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Full Length Article

Growth and Physiological Responses of Two Rice Varieties to Applied Lead in Normal and Salt-Affected Soils

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Abstract

In arid and semi-arid regions of the world, soil salinity and heavy metal pollution occurring through various natural and anthropogenic means, is becoming major threat to safe crop production. The most feasible and effective approach is screening, breeding and cultivation of food crop varieties with low toxic metal accumulation. A pot study was conducted to investigate the growth and physiological responses of rice exposed to applied lead (Pb) in normal and salt-affected soils comprising four levels of Pb (0, 50, 100 and 150 mg kg^{-1} soil), two rice varieties (Shaheen basmati and KS-282) and two types of soil as normal and salt-affected soils. Increasing level of Pb decreased plant height, straw and paddy yields, photosynthetic and transpiration rates and stomatal conductance, more under salt-affected conditions. The cv. Shaheen basmati showed better response for growth, yield, physiological attributes and had lower Pb concentration in straw and paddy compared to cv. KS-282, at all levels of applied Pb in both normal and salt-affected soils. Thus, Shaheen basmati was found to be grown for Pb risk-free rice production and can be a good source for future rice breeding programs with the aim to produce low Pb accumulation and salt-tolerant rice varieties. © 2015 Friends Science Publishers

Keywords: Photosynthetic rate; Transpiration rate; Stomatal conductance; Pb concentration; Rice; Salt-affected soil

Introduction

Contamination of soils by Pb is widespread as a result of anthropogenic activities. These include agricultural and industrial activities such as mining and smelting processes, urban activities, use of Pb in gasoline, batteries, paints, insecticides and raw sewage irrigation (Sharma and Dubey, 2005). Apart from sewage irrigation, it is generally considered that soils of arid and semi-arid regions are often rich in total Cu, Ni, Pb and Zn (Han *et al*., 2002). Compared to most of developing countries of the world, the soils of Pakistan contain relatively high concentration of available Cd, Co, Pb and Se (Saif Ullah *et al*., 2008). Many researchers reported retardation of plant growth and decrease in biomass due to Pb phyto-toxicity. Plant foliage contamination is a significant factor of Pb concentration in above-ground parts (grains and straw) of wheat (Saif Ullah *et al*., 2009) and natural vegetation (Murtaza *et al*., 2012). Physiological effects of Pb phyto-toxicity include small leaves, short black roots, and stunted growth (Li *et al*., 2012). Low rates of photosynthesis, transpiration as well as lower nutrient absorption have also been reported in many plant species as a result of Pb contamination in soils (Sharma and Dubey, 2005; Yu *et al.*, 2014).

Soils irrigated with raw effluent slowly accumulate not only heavy metals but also salts leading to salinity/sodicity problems (Murtaza *et al*., 2008; Abd-Elrahman *et al*., 2012). City and industrial effluent has been found unfit for irrigation owing to high electrical conductivity (EC), sodium adsorption ratio (SAR) and residual sodium carbonate (RSC) (Murtaza *et al*., 2012). The concentration of Cd, Cr, Cu, Ni and Pb in raw sewage (Iqbal *et al*., 2011) were higher than permissible heavy metals limits for irrigation water (WHO, 2007). Qadir *et al*. (2010) also reported that concentration of heavy metals (Cd, Co, Cr, Ni and Pb) in salt-affected soils were increased by waste water irrigation. Salinity-heavy metal interactions are complex and difficult to understand due to several factors being involved in heavy metal absorption by plants, which may vary with soil and water salinity (Ahmad, 2007). In salt-affected soils, heavy metals concentration in plants may increase or decrease depending on the type of plants, salinity, heavy metal ion concentration, and other environmental conditions (Kabata-Pendias and Pendias, 2001).

Rice is an important food crop of Pakistan for both domestic consumption and export (Akram, 2009). Several biotic and abiotic factors including salinity and heavy metal toxicity can decrease rice yield and quality (Ahmad, 2007). Rice is very sensitive to salinity but tolerant to sodicity (Khan and Abdullah, 2003). Salinity upsets growth and physiological processes of plants owing to specific ion effects, nutritional imbalances, low osmotic potential of soil solution (water stress) or a combination of all of these factors (Ashraf and Harris, 2013). There are few examples

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of genetic differences being exploited for salt-tolerance between Shaheen basmati and KS-282 in salt-affected growth mediums (Arshadullah *et al.*, 2011; Jamil *et al.*, 2012).

It is expected that crop species/varieties having better salt tolerance, in general, could absorb and bio-accumulate less metals (Ghafoor *et al.*, 2012). Current information available from laboratory and field experiments has indicated that increasing salinity has the potential to increase heavy metal mobility (Hatje *et al.*, 2003; Pedron *et al.*, 2009). Kadkhodaie *et al.* (2012) reported that with increasing salinity, absorbable forms of heavy metals (Cd, Ni and Pb) increased in soil and consequently concentration of Cd, Ni and Pb increased in sunflower and Sudan grass when grown in such soils. Variation in Pb accumulation and transport of heavy metals amongst rice varieties has been previously observed (Liu *et al.*, 2003; Niaz *et al.*, 2010). Thus, successful rice crop production with better grain quality in saline and Pb stress conditions demands the selection of suitable variety having better salt (Arshadullah *et al.*, 2011) and Pb tolerance (Wang *et al.*, 2011).

