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Full Length Article

Effects of Lead Salts on Growth, Chlorophyll Contents and Tissue Concentration of Rice Genotypes

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Abstract

Lead (Pb) is one of the most abundant heavy metal pollutants and readily absorbed by plants that exerts toxic effects and also gets entered into human food chain. There are very few examples of genetic differences being exploited to produce low Pb rice food through decreased metal uptake from Pb-polluted environment. In this regard, solution culture screening has been proposed as a rapid technique for the identification of prospective rice genotypes. In a hydroponics study, fourteen rice genotypes were tested against varying rates of applied Pb, to investigate the effects of chloride, sulfate and nitrate salts of Pb on growth, chlorophyll contents and tissue concentration of rice genotypes. The plants were exposed to 0, 100 and 200 µM Pb as $PbCl_2$, $PbSO_4$ and $Pb(NO_3)_2$, separately for 42 days. Thus, there were seven treatments arranged in completely randomized design each with four replications. The results showed that at a certain defined Pb rate (100 or 200 µM), the toxic effects of Pb as $Pb(NO_3)_2$ to rice plants were found significantly ($p \leq 0.05$) higher than $PbCl_2$ and $PbSO_4$ treatments. At all applied Pb treatments, the Shaheen Basmati and KS-282 were found tolerant to Pb because of higher growth, total chlorophyll contents, low root and shoot Pb concentration. Therefore, these both genotypes were found to be a good source for future rice breeding programs or grown by the farmers for Pb risk-free rice production in Pb polluted environment. © 2017 Friends Science Publishers

Keywords: Solution culture; Rice varieties; Pb toxicity; Pb accompanying anion effect

Introduction

Lead metal is a major pollutant in the environment due to its impact on human health through the bio-magnification in an ecological system (Sartipnia *et al*., 2013). Major sources of Pb pollution are mining and smelting operations, leakages from storage battery industries, burning of coal, automobile exhausts, agricultural pesticides and the disposal of Pb enriched municipal sewage sludge (Sharma and Dubey, 2005; Pourrut *et al*., 2011; Sartipnia *et al*., 2013). Lead can adversely affect plant growth and physiological processes even when present in very minute amounts. Chlorosis, stunted plant growth and inhibition of root growth are general toxicity symptom of Pb in plants (Sharma and Dubey, 2005). Inhibition of chlorophyll biosynthesis is one of the most Pb-sensitive physiological processes (Li *et al*., 2012). Lead obstructs chlorophyll production by reducing the uptake of essential nutrients such as Mg and Fe by plants (Pourrut *et al*., 2011). At high concentration of Pb eventually might lead to cell death (Seregin and Ivanov, 2001).

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However, the toxic effects of Pb depend on the type and properties of salts, metal concentration, characteristics of soil and species of plants involved (Patra *et al*., 2004). In plant systems in vivo, the water solubility of the Pb salt is of prime importance (Truta *et al*., 2011). The different characteristics of Pb solutions may alter biological features of Pb compounds, and influence its penetration in plant leaves and their lethal efficacy. The chemical forms of Pb also affect the transport of Pb from the medium into the plants (Patra *et al*., 2004; Truta *et al*., 2011). Therefore, the effect of accompanying anions such as Cl^-, NO_3^- or SO_4^{2-} on Pb availability and phytotoxicity can also be a critical factor. Research on these interactions should attract more attention because it relates to crop yield and quality, and ultimately human health.

Among cereal crops, rice (*Oryza sativa* L.) is very important food crop in global agriculture and is ranked second (more than 150 mha) after wheat in the cropped area (Kyuma, 2004). In Pakistan, rice is grown on an area of 2.89 mha and its total production is 6.79 mt with an average yield of 2423 kg ha^{-1} (GoP, 2015). Moreover, rice straw is used for livestock feed and bedding that may indirectly become a human health risk, if having high Pb and As in flooded soil contaminated by lead arsenate pesticide (Codling, 2009). In literature, a very few reports are available regarding the genetic variability among rice genotypes to produce low Pb rice from Pb-polluted soil conditions (Yang *et al*., 2000; Verma and Dubey, 2003). For a wide range of soils, selecting and growing low Pb accumulating rice genotypes/varieties can be a wise choice.

Since there are a few reports that metal tolerance is under genetic control, breeding crops for cultivation in poor drained soils can be beneficial. To enable crops breeding programs in an efficient way, a quick, non-destructive, cheap and repeatable seedling-based bioassay is required for the assortment of tolerant genotypes/species from initial segregating generations (Rout and Das, 2002). The use of solution culture has been recommended as a means of evaluating the plant tolerance to the toxic elements or the efficiency in mineral utilization (Giri and Patel, 2012). Hydroponic cultures allow an easy observation, making quick screening on the basis of relative growth rate and toxicity (Zhivotovsky *et al*., 2011). Therefore, the present study was conducted to recognize the Pb-tolerant rice cultivars based on their growth, chlorophyll contents and Pb concentration in different rice tissues.

