Effects of Lead Toxicity on Plant Growth and Biochemical Attributes of Different Rice (Oryza Sativa L.) Varieties

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Abstract: Rice (Oryza sativa) is one of the principal foods for a large part of the world's human population, but its productivity is reduced by the toxic effects of heavy metals such as lead. In the present study seeds of the rice cultivars KSK-133, NIAB-IR-9, Basmati-385 (B-385) and Shaheen Basmati (SB) were treated with different concentrations of lead chloride (PbCl₂) (0, 250, 500, 1000 and 2000 ppm) to evaluate the effect on germination, seedling growth and some biochemical attributes. Lead had no effect on germination percentage, except cv. SB at 2000 ppm. Germination rate, root and shoot length and dry weight decreased with increasing lead concentrations. A sand culture experiment was conducted to measure the ion contents (Na, K, Ca), photosynthetic pigments, total protein and nitrogen contents. Chlorophyll a, chlorophyll b and carotenoids decreased with the increasing concentration of lead. Three varieties (NIAB-IR-9, KSK-133 and SB) had a decreasing trend in potassium and calcium ions and increasing in lead ion concentration. There was an increase in sodium ion concentration with the increase in lead concentration but B-385 showed contrary results. Results confirm that lead has an inhibitory effect on plant growth and development.

Key words: Oryza sativa L., Lead stress, Germination, Plant growth

Introduction

Rice (Oryza sativa) is one of the most important cereal crops, providing food for nearly a half of the world population (Panich-pat and Srinives, 2009) and contributing with one fifth of the calories consumed by human’s worldwide (Welch and Graham, 2005). About 90 percent of the total rice is cultivated in Asia (Salimet al., 2003). Rice is the second most important food crop of Pakistan; not only because of its local consumption but also in view of large exports (Noor et al., 2005). Abiotic stresses such as the presence of heavy metals at toxic levels adversely affect
growth and grain yield of crops (Salt et al., 1995). Among the various heavy metals, Lead (Pb) is commonly spread throughout the environment, and reveals a comparatively high reactivity to plant cells (Lukaszek et al., 1998). Lead deposition interferes with plant growth and development, and can cause plant death in Pakistan, only 172 billion cubic meters of fresh water are available, which are not adequate to meet the water requirements of the crops (Ibrahim and Salmon, 1992; Qadir et al., 1998). Therefore, sewage waters are used to irrigate rice fields which are usually mixed with irrigated water; however, those are not treated because of the lack of infrastructure and facilities for sewage treatment (Nawaz et al., 2006). Lead is the major component of those nutritional elements by seedlings and plants and causes deficiencies or adverse ion distribution within the plant (Trivedi and Erdei, 1992). Research about the effect of heavy metals on plant growth has been well documented (Qi et al., 2000; Kang et al., 2002), including effects of lead pollution on under extreme conditions (Laraque and Trasande, 2005; Clemens, 2006). In Triticum aestivum and Cucumis sativus, lead toxicity decreases seed germination rate, length and weight of fresh and dry mass of roots and shoots (Munzuroglu and Geckil, 2002). These effects can be attributed to the fact that lead obstructed the absorption of seed germination and seedling growth in rice (He, 1990; Cai et al., 2002). It has been reported that lead deposition in leaves of rice decreased the concentrations of chlorophyll contents. Lead produced highly significant effects on shoot, root lengths and seedling dry biomass of Lythrum salicaria (Jusephet et al., 2002), waste water, which is used for irrigation. In such conditions there is a strong need to investigate the effect of lead on rice seed germination and seedling and plant growth by evaluating various physiological and biochemical attributes of Oryza sativa. It was hypothesized that lead toxicity might induce physiological and biochemical changes in Oryza sativa. To prove this hypothesis, the present study was designed.

