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Original Research Article

Uptake, Translocation of Pb and Chlorophyll Contents of *Oryza Sativa* as Influenced by Soil-Applied Amendments under Normal and Salt-Affected Pb-Spiked Soil Conditions

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Abstract

Heavy metal contamination of the soil environment has become a major source of concern and has posed serious human health related problems in many developing countries particularly Pakistan. Chemical immobilization of heavy metals can be accomplished by the addition of amendments to reduce contaminant solubility and ultimately uptake by the plants. However, a very scarce information is available on the immobilization of Pb with the application of different Ca, S and P sources (gypsum i.e., gyp, rock phosphate i.e., RP and Di-ammonium phosphate i.e., DAP) on rice grown normal and salt-affected Pb-spiked soils. Therefore, a pot trial was conducted to investigate the uptake, translocation of Pb and chlorophyll contents of rice as influenced by soil applied amendments (gyp, RP and DAP) and their variable amounts in normal and salt-affected Pb-spiked soils. The results showed that the Pb and salinity stress induced decrease in chlorophyll contents of rice were significantly ($p \leq 0.05$) counteracted by the applied gyp, RP and DAP. Application of 7.5 g gyp kg^{-1} soil was found the most effective in improving chlorophyll contents, and reducing Pb uptake and translocation both in normal and salt-affected Pb-spiked soils.

Keywords: Rice, lead accumulation, transport, photosynthetic pigments, amendments, saline Pb stressed soil.

Introduction

During the past few years, the rapid industrialization and urbanization have resulted in serious complications of environmental pollution. The natural ecological system has been severely affected due to rise in heavy metals status in soils (Meenakshi et al., 2006). The toxic metals marked a substantial impact on the environmental pollution due to increased human activities including mining and smelting operations, electroplating and energy processes, fuel production from fossils, power transmission setups, intensification of agriculture, sewage sludge dumping operations and military actions (Britto et al., 2011, Rizwan et al., 2016). The presence of heavy metals is a menace for flora and fauna and ultimately to humans. Among heavy metals, lead (Pb) is one of the most abundant toxic elements that cause serious concerns to human health. It affects growth and metabolism of plants, also having visible symptoms such as stunted growth and lesser leaves, as well as membrane disorder and decrease in photosynthetic rate (Sharma and Dubey, 2005). Moreover, inhibition of chlorophyll synthesis is one of the most Pb-sensitive plant physiological feature (Li et al., 2012).

Rice (*Oryza sativa* L.) is the most dominant staple food in the world. According to an estimate, rice denotes 30% of the global cereal food production and will be needed for 4.6 billion people for their daily nutrition in 2025 (Gnanamanickam, 2009). In Pakistan, rice is grown on an area of 2.89 mha and its total production is 6.79 mt by average yield of 2423 kg ha^{-1} (GoP, 2015). Despite higher yield potentials, the average yield of rice in Pakistan is lower than that of other rice-growing countries of the world. Rice varieties of Pakistan are found susceptible to different environmental stresses including soil salinity and toxicity of heavy metals that are responsible for decreasing the rice yield and quality in Pakistan (Ahmad, 2007). A soil containing an excess amount of soluble salts and/or exchangeable Na^+ affect most of the crops adversely (Ghafoor et al., 2004). Rice was marked sensitive to salinity and its sensitivity to salinity differs with stages of growth and development (Maas and Grattan, 1999).

The continuous use of city and industrial effluents is creating soil salinization, sodication and builds up of toxic metals in the surface soils (Murtaza et al., 2008; Abd-Elrahman et al., 2012). Due to high electrical conductivity (EC), sodium adsorption ratio (SAR) and residual sodium carbonate (RSC), the raw effluent has been established unfit for irrigation purposes (Murtaza et al., 2012). The concentration of Pb and other heavy metals in raw sewage (Iqbal et al., 2011) were found greater than permissible heavy metals limits for irrigation water (Iqbal et al., 2015). In case of corn plants, accumulation of Pb in shoots increased in response to high NaCl salinity compared to those grown in low salinity soil (Izzo et al., 1991). Moreover, the Pb uptake and transport in plants depend on the type of soil and plant species. Kabata-Pendias and Pendias (2001) reported that in salt-affected soils, heavy metals uptake by plants might raise or decline conditional to the type of plant, salinity/sodicity, magnitude of metal ion and other environmental circumstances.