Materials and Methods

A solution culture experiment was conducted in the wire-house having a glass covered roof (sides being open and only having iron wire screens with no control over temperature and humidity) at Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Seed of the fourteen rice genotypes were obtained from the Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan. The rice genotypes included were as V_1 = Basmati-2000, V_2 = 99417, V_3 = 99404, V_4 = Super basmati, V_5 = Basmati Pak, V_6 = Shaheen basmati, V_7 = IRRI-6, V_8 = NIAB IRRI-9, V_9 = KS-282, V_{10} = KSK-133, V_{11} = KSK-432, V_{12} = KSK-434, V_{13} = Basmati-370, V_{14} = Basmati-385. Healthy seeds of rice varieties were grown in polythene lined trays containing sand, pre-washed with 0.05 *N* hydrochloric acid (HCl). Yoshida nutrient solution (Yoshida *et al*., 1976) was applied to raise nursery of rice. The rice seedlings were irrigated with distilled water.

Ten days old rice seedlings, three seedlings per hill, were transplanted in polystyrene sheets with foam plugged holes and floating modified Yoshida solution in tubs of 100 L capacity. The Yoshida nutrient solution had the composition (mg L^{-1}): 40 N as NH_4NO_3; 10 P as $NaH_2PO_4.2H_2O$; 40 K as K_2SO_4; 40 Ca as $CaCl_2$; 40 Mg as $MgSO_4.7H_2O$; 0.5 Mn as $MnCl_2.4H_2O$, 0.05 Mo as $(NH_4)_6.MO_7O_{24}.4H_2O$; 0.2 B as H_3BO_3; 0.01 Zn as $ZnSO_4.7H_2O$; 0.01 Cu as $CuSO_4.5H_2O$; and 2 Fe as $FeCl_3.6H_2O$. This treatment solution contained all the nutrients in Yoshida nutrient solution except phosphorus (P), to prevent precipitation of Pb, following Saif Ullah *et al.* (2013). The tubs were arranged in a completely randomized design each with four replications. The pH of the treatment solution in all the tubs was checked and maintained daily at 5.0 ± 0.5 with 1 *N* HCl or NaOH. The treatments included were as T_1 = control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$ and T_7 = Pb at 200 µM as $Pb(NO_3)_2$. The Pb-treated nutrient solution was changed after every week during the study.

After 30 days of rice nursery transplantation, the flag leaf total chlorophyll content (TCC) index in terms of Special Products Analysis Division (SPAD, division of Minolta) value was determined from leaf tip to leaf base via a hand-held SPAD-502 meter (Minolta, Osaka, Japan) and then averaged. It is an economical approach to quantify photosynthetic capability compared to expensive chlorophyll fluorescence (Munns *et al*., 2006).

The plants were harvested after 42 days exposure to Pb-treated solution. Rice shoots and roots were washed sequentially with tap water and distilled water to remove any adhering material. The plant materials were blotted dry with tissue paper and then air-dried for 2 days in the shade followed by oven-drying at 65 ± 5°C for 72 h to obtain oven-dry rice shoot and root weights.

After oven-drying, the plant material was ground to particle size < 1 mm using a mechanical grinder (MF 10 IKA, Werke, Germany). Subsequent to grinding, the samples were uniformly mixed and 1 g portion was digested in 3:1 mixture of nitric acid to perchloric acid at 150°C (AOAC, 1990; Miller, 1998). Concentration of Pb in rice shoot and root filtered digests was determined using flame atomic absorption spectrometry (FAAS;

Model Thermo S-Series, Thermo Electron Corporation, Cambridge, UK).

Statistical Analysis

The data gathered were analyzed statistically following analysis of variance technique (ANOVA) and least significant difference (LSD) test was applied to differentiate the treatment effectiveness (Steel *et al*., 1997) using "Statistix 8.1" statistical computer software package(s).

Results

Rice Growth and Chlorophyll Contents

The application of Pb treatments (T), rice genotypes (V) and their interaction (T × V) had significantly (Table 6, $p \leq 0.05$) affected SDW (Table 1), RDW (Table 2) and TCC (Table 3). These parameters of rice genotypes decreased with applied Pb in growth medium as $PbCl_2$, $PbSO_4$ and $Pb(NO_3)_2$ at both rates i.e., 100 and 200 µM as compared to their respective controls. The treatments effectiveness on SDW, RDW and TCC of rice genotypes was found in decreasing order of $T_1 > T_3 > T_2 > T_4 > T_6 > T_5 > T_7$. The decrease in SDW, RDW and TCC of rice genotypes was more devastating by $Pb(NO_3)_2$ treatments followed by $PbCl_2$ and $PbSO_4$. Among different used rice genotypes, the V_6 had higher SDW, RDW and TCC followed by V_9 at all applied Pb treatments.

The interactive effects of T × V showed that maximum SDW was observed in V_6 and minimum in V_1 at T_1, which was statistically similar (Table 6) with V_4. With the application of Pb at 100 µM in T_2, T_3 and T_4, the SDW was decreased in rice genotypes compared to their respective controls. The maximum SDW was recorded for V_6 and it was 6.0, 6.9 and 5.4 g pot^{-1} with T_2, T_3 and T_4, respectively (Table 1). Whereas, the maximum RDW was observed in V_3 and minimum in case of V_{13} at control treatment. The applied Pb at 100 µM in T_2, T_3 and T_4 reduced the RDW of rice genotypes in comparison with their respective controls. The maximum RDW was observed for V_6 and it was 3.8, 4.4 and 3.4 g pot^{-1} with T_2, T_3 and T_4, respectively (Table 2). While, the maximum TCC were observed in V_4 closely followed by V_6 under control treatment. The application of Pb at 100 µM in T_2, T_3 and T_4 decreased the TCC of rice genotypes than their respective controls. The maximum TCC were observed for V_6 and it was 34.3, 40.9 and 25.9 SPAD-value at T_2, T_3 and T_4, respectively (Table 3).