To date, no reports are available in the literatures which examine the effects of Pb on the growth and physiological responses of rice in normal and salt-affected soils. Therefore, a pot study was conducted to evaluate different growth, physiological and accumulation responses of two rice varieties exposed to exogenous application of Pb in both normal and salt-affected soils.

Materials and Methods

Soil Physico-chemical Properties

Surface soil (0–20 cm) was collected from Land Utilization Farm, University of Agriculture Faisalabad, Pakistan. Soil was air-dried, pulverized with a wooden roller, passed through a 2 mm sieve, thoroughly mixed and stored. Soil samples were analyzed in triplicate to determine physico-chemical properties such as particle size by the hydrometer method (Bouyoucos, 1962) and soil textural class by the United States Department of Agriculture (USDA) textural triangle, saturation paste pH (pH_s), pH of soil-to-water ratio ($pH_{1:2}$), saturation paste extract electrical conductivity (EC_e), sodium adsorption ratio (SAR), cation exchange capacity (CEC), (US Salinity Lab. Staff, 1954), soil organic matter (Jackson, 1962), lime contents ($CaCO_3$) by the calcimeter method (Moodie *et al.*, 1959), extractable Pb concentration by ammonium-bicarbonate-diethylene-triamine-penta-acetic acid (AB-DTPA) (Soltanpour, 1985) and total Pb by Amacher (1996). The properties of soil used for this experiment are presented in Table 1.

Soil Preparation

The pot experiment was conducted in a wire house having a glass covered roof (sides being open and only having iron wire screens with no control over temperature and humidity). The Pb treatments used were as M0 = uncontaminated control, M1 = Pb at 50 mg kg^{-1}, M2 = Pb at 100 mg kg^{-1} and M3 = Pb at 150 mg kg^{-1} of soil, using Pb as $Pb(NO_3)_2$. The same set of Pb treatments were also applied to an artificially made salt-affected soil having EC_e = 6 dS m^{-1} and SAR = 22 (mmol L^{-1})$^{1/2}$. To develop a required salt-affected soil, the amount of salts was used (i.e., NaCl = 0.36, Na_2SO_4 = 0.53, $CaCl_2$ = 0.14 and $MgSO_4$ = 0.04, g kg^{-1} soil), which were calculated following quadratic equation (Muhammed and Ghafoor, 1992). The required amount of normal and salt-affected soils were spiked with three rates of Pb (50, 100, 150 mg kg^{-1}) using $Pb(NO_3)_2$ salt, following the method of Saif Ullah *et al.* (2010). White glazed ceramic pots (30 cm top and 25 cm bottom diameters, and 25 cm high; without any leaching provision, pre-aligned with polythene bags) containing 12 kg processed soil per pot following prescribed treatments layout (total 48 pots comprising of 4 levels of Pb, 2 types of soils, 2 rice varieties and each with 3 replicates) were arranged in a completely randomized design.

Crop Husbandry Practices

Seeds of two salt-tolerant rice varieties (Shaheen basmati and KS-282) were obtained from the Rice Research Institute, Kala Shah Kaku, Punjab - Pakistan. Healthy seeds of both 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 initially applied to germinate the rice and subsequently rice seedlings were irrigated with distilled water. Four week old rice seedlings of both varieties were transplanted with three seedlings per hill and five hills per pot (Ahmad, 2007). Rice was fertilized at 160-100-77-15 kg ha^{-1} of NPK and Zn, respectively. The source fertilizers for NPK and Zn were urea, di-ammonium phosphate (DAP), potassium sulfate (SOP) and $ZnSO_4.7H_2O$. Additional N added through application of varying levels of Pb as $Pb(NO_3)_2$ were accounted so that all pots received the same amount of N during fertilization. All of the nutrients P, K, Zn and half of the N (by making solution in distilled water), were applied at the time of initial pot preparation while the remaining N was applied in two equal splits; 30 and 45 days after transplantation. The pots were flooded with pumped ground water [EC = 0.64 dS m^{-1}, SAR = 1.33 (m mol L^{-1})$^{1/2}$, residual sodium carbonate (RSC) = 0.0 $mmol_c$ L^{-1}], which was found fit for irrigation (Arif *et al.*, 2010).

Rice Crop Physiological and Agronomic Measurements

Measurement of physiological processes like the photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) were made (from 10:00 a.m. to 1:00 p.m.) on flag leaves of three randomly selected rice plants from each pot using a portable narrow chambered infrared gas analyzer (IRGA, LCA-4, Analytical Development Company, Hoddesdon, England) following the method of Saif Ullah *et al.* (2010). At physiological maturity, the rice crop was harvested and plant height, straw dry matter and paddy yield were recorded following Yoshida *et al*. (1976). Rice (straw and paddy) and post-rice soil samples were collected from each pot separately and stored for Pb analysis. Soil samples were taken with a stainless steel sampling tube.