A number of strategies can be used to decrease the soil-plant transfer of Pb to produce crops with the lowest possible Pb uptake especially in edible parts. To decrease the solubility and bioavailability of Pb, chemical immobilization is also one of the promising techniques. The right selection of an amendment at a particular place is subjected to its comparative efficacy as judged from the enhancement of soil characteristics and plant growth, physiological processes, its availability, cost, management and application complications, and period vital to react in the soil (Ghafoor et al., 2004).

Gypsum is used in agriculture as a fertilizer as well as a soil amendment because of having essential plant nutrients (Ca and S) and an inexpensive amendment for reclamation of saline-sodic soils/waters, and for enhancing crop growth and yields (Murtaza et al., 2009). Gypsum dissolution releases its component ions (Ca^{2+} and SO_4^{2-}) into soil solution (Lottermoser, 2007) and consequently, new reactions take place. Sulfates rapidly counter with Pb which results in the formation of greatly insoluble anglesite like minerals (Garrido et al., 2005). Antosiewicz (2005) reported that Ca-regulated Pb deposition in cell walls of plants. It was found that phosphate which is accessible to the roots of crop plants might also be available to toxic heavy metals, conclusively insoluble metal phosphates form. The RP as a primary P source found very operative in diminishing Pb in the soil solution, transferable fraction of contaminated soils (Ma et al., 1995). The soluble fertilizers like DAP not only decrease the metal toxicity in plants but also as an excellent source of nutrients as well to increase the biomass production, which indirectly decreased the metal toxicity (Khan and Jones, 2008, Rehman et al. 2015; Arshad et al., 2016).

Keeping in view the present high costs of fertilizers and amendments detailed investigations are required for the minimum uptake, translocation of Pb and chlorophyll contents of rice as affected by applied amendments with their most appropriate amounts, both in normal and salt-affected Pb-spiked soils.

Materials and Methods

The present pot trial was conducted in wirehouse (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 (UAF). The soil was taken from 0-20 cm depth from the Farms of UAF. Soil was air-dried, minced with wooden roller, passed through 2 mm sieve, systematically mixed and stored. The soil samples were analyzed for physico-chemical properties. The characters of soil used for present study are described in Table 1.

There were ten treatments arranged in completely randomized design each with three replications. The treatments used were as T_1 = Control (without applied Pb), T_2 = 100 mg Pb kg^{-1} soil, T_3 = 100 mg Pb kg^{-1} soil + 2.5 g gyp kg^{-1} soil, T_4 = 100 mg Pb kg^{-1} soil + 5 g gyp kg^{-1} soil, T_5 = 100 mg Pb kg^{-1} soil + 7.5 g gyp kg^{-1} soil, T_6 = 100 mg Pb kg^{-1} soil + 1.5 g RP kg^{-1} soil, T_7 = 100 mg Pb kg^{-1} soil + 3 g RP kg^{-1} soil, T_8 = 100 mg Pb kg^{-1} soil + 4.5 g RP kg^{-1} soil, T_9 = 100 mg Pb kg^{-1} soil + 115 mg DAP kg^{-1} soil, T_{10} = 100 mg Pb kg^{-1} soil + 130 mg DAP kg^{-1} soil.

Similar treatments were investigated in spiked salt-affected soil with EC: SAR = 6 dS m^{-1} : 22 $(mmol\ L^{-1})^{1/2}$. The required amounts of salts were applied by calculating through quadratic equation i.e., NaCl = 0.36, Na_2SO_4 = 0.53, $CaCl_2$ = 0.14 and $MgSO_4$ = 0.04, g kg^{-1} soil (Muhammed and Ghafoor, 1992, Iqbal et al., 2015). Both the normal and salt-affected soils were spiked at 100 mg Pb kg^{-1} soil using $Pb(NO_3)_2$ salt. White glazed ceramic pots containing 12 kg processed soil per pot (total 60 pots), following prescribed treatments layout were used.

Healthy seeds of rice were taken from Rice Research Institute, Kala Shah Kaku, Sheikhupura and then grown in polythene lined trays containing sand. Yoshida nutrient solution was applied to germinate the rice nursery (Yoshida et al., 1976). The twenty-eight days old rice seedlings were transplanted with three seedlings per hill and five hills per pot (Iqbal et al., 2015). Rice crop was fertilized at 80-50-38.5-7.5 mg NPKZn kg^{-1} soil using urea, DAP, sulfate of potash (SOP) and $ZnSO_4.7H_2O$, respectively. The pots were submerged with pumped groundwater (Table 2) upto 2-3 cm throughout the crop growth.