However, the decrease in SDW, RDW and TCC was higher at 200 µM Pb compared to 100 µM Pb treatments. With the applied T_5, T_6 and T_7, the maximum SDW was observed for V_6 and it was 3.9, 5.0 and 3.2 g pot^{-1}, respectively (Table 1). Whereas, the highest RDW was recorded for V_6 and it was 2.3, 2.7 and 1.7 g pot^{-1} at T_5, T_6 and T_7, respectively (Table 2). While, the maximum TCC were observed for V_6 and it was 16.0, 19.7 and 12.0 SPAD-value by T_5, T_6 and T_7, respectively (Table 3). Under applied Pb treatments, the SDW, RDW and TCC of V_9 were lower than V_6 but remained higher than all other tested rice genotypes.

Rice Tissue Pb Concentration

The application of Pb salts with rates (T), rice genotypes (V) and their interaction (T × V) had significantly (Table 6, $p \leq 0.05$) affected the Pb concentration in rice shoot (Table 4) and root (Table 5). The treatments effectiveness on shoot and root Pb concentration of rice genotypes was ranked in decreasing order of $T_7 > T_5 > T_6 > T_4 > T_2 > T_3 > T_1$. The increase in Pb concentration in root and shoot by $Pb(NO_3)_2$ treatments was more followed by $PbCl_2$ and $PbSO_4$ in rice genotypes. Among different rice genotypes, maximum shoot and root Pb concentration was observed in V_3 followed by V_2.

Regarding T × V interactive effects, under control, the shoot Pb concentration was more for V_1, V_2, V_3, V_4, V_5 and V_{14} rice genotypes that was found statistically at par with each other. Whereas, the root Pb concentration was found statistically at par in all rice genotypes, being maximum recorded in V_3 and minimum in case of V_6 at T_1. The application of Pb at 100 µM in T_2, T_3 and T_4, shoot Pb concentration increased significantly (Table 6, $p \leq 0.05$) in all genotypes compared to their respective controls. With T_2, T_3 and T_4, the minimum shoot Pb concentration was observed for V_6 and it was 29.3, 7.0 and 97.1 µg g^{-1} DW, respectively (Table 4). The minimum root Pb concentration was observed for V_6 and it was 565.3, 371.3 and 686.0 µg g^{-1} DW at T_2, T_3 and T_4, respectively (Table 5). While, the application of Pb at 200 µM in T_5, T_6 and T_7, shoot Pb concentration in rice genotypes was increased. With T_5, T_6 and T_7, the minimum shoot Pb concentration was observed for V_6 and it was 232.8, 151.4 and 349.4 µg g^{-1} DW, respectively (Table 4). The minimum root Pb concentration was observed for V_6 and it was 1269.2, 1071.6 and 1387.9 µg g^{-1} DW at T_5, T_6 and T_7, respectively (Table 5). The shoot and root Pb concentration of V_9 was higher than V_6 but significantly lower than other used rice genotypes at all applied Pb rates.

Discussion

In the context of increasing concerns about Pb pollution by natural and anthropogenic activities, the most feasible and effective approach is screening and breeding species/genotypes having low toxic metal accumulation with high tolerance. In this regard, hydroponic screening has been proposed as a rapid and inexpensive technique for the identification of prospective heavy metals remediating species as an alternative to costly field trials (Zhivotovsky *et al*., 2011).

Table 1: Effect of applied Pb on shoot dry weight (SDW, g pot^{-1}) of rice genotypes