Plant and Soil Analyses

Rice samples (straw and paddy) were washed with sequentially 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 weight. After oven-drying, the plant material was ground to a particle size < 1 mm using a mechanical grinder (MF 10 IKA, Werke, Germany). After grinding, the samples were uniformly mixed and 1-g portion was digested in a 3:1 mixture of nitric acid to perchloric acid at 150°C (Miller, 1998). Concentration of Pb in rice straw and paddy filtered digests was determined using flame atomic absorption spectrometry (FAAS; Model Thermo S-Series, Thermo Electron Corporation, Cambridge, UK). Soil samples were air-dried, ground and sieved < 2 mm. The AB-DTPA extractable soil Pb was determined via FAAS, by extracting soil (10 g) with AB-DTPA solution (20 mL) adjusted to pH 7.60 (Soltanpour, 1985).

Statistical Analysis

All the data were analyzed statistically via analysis of variance (ANOVA) and the least significant difference (LSD) test to determine any differences between treatments (Steel *et al*., 1997) using the "Statistix 8.1" statistical computer software package.

Results

Growth Responses

Plant height, straw dry matter and paddy yield (Fig. 1) under salinity and Pb stresses were significantly ($p \leq 0.05$) inhibited, depending on Pb level, salinity and rice variety. At M0, the plant height, straw dry matter and paddy yield of both rice varieties were higher in normal soil than in salt-affected soil. With increasing levels of applied Pb (M1, M2 and M3), the plant height, straw dry matter and paddy yield of rice varieties decreased gradually in both normal and salt-affected soils in deceasing order of M0 > M1 > M2 > M3. Salinity and Pb stress affected both biomass and yield differently between the two rice varieties, with cv. Shaheen basmati had higher values of plant height, straw dry matter and paddy yield compared to cv. KS-282.

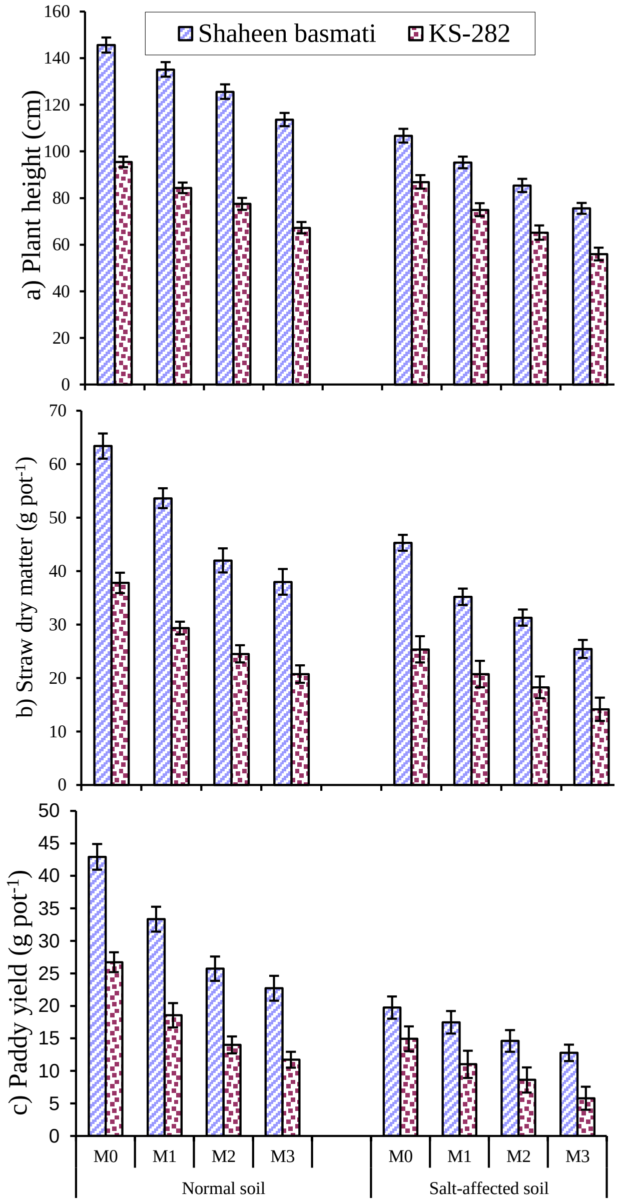

Fig. 1: Effect of Pb treatments on growth responses: a) plant height (cm), b) straw dry matter (g pot^{-1}) and c) paddy yield (g pot^{-1}) of rice varieties in normal and salt-affected soils (Each value is a mean ± SE; n = 3 statistically significant at $p \leq 0.05$)

Treatments: M0 = uncontaminated control, M1 = Pb at 50 mg kg^{-1}, M2 = Pb at 100 mg kg^{-1} and M3 = Pb at 150 mg kg^{-1} of soil

Physiological Responses

Physiological parameters of both rice varieties including photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) (Fig. 2) were significantly ($p \leq 0.05$) affected by Pb treatments in both normal and salt-affected soils. At M0, all three physiological responses (A, E and g_s) of both rice varieties were higher in normal soil than in