Later, sixty days of rice nursery transplantation, total chlorophyll content (TCC) of rice shoots in expressions of SPAD (Special Products Analysis Division, a division of Minolta) value was determined via a portable SPAD-502 meter (Minolta, Osaka, Japan). It is a low-cost mode to quantify plant photosynthetic capacity than expensive chlorophyll fluorescence (Munns et al., 2006). The TCC were determined from the leaf tip to the leaf base and then averaged following Saqib et al. (2012).

At harvest, the data about total biomass, plant height, paddy and straw yields was recorded. The concentration of Pb was determined from straw, paddy and post-experiment soil samples via atomic absorption spectrophotometer.

The translocation factor (TF) of Pb in rice was determined by using the ratio of paddy-Pb concentration to straw-Pb concentration (Majid et al., 2012). The Pb uptake by rice straw or paddy was calculated via Pb concentration in rice straw or paddy × straw dry matter or paddy yield / 1000 (Hadi and Bano, 2010).

The statistical analysis was performed via analysis of variance technique (ANOVA), and the least significant difference (LSD) test was functioned to evaluate the effectiveness of the treatments (Steel et al., 1997) at 5% significance level using "Statistix 8.1" statistical computer-based software package.

Results

Total chlorophyll contents

In the current experiment, the treatments, soil-type and their interaction significantly ($p \leq 0.05$, Table 7) affected the total chlorophyll contents (TCC) of rice. The treatment effectiveness on mean TCC of rice were in the decreasing order of $T_5 > T_4 > T_3 > T_1 > T_{10} > T_9 > T_8 > T_7 > T_6 > T_2$ (Table 3). The mean TCC of rice were found higher in normal soil than salt-affected soil.

In control, TCC were 57.7 and 34.9 SPAD-value, in normal and salt-affected soils, respectively. At T_2, the TCC were 37.4 and 29.5 SPAD-value, respectively. Amendments and their increasing amounts gradually improved TCC in both normal and salt-affected Pb-spiked soils.

In normal Pb-spiked soil, application of gyp resulted in significant enhancement of TCC and these were found as 47.8, 48.5 and 52.6 SPAD-value with T_3, T_4 and T_5, respectively. Therefore, TCC were increased by 29.6, 27.7 and 40.7 % at T_3, T_4 and T_5, respectively over T_2. Moreover, the TCC were greater by 5.6, 10.5 and 15.9 % at T_6, T_7 and T_8, respectively over T_2. However, the TCC were enhanced by 17.5 and 22.6 % at T_9 and T_{10} respectively, over T_2.

In salt-affected Pb-spiked soil, TCC were found as 49.1, 51.1, and 54.5 SPAD-value with T_3, T_4 and T_5, respectively. Therefore, TCC were increased by 66.5, 73.3 and 85.0 % at T_3, T_4 and T_5, respectively over T_2. Furthermore, the TCC were greater by 16.0, 22.8 and 30.2 % at T_6, T_7 and T_8, respectively over T_2.

Moreover, the TCC were enhanced by 41.0 and 50.0 % at T_9 and T_{10}, respectively over T_2.

Pb uptake by rice straw

In the present study, a significant ($p \leq 0.05$, Table 7) effect of treatments, soil-type and their interactive effects was found for Pb uptake by rice straw. The treatment effectiveness on mean Pb uptake by rice straw was in the decreasing order of $T_2 > T_6 > T_7 > T_8 > T_9 > T_{10} > T_3 > T_4 > T_5 > T_1$ (Table 4). The mean Pb uptake by rice straw was found higher in salt-affected soil than normal soil.

In control, Pb uptake by rice straw was 0.320 and 0.242 mg pot^{-1} DM in normal and salt-affected soils respectively. At T_2, Pb uptake by rice straw was 1.002 and 0.791 mg pot^{-1} DM in normal and salt-affected soils respectively. The gyp, RP and DAP with their increasing amounts gradually decreased Pb uptake by rice straw in both normal and salt-affected Pb-spiked soils.