Genotype Name	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Genotype Mean
V_1= Basmati-2000	7.2 E-G	4.5 V-a	5.2 N-U	4.0 Z-h	3.0 m-t	3.6 f-p	2.4 s-u	4.3 EF
V_2= 99417	7.8 B-E	4.6 S-a	5.3 M-T	4.1 Y-g	3.0 n-t	3.6 f-p	2.4 s-u	4.4 E
V_3 = 99404	8.2 A-C	4.8 Q-Y	5.0 P-W	3.8 b-j	2.9 p-u	3.6 f-n	2.3 tu	4.4 EF
V_4 = Super Basmati	7.2 E-G	4.4 V-d	5.1 O-V	4.0 a-i	2.9 p-u	3.5 g-p	2.2 u	4.2 F
V_5 = Basmati Pak	7.6 C-F	4.7 R-Z	5.5 L-P	4.2 X-f	3.1 k-r	3.7 d-m	2.4 r-u	4.5 E
V_6 = Shaheen Basmati	8.7 A	6.0 I-L	6.9 F-H	5.4 L-Q	3.9 a-i	5.0 O-W	3.2 j-q	5.6 A
V_7 = IRRI-6	8.1 A-C	5.3 M-S	6.3 H-K	4.8 Q-Y	3.5 f-p	4.4 V-c	3.0 n-t	5.1 C
V_8 = NIAB IRRI-9	8.2 A-C	5.4 L-Q	6.4 H-J	4.9 P-X	3.6 f-o	4.6 S-a	3.0 l-s	5.2 BC
V_9 = KS-282	8.4 AB	5.7 K-O	6.6 G-I	5.0 O-W	3.7 d-l	4.8 Q-Y	3.1 k-r	5.3 B
V_{10} = KSK-133	8.1 A-C	5.3 N-U	6.2 I-K	4.8 Q-Y	3.5 g-p	4.4 W-e	2.9 o-t	5.0 C
V_{11} = KSK-432	7.7 C-E	5.0 P-W	5.8 J-N	4.5 V-b	3.3 i-q	4.2 X-f	2.7 q-u	4.7 D
V_{12} = KSK-434	7.9 B-D	5.1 O-V	6.0 I-M	4.6 U-a	3.4 h-q	4.1 Z-h	2.7 q-u	4.8 D
V_{13} = Basmati-370	7.3 D-F	4.7 R-Z	5.4 L-Q	4.2 X-g	3.1 k-r	3.7 c-k	2.4 r-u	4.4 E
V_{14} = Basmati-385	7.3 D-F	4.6 T-a	5.3 L-R	4.1 Y-g	3.0 k-s	3.7 e-m	2.4 r-u	4.4 EF
Treatment Mean	7.8 A	5.0 C	5.8 B	4.5 D	3.3 F	4.1 E	2.6 G	

LSD: Genotype (V) = 0.2*; There are 6 groups (A, B, etc.) in which the means are not significantly different from one another
LSD for treatment (T) = 0.1*; All 7 means are significantly different from one another
LSD for V × T = 0.7*; There are 47 groups (A, B, etc.) in which the means are not significantly different from one another
NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)
Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 4)
Note: For simplicity, first and last alphabets of lettering are mentioned with values in all the Tables. e.g. Z-j = Zabcdefghij
Treatments: T_1 = Control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$, T_7 = Pb at 200 µM as $Pb(NO_3)_2$

Table 2: Effect of applied Pb on root dry weight (RDW, g pot^{-1}) of rice genotypes

Genotype Name	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Genotype Mean
V_1= Basmati-2000	4.8 cd	3.5 lm	4.1 h	3.1 pq	1.9 vw	2.5 s	1.5 y	3.1 G
V_2= 99417	5.0 ab	3.6 j-m	4.1 gh	3.1 pq	2.0 v	2.5 rs	1.6 xy	3.1 E-G
V_3 = 99404	5.2 a	3.5 k-m	4.2 f-h	3.0 q	1.9 vw	2.5 s	1.6 xy	3.1 D-F
V_4 = Super Basmati	5.0 ab	3.6 j-l	4.2 e-h	3.1 o-q	2.0 uv	2.6 rs	1.6 xy	3.2 DE
V_5 = Basmati Pak	5.0 a-c	3.6 i-l	4.2 e-h	3.1 o-q	2.0 uv	2.6 rs	1.6 xy	3.2 DE
V_6 = Shaheen Basmati	4.9 b-d	3.8 i	4.4 e	3.4 mn	2.3 t	2.7 r	1.7 wx	3.3 A
V_7 = IRRI-6	4.8 cd	3.7 i-l	4.2 e-h	3.2 n-q	2.1 t-v	2.7 rs	1.6 xy	3.2 CD
V_8 = NIAB IRRI-9	4.9 b-d	3.8 ij	4.3 ef	3.3 n-p	2.2 tu	2.7 rs	1.6 xy	3.2 BC
V_9 = KS-282	4.8 b-d	3.8 ij	4.3 e-g	3.3 no	2.2 tu	2.7 rs	1.7 xy	3.3 B
V_{10} = KSK-133	4.8 cd	3.7 i-l	4.2 f-h	3.2 o-q	2.1 t-v	2.6 rs	1.6 xy	3.2 DE
V_{11} = KSK-432	4.9 b-d	3.8 ij	4.3 ef	3.2 n-p	2.1 t-v	2.7 rs	1.6 xy	3.2 BC
V_{12} = KSK-434	4.9 b-d	3.7 i-k	4.3 e-g	3.2 n-q	2.1 t-v	2.7 rs	1.6 xy	3.2 BC
V_{13} = Basmati-370	4.8 d	3.6 j-m	4.1 f-h	3.1 pq	2.0 uv	2.6 rs	1.5 xy	3.1 FG
V_{14} = Basmati-385	4.9 b-d	3.6 j-l	4.2 e-h	3.1 o-q	2.0 uv	2.6 rs	1.6 xy	3.2 DE
Treatment Mean	4.9 A	3.7 C	4.2 B	3.2 D	2.1 F	2.6 E	1.6 G	

LSD for genotype (V) = 0.06*; There are 7 groups (A, B, etc.) in which the means are not significantly different from one another
LSD for treatment (T) = 0.04*; All 7 means are significantly different from one another
LSD for V × T = 0.20*; There are 25 groups (A, B, etc.) in which the means are not significantly different from one another
NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)
Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 4)
Small letters represent comparison among interaction means and capital letters are used for overall mean
Note: For simplicity, first and last alphabets of lettering are mentioned with values in all the tables. e.g. Z-j = Zabcdefghij
Treatments: T_1 = Control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$,T_7 = Pb at 200 µM as $Pb(NO_3)_2$