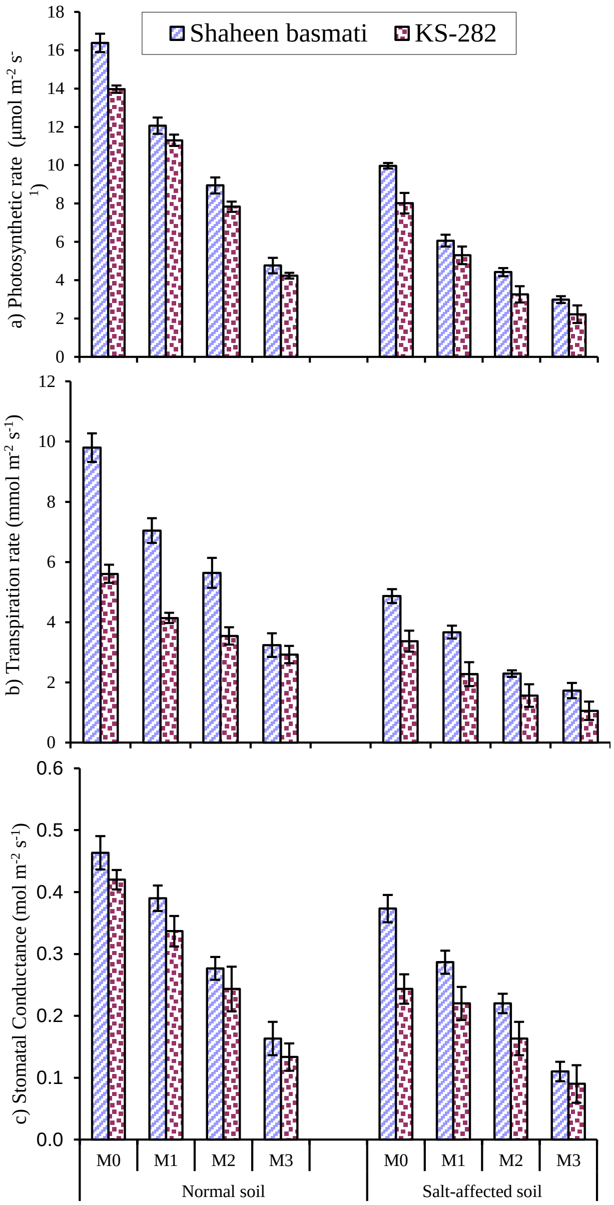

Fig. 2: Effect of Pb treatments on physiological responses: a) photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), b) transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and 3) stomatal conductance (g_s, mol m^{-2} s^{-1}) of rice varieties in normal and salt-affected soils (Each value is mean ± SE; n = 3 statistically significant at $p \leq 0.05$)

Treatments: M0 = uncontaminated control, M1 = Pb at 50 mg kg^{-1}, M2 = Pb at 100 mg kg^{-1} and M3 = Pb at 150 mg kg^{-1} of soil

salt-affected soil. Thereafter, physiological responses of both rice varieties decreased with increasing Pb application rates in the order of M0 > M1 > M2 > M3 for both normal and salt-affected soils. Thus, both salinity and Pb stresses lowered A, E and g_s of both rice varieties significantly depending on the treatment. Between the two tested rice varieties, maximum A, E and g_s was recorded for Shaheen basmati and the minimum for KS-282.

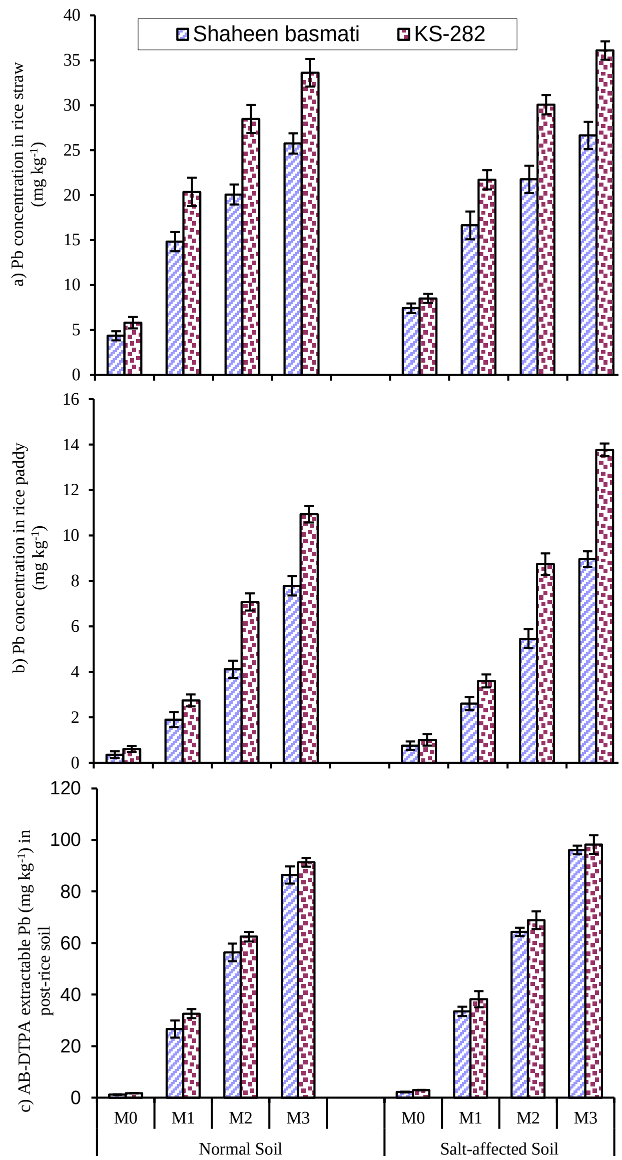

Fig. 3: Effect of Pb treatments on concentration (mg kg^{-1}) of Pb in a) rice straw, b) rice paddy and c) AB-DTPA extractable Pb in post-rice soil both in normal and salt-affected conditions (Each value is mean ± SE; n = 3 statistically significant at $p \leq 0.05$)

