial purpose.

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