In present solution culture study, results showed that the rice growth (SDW and RDW), physiological (chlorophyll contents) and biochemical (shoot and root Pb concentration) responses of tested fourteen rice genotypes were significantly ($p \leq 0.05$) affected by different applied Pb salts i.e., $PbCl_2$, $PbSO_4$ and $Pb(NO_3)_2$ and their increasing application rates (0, 100 and 200 µM). The trend in observations were found in agreement with the previous work of various researchers for different rice varieties (Mishra and Choudhuri, 1998; Yang *et al*., 2000; Verma and Dubey, 2003), cereals (Mahmood *et al*., 2007), castor bean (Romeiro *et al*., 2006), maize (Gupta *et al*., 2009) and willows (Zhivotovsky *et al*., 2011). In response to Pb absorption, root growth was reduced and branching pattern was changed. The inhibition of root growth can be due to a decrease in Ca in root tips, leading to a decrease in cell division or cell elongation (Eun *et al*., 2000). Root growth was considered a good parameter to study the relative tolerance or sensitivity of plants to heavy metals (Niaz *et al*., 2010).

Table 3: Effect of applied Pb on total chlorophyll contents (TCC, SPAD-value) of rice genotypes

Genotype Name	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Genotype Mean
V_1= Basmati-2000	59.4 AB	28.7 M-P	36.0 E-H	18.3 T-W	7.8 k-o	12.6 a-g	5.2 o-r	24.0 D
V_2= 99417	58.8 A-C	26.3 O-Q	32.3 I-L	16.9 V-Z	4.3 p-r	11.0 e-k	3.6 qr	21.9 FG
V_3 = 99404	58.0 A-C	25.4 PQ	30.7 K-N	15.8 W-b	3.9 qr	10.6 f-l	2.9 r	21.0 G
V_4 = Super Basmati	60.2 A	27.6 N-P	34.0 H-K	17.6 U-Y	4.8 o-r	11.5 d-j	4.0 p-r	22.8 EF
V_5 = Basmati Pak	55.7 C	26.5 O-Q	32.5 I-L	16.9 V-Z	7.0 m-q	11.6 d-j	4.8 o-r	22.1 F
V_6 = Shaheen Basmati	60.0 AB	34.3 G-J	40.9 D	25.9 O-Q	16.0 W-a	19.7 S-V	12.0 c-i	29.8 A
V_7 = IRRI-6	56.8 A-C	30.3 L-N	37.4 E-H	21.3 R-T	11.9 c-i	14.5 Y-d	6.9 m-q	25.6 C
V_8 = NIAB IRRI-9	56.7 BC	31.2 J-M	37.6 D-G	21.8 RS	12.5 b-h	15.1 W-c	7.4 l-p	26.0 C
V_9 = KS-282	57.9 A-C	32.1 J-M	38.5 D-F	24.1 QR	14.5 Y-d	16.8 V-Z	9.1 h-m	27.6 B
V_{10} = KSK-133	59.2 AB	31.2 J-M	38.8 DE	21.9 RS	10.1 g-m	14.8 X-d	6.9 m-q	26.1 C
V_{11} = KSK-432	59.4 AB	30.9 J-N	38.4 D-F	21.8 RS	9.7 g-m	14.5 Y-d	6.7 m-q	25.9 C
V_{12} = KSK-434	58.9 A-C	30.5 K-N	37.5 D-G	21.0 R-U	9.6 g-m	14.1 Z-e	5.6 n-r	25.3 C
V_{13} = Basmati-370	59.8 AB	30.7 K-N	37.5 D-G	19.8 S-V	8.9 i-n	13.7 Z-f	5.5 n-r	25.1 C
V_{14} = Basmati-385	57.3 A-C	29.1 L-O	35.0 F-I	18.2 T-X	8.2 j-o	12.6 a-g	5.1 o-r	23.6 DE
Treatment Mean	58.4 A	29.6 C	36.2 B	20.1 D	9.2 F	13.8 E	6.1 G	

LSD for genotype (V) = 1.0*; There are 7 groups (A, B, etc.) in which the means are not significantly different from one another
LSD for treatment (T) = 0.6*; All 7 means are significantly different from one another
LSD for V × T = 3.4*; There are 44 groups (A, B, etc.) in which the means are not significantly different from one another
NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)
Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 4)
Note: For simplicity, first and last alphabets of lettering are mentioned with values in all the tables. e.g. Z-j = Zabcdefghij
Treatments: T_1 = Control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$,T_7 = Pb at 200 µM as $Pb(NO_3)_2$

Table 4: Shoot Pb concentration (µg g^{-1} DW) in rice as affected by application of Pb