Treatments: M0 = uncontaminated control, M1 = Pb at 50 mg kg^{-1}, M2 = Pb at 100 mg kg^{-1} and M3 = Pb at 150 mg kg^{-1} of soil

Concentration of Pb

Total Pb concentration in rice straw and paddy, and AB-DTPA extractable Pb in post-rice soil (Fig. 3) were significantly ($p \leq 0.05$) affected by Pb treatments both in normal and salt-affected soils. At M0, Pb concentration in rice straw and paddy, and AB-DTPA extractable Pb in post-rice soil were lower in normal soil compared to salt-affected soil. As Pb application rate increased from 50 to 150 mg kg^{-1}, KS-282 accumulated more Pb in its straw and paddy compared to Shaheen basmati. Salinity significantly enhanced Pb concentration in rice straw and paddy. At a certain defined level (0, 50, 100 or 150 mg kg^{-1}), the concentration of Pb in rice straw and paddy was significantly (Table 2) higher in salt-affected Pb-contaminated soil compared to normal Pb-contaminated soil. With increasing rates of Pb application, the AB-DTPA extractable Pb in post-rice soil also increased for both normal and salt-affected soils in the increasing order of M0 < M1 < M2 < M3. The maximum concentration of Pb in rice straw and paddy was observed for KS-282 and the minimum Pb concentration for Shaheen basmati in both normal and salt-affected soils.

Discussion

Today, there is an increasing concern regarding how to produce safe agricultural products in moderately salt-affected and heavy metal contaminated soils. For this purpose, effective evaluation and identification of salt-tolerant and low Pb accumulating crop species and/or genotypes are a pre-requisite for successful development of safer food production (Zeng *et al*., 2008; Arshadullah *et al*., 2011; Manzoor *et al.*, 2014). Thus, the assessment of crop genotypes within the same genus for heavy metal tolerance is an important tool in defining whether growth and physiological traits are related to heavy metal tolerance (Zhivotovsky *et al*., 2011). Generally, soil properties such as soil pH, redox status and salinity, SOM, $CaCO_3$ contents and texture are considered important factors in describing bioavailability of heavy metals including Pb in soil (Connell, 2005). In particular, anions have been recognized to greatly reduce sorption and enhanced mobility of Cd and Pb in soils by complexation of Cl^- with these metals (Usman *et al.*, 2005; Ghallab and Usman, 2007). Salinity change the bioavailability of heavy metals such as Cd and Pb in soil, irrespective of soil pH (Weggler *et al.*, 2004) and it is found to be one of the key features in translocating metals from roots to foliar parts of the plants (Fitzgerald *et al.*, 2003; Manousaki *et al.*, 2009). Moreover, Sari and Din (2012) reported that physiological mechanisms in addition to metals speciation control the Cd and Pb uptake by *Avicennia alba* roots and further translocation to the stems and leaves in saline conditions. Worldwide rice is grown under many different diverse climatic, hydrological and edaphic conditions (Zayed *et al*., 2014) and sometimes critical problems in rice production occur due to a combination of salinity and heavy metal toxicity (Ahmad, 2007). Among heavy metals, Pb severely inhibited both plant growth and physiological processes of rice (Sharma and Dubey, 2005). In present study, there was also decrease in growth and physiological responses of both rice varieties observed, with application of Pb in both normal and salt-affected soils.

Table 1: Properties of soil used for pot experiment (mean ± standard deviation, n=3)

Parameter	Value
Textural class	Sandy loam
Sand (%)	69.70 ± 0.30
Silt (%)	14.10 ± 0.10
Clay (%)	16.20 ± 0.20
pH_s	7.62 ± 0.12
$pH_{1:2}$	8.11 ± 0.13
EC_e (dS m^{-1})	[a]1.11 ± 0.01 (6 ± 0.01)
TSS ($mmol_c$ L^{-1})	111 ± 0.58
SAR	[a]3.28 ± 0.12 (22 ± 0.12)
Saturation percentage (%)	29.34 ± 1.06
CEC ($cmol_c$ kg^{-1})	5.42 ± 0.12
Organic matter (%)	0.81 ± 0.04
$CaCO_3$ (%)	1.72 ± 0.06
AB-DTPA extractable Pb (mg kg^{-1})	2.91 ± 0.06
Total Pb (mg kg^{-1})	18.82 ± 0.74

[a]Initial EC_e, SAR of the soil while values in parentheses represent artificially made salt-affected soil as described by Muhammed and Ghafoor (1992)

Table 2: F-value of three-way ANOVA for the effect of Pb treatments on growth and physiological responses of two rice varieties in normal and salt-affected soils