**Materials and Methods**  
**Plant material**  
Seeds of the rice varieties NIAB-IR-9, KSK-133, B-385 and SB were obtained from National Agricultural Research Centre (NARC) Islamabad.  
**Seed germination**  
Prior to the tests, seeds were surface sterilized in a 0.5 % sodium hypochlorite (NaOCl) solution for 10 minutes to remove dirt and fungal traces. The seeds were then rinsed with distilled water. Ten seeds were placed per Petri plate on Whatman No.2 filter paper discs. Afterwards, they were moistened with 10 mL of either 0, 250, 500, 1000, or 2000 ppm PbCl$_2$ solution. There were five replications per concentration and variety. The plates were then covered and kept in the dark at 30 ºC for 4 d in growth chamber (K-HB 3015, Korea). The seeds during that time, every seed that reached 2 mm radical growth were considered as germinated. Seed germination in each set was recorded every 12 h up to 4 d and germination rate calculated as 1/t$_{50}$ (where t$_{50}$ is the time to 50 % of germination). After 4 d of germination, the seeds were provided with proper light and dark periods (16/8 hr) and allowed to grow for 6 d more. After 10 d, the lengths of fresh shoots and roots of all the seeds in all replicates were measured. After the measurement, the roots and shoots were excised from the seeds and their fresh weights were taken using a digital balance (ALS 120-4, Germany). After taking fresh weights, the roots and shoots of all the seeds were placed in an incubator (Memmert, Model 100-800, Germany) in Petri plates at
Effects of Lead Toxicity on Plant Growth

80 °C for 48 h. After that, the dry weights of roots and shoots were taken.

Sand culture

Seeds of four varieties were grown in distill water. After 15 d, plants seedlings were transferred to sand culture in clean pots and were supplied with Hoagland's solution for 14 d. The pH was maintained at 5.8. After 14 d, a solution containing PbCl₂ in combination with Hoagland's solution was supplied to the plants for 7 d. Three replicates of each variety for every concentration (0, 250, 500, 1000, or 2000 ppm) were used. Twelve pots (three of each variety) were supplied with Hoagland's solution only and were used as control. After 36 d, the plants were harvested, dried at 80 °C in an incubator and hand crushed for biochemical tests.

Determination of Chlorophyll a, b and Carotenoids

For the chlorophyll test, 25 mg of crushed plant material from each replicate of four varieties was taken in test tubes. To protect chlorophyll from degradation by light, 25 mg of magnesium oxide were added to each tube. Then, 5 ml of methanol were added and the tubes were placed on a shaker at 200 g for 2 h. After that, the material was centrifuged (5810R Eppendrof, Germany) for one minute at 4000 g and the absorbance of the liquid at three different wavelengths (470, 653 and 666 nm) was checked by using a spectrophotometer (BMS UV-1900, Canada). From the values of absorbance, chlorophyll a, chlorophyll b and carotenoids contents were calculated by the following formula proposed by Lichtenthaler and Wellburn (1985):

Chlorophyll a = 15.65 × absorbance at 666 nm – 7.340 × absorbance at 653 nm
Chlorophyll b = 27.05 × absorbance at 653 nm – 11.21 × absorbance at 666 nm
Carotenoid (cx+c) = 1000 × absorbance at 470 nm – 2.86 × ch. a – 129.2 × ch. b/245

Determination of ions

For ion analysis, 25 mg of plant material of both varieties (for each replicate) was digested. For digestion, 2 mL of sulphuric acid (H₂SO₄) were added to the beaker and the beakers were placed on a hot plate at 150 °C. When black slurry was formed, one milliliter of H₂O₂ was added and again heated until it all evaporated and only a few drops were left. Then this was dissolved in 30 mL of distilled water and then filtered. Sodium, potassium and calcium were analyzed in these samples using a flame photometer while lead concentration was determined using atomic absorption (AAAnalyst 400, Perkin Elmer, USA).