In normal Pb-spiked soil, applied gyp resulted in significant decreased of Pb uptake by rice straw, and it was found as 0.565, 0.471 and 0.393 mg pot^{-1} DM with T_3, T_4 and T_5 respectively. Therefore, Pb uptake was decreased by 43.5, 53.0 and 60.7 % at T3, T_4 and T_5 respectively over T_2. However, the Pb uptake by rice straw was reduced by 13.2, 15.2 and 23.1 % with T_6, T_7 and T_8 respectively over T_2. Moreover, the Pb uptake by rice straw was declined by 29.1 and 42.4 % with T_9 and T_{10}, respectively over T_2.

In salt-affected Pb-spiked soil, Pb uptake by rice straw was found as 0.725, 0.624 and 0.585 mg pot^{-1} DM with T_3, T_4 and T_5, respectively. Therefore, Pb uptake was decreased by 8.3, 21.1 and 26.0 % at T_3, T_4 and T_5 respectively over T_2. Furthermore, the Pb uptake by rice straw was reduced by 1.0, 1.2 and 1.5 % at T_6, T_7 and T_8 respectively over T_2. However, the Pb uptake by rice straw was declined by 1.8 and 8.3 % at T_9 and T_{10} respectively over T_2.

Pb uptake by rice paddy

A significant ($p \leq 0.05$, Table 7) effect of treatments was noted for Pb uptake by rice paddy. The treatment effectiveness on mean Pb uptake by rice paddy was in the decreasing order of $T_2 > T_7 > T_6 > T_8 > T_9 > T_{10} > T_3 > T_4 > T_5 > T_1$ (Table 5). The mean Pb uptake by rice paddy was found higher in salt-affected soil than normal soil.

In control, Pb uptake by rice paddy was 0.009 and 0.011 mg pot^{-1} DM in both normal and salt-affected soils respectively. At T_2, Pb uptake by rice paddy was 0.095 and 0.137 mg pot^{-1} DM in normal and salt-affected soils respectively. Gyp, RP and DAP with their increasing amounts gradually decreased Pb uptake by rice paddy in both normal and salt-affected Pb-spiked soils.

In normal Pb-spiked soil, applied gyp resulted in significant decreased of Pb uptake by rice paddy and it was found as 0.085, 0.057 and 0.041 mg pot^{-1} DM with T_3, T_4 and T_5, respectively. Therefore, Pb uptake was decreased by 10.4, 40.5 and 56.2 % at T_3, T_4 and T_5 respectively over T_2. Moreover, the Pb uptake by rice paddy was declined by 8.0, 8.3 and 14.6 % with T_6, T_7 and T_8 respectively over T_2. However, the Pb uptake by rice paddy was reduced by 1.8 and 11.8 % with T_9 and T_{10} respectively over T_2.

In salt-affected Pb-spiked soil, Pb uptake by rice paddy was found as 0.076, 0.056 and 0.036 mg pot^{-1} DM with T_3, T_4 and T_5 respectively. Therefore, Pb uptake decreased by 44.5, 59.1 and 73.4 % at T_3, T_4 and T_5 respectively over T_2. Furthermore, the Pb uptake by rice paddy was declined by 15.5, 14.8 and 19.7 % at T_6, T_7 and T_8 respectively over T_2. Likewise, the Pb uptake by rice paddy was reduced by 27.0 and 35.2 % at T_9 and T_{10} respectively over T_2.

Translocation factor of Pb

For Pb translocation factor (TF) from rice shoot to paddy, a significant ($p \leq 0.05$, Table 7) effect of treatments, soil-type and their interaction was found. The treatment effectiveness on mean TF was in the decreasing order of $T_2 > T_6 > T_7 > T_8 > T_9 > T_{10} > T_3 > T_4 > T_5 > T_1$ (Table 6). The mean Pb TF was found higher in salt-affected soil than normal soil.

In control, Pb TF was 0.050 and 0.057 in normal and salt-affected soils respectively. At T_2, TF was 0.238 and 0.244 in normal and salt-affected soils respectively. The gyp, RP and DAP with their increasing amounts gradually decreased Pb TF in normal and salt-affected Pb-spiked soils.