Genotype Name	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Genotype Mean
V_1= Basmati-2000	3.9 wx	144.5 f-k	120.1 j-o	210.5 Z-b	536.7 IJ	416.9 N-P	764.9 E	313.9 E
V_2= 99417	5.0 wx	188.7 b-e	145.6 f-k	247.9 XY	725.3 F	530.3 IJ	918.7 B	394.5 B
V_3 = 99404	6.1 wx	209.5 Z-b	157.0 e-i	261.8 W-Y	790.5 DE	585.0 H	958.2 A	424.0 A
V_4 = Super Basmati	4.8 wx	175.2 b-g	142.3 g-l	237.6 YZ	649.7 G	503.3 JK	868.2 C	368.7 C
V_5 = Basmati Pak	4.3 wx	161.4 d-i	134.0 h-m	225.7 Y-a	590.0 H	468.4 K-M	818.1 D	343.1 D
V_6 = Shaheen Basmati	0.2 x	29.3 t-x	7.0 v-x	97.1 n-q	232.8 YZ	151.4 f-j	349.4 ST	123.9 N
V_7 = IRRI-6	1.1 x	64.9 q-t	25.3 u-x	133.4 i-n	326.5 TU	232.5 YZ	447.3 L-N	175.9 K
V_8 = NIAB IRRI-9	0.8 x	57.4 r-u	15.4 v-x	118.8 i-o	307.9 UV	204.7 Z-c	424.1 N-P	161.3 L
V_9 = KS-282	0.3 x	43.0 s-v	9.3 v-x	106.6 l-p	278.0 V-X	179.1 b-f	387.6 P-R	143.4 M
V_{10} = KSK-133	1.4 x	74.6 p-s	39.4 s-w	140.9 g-l	370.7 Q-S	250.8 W-Y	520.1 IJ	199.7 J
V_{11} = KSK-432	1.7 x	84.4 o-r	56.6 r-u	153.7 e-j	400.2 O-Q	286.6 VW	545.6 I	218.4 I
V_{12} = KSK-434	2.1 x	100.0 m-q	70.1 p-s	170.7 c-h	432.4 M-O	336.3 S-U	588.9 H	242.9 H
V_{13} = Basmati-370	2.5 x	111.7 k-o	84.7 o-r	181.0 b-f	478.8 KL	360.1 R-T	681.2 G	271.4 G
V_{14} = Basmati-385	2.8 wx	131.8 i-n	102.4 m-p	195.3 a-d	518.0 IJ	388.0 P-R	721.2 F	294.2 F
Treatment Mean	2.6 G	112.6 E	79.2 F	177.2 D	474.1 B	349.5 C	642.4 A	

LSD for genotype (V) = 10.8*; All 14 means are significantly different from one another
LSD for treatment (T) = 6.7*; All 7 means are significantly different from one another
LSD for V × T = 36.8*; There are 50 groups (A, B, etc.) in which the means are not significantly different from one another
NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)
Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 4)
Note: For simplicity, first and last alphabets of lettering are mentioned with values in all the tables. e.g. Z-j = Zabcdefghij
Treatments: T_1 = Control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$,T_7 = Pb at 200 µM as $Pb(NO_3)_2$

The present data showed that V_6 had greater SDW (Table 1), RDW (Table 2) and TCC (Table 3) at all applied Pb treatments closely followed by V_9. The increasing concentration of Pb from 100 to 200 µM also adversely decreased the shoot, root biomass and TCC of rice genotypes. With the exception of Shaheen basmati, all other tested basmati rice genotypes were found as sensitive to Pb and coarse rice genotypes were proved Pb-tolerant. Similarly, Niaz *et al.* (2010) also reported that Shaheen basmati produced the highest root and shoot weights followed by KS-282, IRRI-6 and Super basmati under Cd stressed hydroponic culture. The Pb inhibit the growth of rice shoot due to its harmful effects on photosynthesis, mineral nutrition, water balance, hormonal status and membrane structure and permeability (Sharma and Dubey, 2005). Verma and Dubey (2003) found that with increasing Pb rates, the root and shoot growth was reduced gradually over control. Mahmood *et al.* (2007) reported that the wheat shoot height decreased by 15% with increasing Pb from 0 to 10 µM. Similarly, the presence of 10 µM Pb in the growth medium decreased dry masses of shoots and roots of rice cvs. Ratna and IR36 (Mishra and Choudhuri, 1998).

Table 5: Root Pb concentration (µg g^{-1} DW) in rice as affected by application of Pb