Parameter	Soil types (S)	Rice varieties (V)	Pb treatments (T)	S × V	S × T	V × T	S × V × T
Plant height	8589.54**	16460.16**	2358.70**	2901.03**	3.78*	4.10*	3.05*
Straw dry matter	1143.16**	2678.91**	526.03**	87.59**	21.49**	32.06**	2.86*
Paddy yield	1943.88**	1436.79**	477.82**	205.36**	74.23**	3.40*	9.75**
Photosynthetic rate	4106.49**	186.54**	2516.73**	5.42*	186.03**	40.09**	8.36**
Transpiration rate	1188.13**	507.35**	418.39**	72.28**	25.56**	43.34**	16.85**
Stomatal conductance	227.59**	82.44**	329.38**	5.64*	10.50**	4.74*	3.00*
Pb concentration in straw	3043.94**	27935.99**	85971.81**	5.49*	78.51**	2373.17**	46.61**
Pb concentration in paddy	206.01**	642.50**	2723.65**	10.67**	19.36**	116.90**	5.41**
AB-DTPA extractable Pb in post-rice soil	1511.08**	638.89**	71320.66**	21.80**	118.62**	57.86**	4.97*

NS = Non-significant (P > 0.05); * = Significant (P ≤ 0.05); ** = Highly significant (P ≤ 0.01)

In the present study, a gradual decrease in plant height, straw dry matter and paddy yield (Fig. 1) was observed in both normal and salt-affected soils with increasing concentration of applied Pb (0, 50, 100 and 150 mg kg^{-1}). For both rice varieties, the maximum plant height, straw dry matter and paddy yield were observed in normal soil and the minimum values in salt-affected Pb-contaminated soil when Pb was applied at 150 mg kg^{-1} soil. This greater restriction of plant growth in salt-affected Pb-contaminated soil was attributed to synergistic (Table 2) adverse effects of both salinity and Pb toxicity. Both rice varieties differed in their potential to maintain growth in Pb-contaminated soils, with a greater reduction being observed in KS-282 compared to Shaheen basmati. Reduction in plant growth can be directly attributed to retardation of cell division by applied Pb, a result which supported by previous research. Liu *et al*. (2003) previously reported a significant decrease in straw and grain yields of rice cultivars in response to Pb stress, when studying Pb toxicity, uptake and translocation in different rice cultivars at varying levels of applied Pb in contaminated soil.

In present study, greater yield reduction in KS-282 compared to Shaheen basmati was attributed to cv. Shaheen basmati exhibiting previously observed ion selectivity mechanism of heavy metals tolerance when grown in a saline medium (Hussain *et al*., 2003; Abbas *et al*., 2007). Such tolerance of Pb and salinity by this variety can be due to inherent capacity and the presence of more tolerant genes to confer stress. Patra *et al.* (2004) described that plant species/genotypes showed differences in tolerance to heavy metal depending on number of metacentric or diploid chromosomes and total length of diploid complement. The other reason can be differential physiological association of tolerance mechanism and growth of plants (Kabir *et al.*, 2010). The Shaheen basmati showed better growth than KS-282 attributed to reduced Pb accumulation and possibly mobilization of the defense mechanisms including antioxidative enzymes such as superoxide dismutase, catalase, guaiacol peroxidase, ascorabte peroxidase and glutathione reductase (Pourrut *et al.*, 2011), which might suppressed the Pb transport to further tissues (Sekara *et al.*, 2005). It is also well known that plants exhibit differences in growth and yield due to variable rates of metal uptake and absorption amongst plant species and varieties (Hong *et al*., 2008).

It has been reported earlier that Pb phytotoxicity affected plant growth and several metabolic activities in different cell components including decreased seed germination, length and biomass of roots and shoots, disruption in mineral nutrition, reduction in cell division, inhibition of photosynthetic pigment contents (Sharma and Dubey, 2005) and enzymatic reactions, as well as several other physiological effects (Pourrut *et al.*, 2011). In present study, a substantial decrease in straw dry matter (Fig. 1) of both rice varieties by applied salinity and Pb can be attributed to production of reactive oxygen species (ROS) and resultant lipid peroxidation, disrupt membranes integrity and reduced A, E or g_s of rice plants and very low rate of leaf growth (Pourrut *et al.*, 2011). Furthermore, in consonance with present findings (Fig. 1), Fritioff *et al.* (2005) reported that biomass of two submersed plants (*Elodea canadensis* and *Potamogeton natans*) affected the metals (Cd, Cu, Pb and Zn) uptake under saline conditions; with less biomass plants having higher metal concentration than did high biomass plants. The differences among rice varieties for decreased paddy yield (Fig. 1) can be reasoned by the fact that Pb toxicity variably affected yield components particularly number of filled grains and filled spikelet percentage by causing sterility at anthers synthesis (Liu *et al.*, 2003).