In normal Pb-spiked soil, applied gyp resulted in significant decreased of Pb TF and it was found as 0.157, 0.120 and 0.061 with T_3, T_4 and T_5, respectively. Therefore, Pb TF was decreased by 34.0, 49.4 and 74.3 % at T_3, T_4 and T_5 respectively over T2. Moreover, the Pb TF was declined by 9.6, 16.8 and 19.7 % with T_6, T_7 and T_8 respectively over T_2. Nevertheless, the Pb TF was reduced by 22.6 and 24.9 % with T_9 and T_{10} respectively over T_2.

In salt-affected Pb-spiked soil, Pb TF was found as 0.162, 0.124 and 0.090 with T_3, T_4 and T_5 respectively. Therefore, Pb TF was decreased by 33.6, 49.3 and 63.1

% at T_3, T_4 and T_5 respectively over T_2. Likewise, the Pb TF was declined by 8.7, 17.2 and 19.6 % at T_6, T_7 and T_8 respectively over T_2. Similarly, the Pb TF was reduced by 20.9 and 22.9 % at T_9 and T_{10} respectively over T_2.

Table 1: Physico-chemical characteristics of soil used for pot trial

Parameter	Value
Textural class	Sandy loam
Sand (%)	69.20
Silt (%)	14.50
Clay (%)	16.30
pH_s	7.66
EC_e (dS m^{-1})	[a]1.11 (6)
TSS ($mmol_c$ L^{-1})	111
SAR (mmol $L^{-1})^{1/2}$	[a]3.28 (22)
Saturation percentage (%)	29.36
CEC ($cmol_c$ kg^{-1})	5.40
Organic matter (%)	0.83
$CaCO_3$ (%)	1.74
AB-DTPA extractable Pb (mg kg^{-1})	2.95
Total Pb (mg kg^{-1})	18.90

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

Table 2: Composition of pumped ground water used for irrigation

Parameter	Value
pH	7.75
EC (dS m^{-1})	0.67
TSS ($mmol_c$ L^{-1})	6.70
CO_3^{2-}	Absent
HCO_3^- ($mmol_c$ L^{-1})	3.1
Cl^- ($mmol_c$ L^{-1})	2.60
SO_4^{2-} ($mmol_c$ L^{-1})	0.10
Ca^{2+} + Mg^{2+} ($mmol_c$ L^{-1})	4.73
Na^+ ($mmol_c$ L^{-1})	1.97
RSC	Nil
SAR (mmol $L^{-1})^{1/2}$	1.28
Pb (mg L^{-1})	Traces

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

Treatment	Normal Soil	Salt-Affected Soil	Mean
T_1 = Control	57.7 a	34.9 mn	46.3 C
T_2 = 100 mg Pb kg^{-1} soil	37.4 klm	29.5 o	33.4 H
T_3 = 100 mg Pb kg^{-1} soil + 2.5 g gyp kg^{-1} soil	47.8 ef (27.7)	49.1 de (66.5)	48.4 B (47.1)
T_4 = 100 mg Pb kg^{-1} soil + 5 g gyp kg^{-1} soil	48.5 def (29.6)	51.1 cd (73.3)	49.8 B (51.4)
T_5 = 100 mg Pb kg^{-1} soil + 7.5 g gyp kg^{-1} soil	52.6 bc (40.7)	54.5 b (85.0)	53.6 A (62.8)
T_6 = 100 mg Pb kg^{-1} soil + 1.5 g RP kg^{-1} soil	39.5 jk (5.6)	34.2 n (16.0)	36.8 G (10.8)
T_7 = 100 mg Pb kg^{-1} soil + 3 g RP kg^{-1} soil	41.3 ij (10.5)	36.2 lmn (22.8)	38.8 F (16.6)
T_8 = 100 mg Pb kg^{-1} soil + 4.5 g RP kg^{-1} soil	43.4 ghi (15.9)	38.4 kl (30.2)	40.9 E (23.1)
T_9 = 100 mg Pb kg^{-1} soil + 115 mg DAP kg^{-1} soil	44.0 ghi (17.5)	41.6 hij (41.0)	42.8 D (29.3)
T_{10} = 100 mg Pb kg^{-1} soil + 130 mg DAP kg^{-1} soil	45.9 fg (22.6)	44.2 gh (50.0)	45.0 C (36.3)
LSD	2.8		1.7
Mean	45.8 A	41.4 B	
LSD	0.47		