Determination of nitrogen and protein content

Protein content was determined by using the BUCHI Distillation Unit k-350 micro-Kjeldahl apparatus. Dried plant material (50 mg) was taken and transferred to the digestion flask with 0.1 g of digestion mixture (copper sulphate, iron sulphate and potassium sulphate) and followed by the addition of 2 mL of concentrated H₂SO₄. The solution was heated until it became clear. After cooling, it was diluted up to 10 mL with distilled water. The digest was transferred to the distillation assembly and 10 mL of 50 % sodium hydroxide (NaOH) solution were added to it. The distillation was completed in 6 minutes, noticed by the change of color of boric acid to yellow due to the formation of ammonium borate indicating the trapping of nitrogen in the form of ammonium hydroxide. The boric acid having the trapped ammonia was titrated with 0.1 N sulphuric acid. Titration was done by methyl red indicator for which about 40 ml of boric acid were taken in a volumetric flask and the methyl red indicator was added, resulting in a pink colored solution. During titration, the color of boric acid having ammonia changed again into pink. The protein content was
calculated by first calculating the total nitrogen and protein content using the formula’s recommended by Pellett and Young (1980).

\[
\text{Nitrogen (g/g)} = \frac{(\text{Titrant volume for sample} - \text{Titrant volume for blank}) \times 1.4007 \times 0.1}{\text{Dry weight of sample} \times 100}
\]

Where, 1.4007 = Nitrogen factor.

Now for protein, we used:

\[
\text{Protein (g/g)} = \text{Calculated nitrogen} \times 6.25
\]

Where 6.25 is Protein factor

**Statistical analysis**

A completely randomized design with two factors was used in the experiment. Analysis of variance was performed by using MS-Excel. Mean comparisons, where applicable, were conducted using Tukey’s least significant difference (Li, 1964).

**Results and Discussion**

**Seed germination**

Germination of seeds started at 24 hours after planting in NIAB-IR-9, KSK-133 and B-385 but in SB it started at 60 hours. Germination was higher in the control and it was reduced at 2000 ppm of lead concentration in SB. Lead chloride at relatively low concentrations (250 ppm) had no significant effect on seed germination of all the varieties, since the germination was almost the same as the control. Lead chloride at 2000 ppm significantly reduced germination but after three days all the seeds germinated excepting SB (Figure 1). Entrance of lead to seed caused delay in germination because the seed membrane has a selective permeability to lead ions (Wierzbicka and Obidziska, 1998). It was evident that increasing lead concentration decreased the germination rate in all varieties, and that the effect was more drastic when concentrations were 500 or 1000 ppm (Figure 1B). The effect of lead on germination percentage and rate was found to be greater in SB than in NIAB-IR-9, KSK-133 and B-385 (Figures 1 A and B). This might be a situation of tolerance and susceptibility to lead because all of these varieties belong to different genetic background. The decrease in seed germination could be explained for what was observed in Albezielebeck where the application of lead caused the rapid breakdown of stored food material in seeds (Farooqi et al., 2009). The effects of lead on seedling growth seem to be different with regard to plant species, cultivars and organs (Sharma and Dubey, 2005). Lead toxicity causes a decline in the seed germination percentage and germination rate (Munzuroglu and Geckil, 2002). Our result coincides with Bhardwaj et al. (2009) who also observed a decline in germination rate of *Phaseolus vulgaris* with increasing concentration of lead.

**Root and shoot length**

Root and shoot elongation of rice seedlings of all the varieties was greatly inhibited by PbCl\(_2\); the degree of inhibition increased as the PbCl\(_2\) level increased. Root growth was more affected than shoot growth (Figs. 2A and 2B). At 1000 and 2000 ppm of PbCl\(_2\) the root of several seeds of all the varieties emerged through the seed coat, but failed to elongate. In contrast, the respective shoots were relatively longer at these concentrations. This response had already been observed by Sheng et al. (2005), who stated that lead significantly reduces the root length and shoot length of rice seedlings, and that the degree of inhibition increased with the increase of Pb concentration. The primary effect of Pb toxicity in plants is a rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip (Eun et al., 2000).
Effects of Lead Toxicity on Plant Growth

Fig. 1. Effect of various concentrations of lead chloride on seed germination (A) and germination rate (B) of different varieties of rice. Vertical bars are the means of five replications±SD.