In the present study, reduction of plant height, straw dry matter and paddy yield (Fig. 1) by Pb was greater in salt-affected Pb-contaminated soil compared to normal Pb-contaminated soil. In studies of the effect of sewage water, contaminated with trace elements, on the performance of rice varieties in saline soil, Abbas *et al.* (2007) reported that Shaheen basmati had a higher yield compared to others rice varieties because of its relatively better salt tolerance and potential yield. However, contemporary reports shown that KS-282 also proved to be high yielding with better growth under saline and Cd stressed conditions (Niaz *et al.*, 2010; Arshadullah *et al.*, 2011). It is generally believed that salinity affected heavy metal mobilization is related to the phyto-toxicity of metals (Acosta *et al*., 2011). For example, exposure of plants to Pb results in decreased root growth, inhibition of chlorophyll biosynthesis and a reduction in photosynthesis and respiration due to inhibition of electron transport mechanisms and enzymes synthesis as well as cell disturbances and chromosomal lesions (Patra *et al*., 2004; Sharma and Dubey, 2005) resulting in growth inhibition and plant damage. Accumulation of Pb reduced the uptake of nutrients and synergistically enhanced the detrimental effects of salinity to disrupt plant growth (Kadukova *et al*., 2008).

Decrease in physiological processes such as A, E and g_s (Fig. 2) were greater in rice plants grown in normal and salt-affected Pb-contaminated soils compared to plants grown in uncontaminated soils. However, for the same level of Pb contamination, the decrease in physiological parameters was greater in salt-affected soil compared to normal soil. Between the two used rice varieties, Shaheen basmati had higher A, E and g_s, whereas the lowest A, E and g_s was observed in both varieties at the highest applied Pb (150 mg kg^{-1}) in the presence of salinity. The decrease in A (Fig. 2) with salinity and Pb stress might have resulted from reduced synthesis of chlorophyll and plastoquinone, distorted chloroplast ultra-structure, blocked electron transport and CO_2 deficiency due to stomatal closure, all were occurring simultaneously (Sharma and Dubey, 2005; Ashraf and Harris, 2013). Similar, influences of Pb and salinity stresses on photosynthesis, chlorophyll contents and other physiological parameters were also reported by Manousaki *et al.* (2009) and Pourrut *et al.* (2011).

The highest decreases in A, E and g_s were found in salt-affected Pb-contaminated soils indicating a potential synergistic effect between Pb and salinity. The presence of salinity further aggravated the toxic effects of Pb. Moreover, salinity as a stressor causes a wide range of physiological changes such as disruption of membranes, diminished mineral nutrition, damages the ability to detoxify ROS, alterations in the antioxidant enzymes and reduced photosynthetic activity by decreasing g_s, E and biosynthesis of photosynthetic pigments (Gupta and Huang, 2014), all these modifications results in severe reduction in both dry biomass and crop yields. Kadukova *et al.* (2004) described that it is not easy to separate the harmful effects of salinity and Pb accumulation and believed that the synergistic combination of both these factors caused drying of *Atriplex halimus* plants. Similar to present study results, Khan and Abdullah (2003) also found that A, E and g_s were reduced in sensitive rice varieties grown in salt-affected soil, where the reduction of A in sensitive cultivars was mainly due to reduced g_s.

Concentration of essential and toxic elements in plant tissues is considered a good criterion for recognizing the genetic variability among cultivars (Tiryakioglu *et al.*, 2014). The toxic soil Pb levels to different plant species are not easy to estimate but mostly soil Pb concentration of 30–300 mg kg^{-1} considered excessive/toxic to plants (Kabata-Pendias and Pendias, 2001). According to WHO (2007), the maximum Pb permissible concentration in rice grain is 0.2 mg kg^{-1}. Codling (2009) pointed that a higher Pb concentration in rice straw indirectly also become risk to human health because of common usage for livestock feed and bedding. In the present study, the concentration of Pb in rice straw and paddy (Fig. 3) of both rice varieties increased with applied Pb in both normal and salt-affected soils. For the same level of contamination, the concentration of Pb in rice straw and paddy was higher in salt-affected soil compared to normal soil. Regardless of the level of applied Pb, the Pb concentration in rice straw and paddy were consistently higher in KS-282 compared to Shaheen basmati. This can be due to the reason that KS-282 with higher concentration of low-molecular-weight organic acids in soil and stronger root oxidation abilities accumulated more Pb in plant parts (Liu *et al.*, 2009). Shaheen basmati might have adopted avoidance mechanism for reduce metal uptake by chelate secretion or deposition in cell wall components (Meharg, 2005). Yang *et al.* (2000) found that tolerant rice varieties regulate oxalate synthesis and secretion that precipitates Pb; thus decreasing its uptake by plant roots. Previous studies have also shown that concentration of Pb in rice plants grown on Pb-contaminated soil was positively correlated with the total soil Pb concentration (Yang *et al*., 2004) and that the concentration of Pb in shoots of *Atriplex halimus* increased with the level of applied Pb in a growth medium in the presence of NaCl (Kadukova *et al*., 2004).