Genotype Name	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Genotype Mean
V_1= Basmati-2000	13.7 B	749.3 i-m	598.6 r-u	889.9 c-f	1747.2 I-K	1451.6 T-W	1957.9 EF	1058.3 E
V_2= 99417	16.5 B	808.6 g-j	629.0 p-s	933.6 cd	1963.9 EF	1595.6 N-Q	2199.5 AB	1163.8 B
V_3 = 99404	17.9 B	829.6 f-i	644.3 o-s	950.2 c	2023.5 DE	1669.6 K-N	2256.7 A	1198.8 A
V_4 = Super Basmati	15.6 B	791.3 g-k	621.5 p-s	921.7 c-e	1850.3 H	1546.3 P-S	2120.5 BC	1123.9 C
V_5 = Basmati Pak	14.5 B	774.3 h-l	616.5 q-t	916.3 c-e	1761.6 IJ	1504.1 R-U	2053.5 CD	1091.5 D
V_6 = Shaheen Basmati	4.2 B	565.3 s-v	371.3 A	686.0 m-q	1269.2 Y	1071.6 b	1387.9 WX	765.1 M
V_7 = IRRI-6	7.3 B	670.7 m-r	448.3 x-A	792.0 g-k	1431.7 U-X	1209.3 Y-a	1526.8 Q-T	869.4 J
V_8 = NIAB IRRI-9	6.4 B	633.6 o-s	440.0 y-A	770.6 h-l	1403.0 V-X	1161.3 Z-a	1483.9 S-V	842.7 K
V_9 = KS-282	5.6 B	612.6 q-t	393.7 z-A	742.3 j-n	1357.3 X	1129.4 ab	1421.6 V-X	808.9 L
V_{10} = KSK-133	8.5 B	663.3 n-r	472.0 w-z	805.3 g-j	1522.9 Q-T	1234.5 YZ	1647.2 L-O	907.7 I
V_{11} = KSK-432	9.5 B	679.6 m-r	501.3 v-y	817.6 f-j	1574.8 O-R	1265.2 Y	1682.4 J-M	932.9 H
V_{12} = KSK-434	10.7 B	702.3 l-p	527.6 u-x	831.3 f-h	1614.4 M-P	1351.1 X	1722.4 I-L	965.7 G
V_{13} = Basmati-370	11.6 B	711.3 k-o	541.3 t-w	843.6 e-h	1697.7 I-L	1386.1 WX	1871.7 GH	1009.0 F
V_{14} = Basmati-385	12.5 B	739.3 j-n	570.6 s-v	864.6 d-g	1766.3 I	1415.3 V-X	1932.9 FG	1043.1 E
Treatment Mean	11.0 G	709.4 E	526.9 F	840.4 D	1641.7 B	1356.5 C	1804.6 A	

LSD for genotype (V) = 24.0*; There are 13 groups (A, B, etc.) in which the means are not significantly different from one another
LSD for treatment (T) = 14.9*; All 7 means are significantly different from one another
LSD for V × T = 81.2*; There are 54 groups (A, B, etc.) in which the means are not significantly different from one another
NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)
Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 4)
Note: For simplicity, first and last alphabets of lettering are mentioned with values in all the tables. e.g. Z-j = Zabcdefghij
Treatments: T_1 = Control (without applied Pb), T_2 = Pb at 100 µM as $PbCl_2$, T_3 = Pb at 100 µM as $PbSO_4$, T_4 = Pb at 100 µM as $Pb(NO_3)_2$, T_5 = Pb at 200 µM as $PbCl_2$, T_6 = Pb at 200 µM as $PbSO_4$, T_7 = Pb at 200 µM as $Pb(NO_3)_2$

Table 6: F-values of two-way ANOVA regarding the effect of Pb salts on growth, chlorophyll contents and tissue concentration of rice genotypes

Parameter	Rice genotypes (V)	Pb treatments (T)	V × T
Shoot dry weight (SDW)	105.6**	3137.6**	1.8**
Root dry weight (RDW)	30.7**	17160.8**	3.7**
Total chlorophyll contents (TCC)	123.6**	14601.0**	5.3**
Shoot Pb concentration	1784.1**	20681.6**	150.2**
Root Pb concentration	717.0**	32361.8**	58.3**

NS = Non-significant ($P > 0.05$); * = Significant ($P \leq 0.05$); ** = Highly significant ($P \leq 0.01$)

Moreover, Zheng *et al*. (2006) also reported that chlorophyll contents decreased gradually with increasing rates of Pb in soil-Pb-rice system. This can happened due to the buildup of Pb inside leaves in enough concentration to further inhibit chlorophyll synthesis by reduced uptake of Fe and Mg. Li *et al*. (2012) also reported that the chlorophyll contents in rice leaves were significantly decreased with increasing Pb concentration. Ghani (2010) reported from the results of chlorophyll analyses of maize varieties that Pb toxicity affected the photosynthetic efficiency of Pb sensitive variety, while no significant effect was observed in tolerant variety of maize.

The tolerance, accumulation and transport of Pb varied greatly among rice varieties (Yang *et al*., 2000; Verma and Dubey, 2003; Liu *et al*., 2003). In present hydroponics experiment, when rice genotypes were raised against increasing rates of Pb, rice plants accumulated significant amounts of Pb in concentration-dependent manners. The absorbed Pb was localized to a greater extent in roots than in shoots of rice genotypes. Verma and Dubey (2003) reported that the rice seedlings grown in sand culture under 1000 µM Pb for 20 days showed up to 1.3065 mmol g^{-1} DW Pb absorbed in roots, while it was up to 0.8008 mmol g^{-1} DW Pb absorbed in shoots. However, Saif Ullah *et al*. (2013) illustrated that under 1000 µM Pb for 10 days of exposure, hydroponically grown wheat absorbed more Pb in root (9137.4 µg g^{-1} DM) than shoot (98.27 µg g^{-1} DM). Similarly, Gupta *et al*. (2009) found Pb concentration of 70,425 µg g^{-1} DW in roots and 995 µg g^{-1} DW in shoots of maize at 200 µM Pb, in the solution culture experiment. Hence, translocation of Pb from root to shoot was very low. The reason behind these effects was the binding of Pb to ion exchangeable sites on root cell wall and extracellular precipitation as Pb carbonates (Sharma and Dubey, 2005). A strong ability of Pb to bind with carboxyl groups of galacturonic and glucuronic acids in cell wall limited its apoplastic transport (Seregin and Ivanov, 2001). Using the radio-autographic technique, Sharma and Dubey (2005) speculated that Pb was transported along both apoplast and symplast pathways. In addition, casparian strip of root endodermis also restricted Pb transport across the endodermis into other plant tissues (Seregin and Ivanov, 2001). This barrier was reported to be broken at lethal concentration and the flux of Pb entered the vascular tissues (Sharma and Dubey, 2005).