The justification for the behavior of root and shoot growth to lead is not known, but it might be due in part to a quicker deposition of lead in the roots than in the shoots (Al-Helal, 1995). Schulz-Baldes and Lewin (1976) reported that Pb reduced cell division and uptake of crucial elements as it accumulate on the cell membrane and hence inhibited seedling growth. Yang et al. (2000) found that the oxalate content in the root and root exudates decreases upon lead treatment in the sensitive varieties, and proposed that compounds such as oxalate secreted from the root may decrease the bio-availability of lead. The reduction in root growth by Pb toxicity is most possibly from the result of a non-selective suppression of both cell division and cell elongation of the seedlings (Ivanov et al., 1988; Eun et al., 2000). The small and poorly developed root system of rice seedlings at elevated Pb concentrations might also be associated to a disorder of metabolic processes (Dinev, 1988; Pahlsson, 1989; Obroucheva et al., 1998).

Fig. 2. Effect of various concentrations of lead chloride on shoot length (A) and root length (B) of different varieties of rice. Vertical bars are the means of three replications±SD.
Fresh and dry weight of root and shoot

Lead toxicity strongly affected the fresh and dry root weights in all the varieties. The effect of Pb was more on roots compared to shoots and weight was even zero at higher concentrations of PbCl$_2$ (Figures 2; A and B). Fresh and dry root and shoot weights significantly decreased at 1000 and 2000 ppm Pb concentrations (Figures 3). This is evident from these results that lead stress predominantly affects root growth. Consequently, Pb stored primarily in the roots because shoots were less affected in all varieties except at 1000 and 2000 ppm lead stress. Lead stress negatively affected the fresh and dry weight of shoots and roots with increasing Pb application (Kibria et al., 2010). In the present study, increasing concentration of lead caused the decrease of the fresh and dry weight of roots and shoots of all the four varieties (Figure 3). The decreased shoot and root biomass of rice seedlings might be due to interference of Pb with the physiological processes of the plant, as Lead phytotoxicity involves the decrease of enzyme activities, disturbed mineral nutrition, water imbalance, and alteration in hormonal status and variation in membrane permeability (Sharma and Dubey, 2005). The decline of biomass by Pb toxicity could be the direct consequences of the inhibition of chlorophyll synthesis and photosynthesis (Chatterjee et al., 2004).

Ion analysis

The Pb ion concentration increased with increasing level of Pb (Figure 4). Similarly, concentration of Na ions of NIAB-IR-9, KSK-133, and S.B. was observed to be increased while that of B-385 decreased with the increasing concentration of Pb (Figure 5). The concentration of K and Ca ions decreased with the increasing concentration of Pb but K ion concentration of NIAB-IR-9 at 1000 and 2000 ppm showed an increasing pattern and K ion concentration of KSK-133 increased only at 2000 ppm. Bas-385 variety showed an overall increasing pattern for K and Ca ion content (Figures 5; A and B). Lead actually blocks the entrance of many ions from absorption sites of the roots (Goldbold and Kettner, 1991). Our result showed the increasing pattern of Na ion content in NIAB-IR-9, KSK-133 and SB but irregular pattern in B-385. A decrease in K and Ca ion content with the increasing concentration of Pb in NIAB-IR-9, KSK-133 and SB is observed, except B-385 where these ions increased (Figures 5). This might be due to a direct competition between Pb and other important nutrients for same site (Chatterjee et al., 2004). It is presumed that the increase of some nutrient ions in plant tissues might be due to the synergistic effects of Pb with those ions via diverse mechanisms. Further research should be done for the understanding (Kibria et al., 2010). Similar results were found by Huang and Cuningham (1996) with corn, where the calcium concentration in corn shoots decreased after Pb treatment. However, in their study, the same Pb treatment did not significantly affect Ca concentration in shoots of ragweed. The results of Bas-385 (increase in K ion content) show similarity with the work of Kibria et al. (2009). They reported that lead application significantly increased K concentrations in both shoots and roots of *Amaranthus oleracea*. 
Effects of Lead Toxicity on Plant Growth