Soil salinity also had a significant role in increasing Pb concentration in the shoot of *Aster tripolium* and *Plantago maritime* (Fitzgerald *et al*., 2003) by influencing the translocation process in plant parts due to a direct gradient in metal concentration from rhizosphere to roots and further to the aerial parts of plant. Sari and Din (2012) proposed that the greater mobility of metal under saline conditions can be due to higher water uptake via increased transpiration which resulted in higher metal flux in plants. In present rice flooded conditions, pH of soil might shifted towards neutral or decreased with changed redox potential (Zheng and Zhang, 2011) and consequently with increasing accessibility of Pb, rice plants accumulated significant amounts of Pb in concentration-dependent manners (Gupta *et al*., 2009). However, soil pH was reported to be unaffected by the salt treatments (Khoshgoftar *et al*., 2004; Weggler *et al*., 2004). Acosta *et al*. (2011) reported that the soil salinity resulted in enhanced heavy metal mobilization. This can be due to the reasons that heavy metals complex formation occurred with highly mobile inorganic ligands such as Cl^- in soil solution (Usman *et al*., 2005). The subsequent association has a lesser positive charge than the free metal ions, and may be uncharged or carry a net negative charge, thereby has higher metal mobility (McLean and Bledsoe, 1992). Similarly, Ghallab and Usman (2007) also reported that salinity induced metal–chloride complexes resulted in decreasing positive charge and conveying metals from solid to solution phase. Recently, Usman (2014) proposed that Na^+ associated with salinity can also contribute to boost metal solubility via ion exchange of Na^+ for metal on soil exchange sites. Connell (2005) described that increasing salt concentration make more competition between cations and metals for binding sites of soil. Therefore, metals will be driven off by mass action forces into the soil solution.

According to Acosta *et al.* (2011) and Sari and Din (2012), one of the main mechanisms known to regulate Pb mobility is the formation of Pb-chloro-complexes. The presence of salts can influence the uptake and accretion of heavy metals in plants either by modifying the root functions or by increasing the mobility of metals (Zurayk *et al*., 2001). The mechanisms related to metal uptake by roots, which can be altered by salinity involving the negative charges on roots cell wall that can trap more Pb (Pourrut *et al.* 2011), the role of senescing roots as sites for metal influx into plants (Zurayk *et al.*, 2001), release of root exudates (Liu *et al.*, 2009), root surface area, root-microbial interactions, mycorrhizal associations (Sharma and Dubey, 2005), length and configuration of root system, soil rooting density, passive permeability of root membranes, binding and dynamics of metals in root apoplast and xylem, synthesis and turnover of organic carriers, transpiration and growth rate, and sink activity of shoot (Helal *et al*., 1998). Sari and Din (2012) recently recognized that higher heavy metal accumulation under saline conditions was related to the maintenance of cell osmosis mechanisms by plants.

Usman *et al*. (2005) found that increased concentration of Pb in wheat shoot in the presence of salinity were the result of increased Pb mobility and availability due to the formation of Pb-Cl complexes. Under saline conditions, the Cl^- more considerably increased the mobility of Pb than SO_4^{2-} due to the formation of soluble ion pairs like $PbCl^+$ (Acosta *et al.*, 2011). The Cd and Pb speciation in soil solution reported to be altered, added Na^+ compete with other cations for sorption sites, extra Cl^- form complex with Cd and Pb, and ionic strength increased which affects activity coefficients along with the degree of preference of divalent over monovalent cations by exchange complex (Khoshgoftar *et al.*, 2004; Usman *et al.*, 2005). The metals-chloro complexes as dominant species resulted in increased diffusion of Cd and Pb via soil solution to plant roots or greater uptake of Cd and Pb if metal–chloride complexes were transported through the root membrane (Usman *et al.*, 2005). Khamis *et al.* (2014) also described that with increasing soluble forms of Pb in solution, plant roots were able to take up more amounts of Pb; consequently toxic accumulation of Pb cause inhibitory impacts on plant metabolic activities.

Several studies have already shown that increasing salinity increased the metals mobility (Hatje *et al*., 2003; Pedron *et al*., 2009). In addition, increased solubility of Pb in soil will result in enhanced bioavailability and hence increased uptake of Pb by plants. In this study, applied Pb significantly increased AB-DTPA extractable Pb in post-rice soils, being maximum in Pb-contaminated salt-affected soil and minimum in uncontaminated normal soil (Fig. 3). Similar trends were reported for AB-DTPA Pb extractability in post-rice and wheat soil both in normal and salt-affected soils (Ahmad, 2007; Ahmad *et al*., 2011). In general, the total acid and AB-DTPA extractable Pb of 50 and 13 mg kg^{-1} respectively, were reported to critical levels of Pb in soils with respect to human health (Rowell, 1994). Such a high magnitude of Pb in present results attributed to exogenous application of Pb decreased soil pH which further increased Pb bioavailability in soil, therefore causing increased Pb accretion in the edible parts of rice (Li *et al*., 2007).

Conclusion

Decrease in rice growth, yield and physiological attributes were greater in salt-affected Pb-contaminated soil compared to normal Pb-contaminated soil. Plant height, straw and paddy yields, photosynthetic and transpiration rates, stomatal conductance decreased, and Pb concentration in straw and paddy of both rice varieties and AB-DTPA-extractable-Pb in post-rice soil increased with soil applied Pb in both normal and salt-affected soils. Increased soil salinization resulted in increased mobility of Pb in soils and plants; which indicates that increased salinity has the potential to become a significant adverse phenomenon for safe crop production. At all levels of applied Pb in both normal and salt-affected soils, cv. Shaheen basmati performed better than cv. KS-282 in terms of growth, yield, physiological attributes and lower Pb concentration in straw and paddy. Therefore, for safe rice production Shaheen basmati should be cultivated in saline environments and used in advanced rice breeding programs to increase salinity and Pb tolerance.

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