In present trial, among all the rice genotypes exposed to different types of Pb salts and rates of Pb, the genotype 99404 (V_3) had the highest shoot and root Pb concentration,

while Shaheen basmati had the lowest Pb concentration because of its genotypic tolerance mechanism. This can be due to the reason that genotype with higher root secretions of organic acids (Yang *et al*., 1993), stronger root oxidation ability, solubilize more Pb in rhizosphere, resultantly more Pb was taken up by rice plants (Liu *et al*., 2009). The present information seems very useful for rice breeders. It can be inferred that shoots of salt-tolerant Shaheen basmati (Shabbir *et al.*, 2001) and KS-282 (Khan and Abdullah, 2003) contained less shoot Pb, perhaps through controlled over its translocation. In general, it was also observed that rice plant biomass also affected by Pb uptake, with low biomass plants had higher Pb concentration than did high biomass plants. Similar trend was reported in case of two submerged plant species (*Elodea canadensis* L. and *Potamogeton natans* L.) by Fritioff *et al.* (2005).

It was reported that the Pb-induced toxic effects depend on Pb compound type, properties of salts, pH of solution, metal oxidizing state, metal concentration, exposure duration, the plant parts and plant species (Patra *et al*., 2004; Azmat and Haider, 2007). In present study, the SDW (Table 1), RDW (Table 2), TCC (Table 3), Pb concentration in shoot (Table 4) and root (Table 5) of rice genotypes were also significantly (Table 6, $p \leq 0.05$) affected by the types of applied Pb salts. The results described that Pb bioavailability/phyto-toxicity also increased with increasing differently applied Pb forms and concentrations. The Pb toxicity due to various inorganic Pb salts was found in the decreasing order of $Pb(NO_3)_2 > PbCl_2 > PbSO_4$. The toxicity of Pb to plants was higher even with Pb at 100 µM as $Pb(NO_3)_2$ than same concentration of applied Pb as $PbCl_2$ and $PbSO_4$, separately. John and Laerhoven (1972) found that added Pb, as $PbCl_2$ and $Pb(NO_3)_2$ reduced lettuce plant weight by 25 and 35%, respectively. Zimdahl *et al.* (1978) showed no difference between sulfate and nitrate source of Pb for corn shoot uptake; however the sugar beet, bean and wheat crops taken up more Pb from the nitrate source. They also reported that the roots of all crops statistically showed a difference between the two Pb salts and in each case the nitrate source had a greater effect. In plant systems *in vivo*, water solubility of Pb salt was of primary importance (Truta *et al*., 2011). In present solution culture experiment, one of the possible reasons for the differences in observations, based on different properties of Pb salts like solubility in water and nature of accompanying ion etc. can be involved in Pb movement through transpiration stream. The chemical forms of Pb affect the transport of Pb from the medium into the plants. According to Truta *et al.* (2011), $Pb(NO_3)_2$ is considered as a weak mutagen. However, they reported that $Pb(NO_3)_2$ induced chromosome aberrations in wheat were higher than those persuaded by lead acetate. The concentration of heavy metals in solution was influenced by the nature of inorganic ligand ions (e.g., HPO_4^{2-}, NO_3^-, Cl^- and SO_4^{2-}) and pH, which ultimately govern the heavy metals sorption processes (Scheffer and Schachtschabel, 2002; Bolan *et al*., 2003). The presence of complexing ligands can increase the metal retention or greatly increase the metal mobility. Ghafoor *et al*. (2001) described that the soil retained more Pb and Cr ions for added as Cl^- salts of metals compared to SO_4^{2-} source. Due to the SO_4^{2-} ions had inhibitory effect on Pb and Cr mobility in soil. The Cl^- ions promoted Pb mobility by formation of ion pairs like $PbCl^+$ (Usman *et al*., 2005). The Cl^- increased the mobility of Pb and Cd more than sulfate did (Acosta *et al*., 2011). Mahmood *et al.* (2007) reported that the inhibitory effects on some cereal crops (i.e., barely, wheat and rice) seedlings was more pronounced by Pb and other metals (Cu and Zn) and was not due to SO_4^{2-} or Cl^-.

Conclusion

In present solution culture study, the tested fourteen rice genotypes showed significant differences in concentration of Pb, when exposed to different applied Pb salts i.e., $PbCl_2$, $PbSO_4$ and $Pb(NO_3)_2$ and their increasing application rates (0, 100 and 200 µM). The Shaheen basmati and KS-282 rice varieties were found tolerant to Pb because of better shoot and root growth, total chlorophyll contents, low root and shoot Pb concentration. However, these results need to be confirmed under field conditions and economic feasibility should be worked out. Thus, development of the rice varieties with low Pb particularly in edible parts can be considered as an effective approach for solving the issues and risks related to Pb. It can also be concluded that one should take into consideration the chemical properties of Pb salts like solubility and chemistry of ions etc. while working in solution culture experiments.

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