Fig. 3. Effect of various concentrations of lead chloride on fresh shoot weight (A) fresh root weight (B) dry shoot weight (C) and dry root weight (D) of different varieties of rice. Vertical bars are the means of three replications±SD.

Fig. 4. Effect of various concentrations of lead chloride on lead content of different varieties of rice. Vertical bars are the means of three replications±SD.

Chlorophyll content

Chlorophyll a, chlorophyll b and carotenoid content of all the varieties were decreased with the increasing level of lead as compared to control (Figure 6). It shows that Pb negatively affects the chlorophyll and carotenoids content and so decreases the photosynthesis rate in contaminated plants. The process of photosynthesis is badly affected by Pb toxicity (Sharma and Dubey, 2005). Under the metal stress, the levels of photosynthetic pigments, namely Chlorophyll 'a' and Chlorophyll 'b' and Carotenoids decrease as the concentrations of Pb in soil increases. (Bhardwaj et al., 2009). Our results show a decline in chlorophyll a, b and carotenoid pigment content in shoots of all the four varieties under lead stress (Figures 6). Plants under Pb stress show a reduction in photosynthetic rate because of the distorted chloroplast ultrastructure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, reduced activities of Calvin cycle enzymes, as well as deficiency of CO₂ as a consequence of
stomatal closure (Sharma and Dubey, 2005). Inhibition of chlorophyll synthesis by Pb is due to impaired uptake of important elements such as Mg and Fe by plants (Burzynski, 1987). An improvement of chlorophyll degradation occurs in Pb-stressed plants due to increased chlorophyllase activity is also observed (Drazkiewicz, 1994).

Fig. 5. Effect of various concentrations of lead chloride on sodium (A) potassium (B) and calcium (C) contents of different varieties of rice. Vertical bars are the means of three replications±SD.

Fig. 6. Effect of various concentrations of lead chloride on chlorophyll a (A) chlorophyll b (B) and carotene (C) contents of different varieties of rice. Vertical bars are the means of three replications±SD.

Nitrogen and protein content
Total nitrogen and protein content of all the varieties showed a decreasing trend with the increase in concentration of lead. Nitrogen and protein content of NIAB-IR-9 was highest followed by KSK-133. At 2000 ppm PbCl₂ concentration, B-385 and SB
showed lowest nitrogen and protein content (Figure 7). Lead treatment results in decline in total protein content (Neelofer et al., 2010). Lead stress may restrain a synthesis of some proteins and promote others with a general trend of decrease in the overall content (Ericson and Alfinito, 1984). Our results showed a decreasing trend in total protein and nitrogen content of all the varieties with the increase in the concentration of lead (Figures 7). The reduction in protein content may be caused by the increased protein degradation process as a consequence of enhanced protease activity that is found to increase under stress condition (Palma et al., 2002). It is also likely that lead may have induced disintegration of proteins due to the toxic effects of reactive oxygen species that led to reduce the protein content (Davies et al., 1987). Protein content under lead stress may be affected due to enhanced protein hydrolysis resulting in decreased concentration of proteins (Melnichuk et al., 1982) and catalytic activity of lead (Bhattacharya and Choudhuri, 1997).

### Conclusion

Lead stress negatively affected the vigor of seedlings and biochemical attributes such as ion, chlorophyll, nitrogen and protein contents. All the varieties can tolerate low concentrations of Pb but at higher concentration of 500 and 1000 ppm, it causes inhibitory effects. The effect of Pb is different in each variety. SB is found to be more sensitive at higher Pb concentrations.

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