Values in parenthesis are percent increase (+) or decrease (-) over that 100 mg Pb kg^{-1} soil (T_2) treatment. Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 3).
Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: **Effect of applied amendments on Pb uptake (mg pot^{-1} DM) by rice straw**

Treatment	Normal Soil	Salt-Affected Soil	Mean
T_1 = Control	0.320 jk	0.242 k	0.281 F
T_2 = 100 mg Pb kg^{-1} soil	1.002 a	0.791 bcd	0.896 A
T_3 = 100 mg Pb kg^{-1} soil + 2.5 g gyp kg^{-1} soil	0.565 gh (-43.5)	0.725 cde (-8.3)	0.645 D (-25.9)
T_4 = 100 mg Pb kg^{-1} soil + 5 g gyp kg^{-1} soil	0.471 hi (-53.0)	0.624 efg (-21.1)	0.547 E (-37.0)
T_5 = 100 mg Pb kg^{-1} soil + 7.5 g gyp kg^{-1} soil	0.393 ij (-60.7)	0.585 fgh (-26.0)	0.489 E (-43.4)
T_6 = 100 mg Pb kg^{-1} soil + 1.5 g RP kg^{-1} soil	0.869 b (-13.2)	0.783 bcd (-1.0)	0.826 AB (-7.1)
T_7 = 100 mg Pb kg^{-1} soil + 3 g RP kg^{-1} soil	0.849 bc (-15.2)	0.781 bcd (-1.2)	0.815 AB (-8.2)
T_8 = 100 mg Pb kg^{-1} soil + 4.5 g RP kg^{-1} soil	0.770 bcd (-23.1)	0.779 bcd (-1.5)	0.774 BC (-12.3)
T_9 = 100 mg Pb kg^{-1} soil + 115 mg DAP kg^{-1} soil	0.710 def (-29.1)	0.776 bcd (-1.8)	0.743 C (-15.5)
T_{10} = 100 mg Pb kg^{-1} soil + 130 mg DAP kg^{-1} soil	0.576 gh (-42.4)	0.721 de (-8.3)	0.648 D (-25.6)
LSD	0.13		0.08
Mean	0.650 B	0.68 A	
LSD	0.02		

Table 5: **Effect of applied amendments on Pb uptake (mg pot^{-1} DM) by rice paddy**

Treatment	Normal Soil	Salt-Affected Soil	Mean
T_1 = Control	0.009 f	0.011 f	0.011 E
T_2 = 100 mg Pb kg^{-1} soil	0.095 abc (86.2)	0.137 a (108.8)	0.116 A (97.5)
T_3 = 100 mg Pb kg^{-1} soil + 2.5 g gyp kg^{-1} soil	0.085 bcde (-10.4)	0.076 bcde (-44.5)	0.081 BC (-27.5)
T_4 = 100 mg Pb kg^{-1} soil + 5 g gyp kg^{-1} soil	0.057 cde (-40.5)	0.056 cdef (-59.1)	0.057 CD (-49.8)
T_5 = 100 mg Pb kg^{-1} soil + 7.5 g gyp kg^{-1} soil	0.041 def (-56.2)	0.036 ef (-73.4)	0.039 DE (-64.8)
T_6 = 100 mg Pb kg^{-1} soil + 1.5 g RP kg^{-1} soil	0.088 bcd (-8.0)	0.116 ab (-15.5)	0.101 AB (-11.8)
T_7 = 100 mg Pb kg^{-1} soil + 3 g RP kg^{-1} soil	0.087 abcd (-8.3)	0.117 ab (-14.8)	0.102 AB (-11.6)
T_8 = 100 mg Pb kg^{-1} soil + 4.5 g RP kg^{-1} soil	0.081 bcde (-14.6)	0.111 ab (-19.7)	0.097 AB (-14.4)
T_9 = 100 mg Pb kg^{-1} soil + 115 mg DAP kg^{-1} soil	0.093 abc (-1.8)	0.100 abc (-27.0)	0.096 AB (-17.2)
T_{10} = 100 mg Pb kg^{-1} soil + 130 mg DAP kg^{-1} soil	0.084 bcde (-11.8)	0.088 abcd (-35.2)	0.086 A-C (-23.5)
LSD	0.05		0.03
Mean	0.072 B	0.085 A	
LSD	0.0008		

Values in parenthesis are percent increase (+) or decrease (-) over that 100 mg Pb kg^{-1} soil (T_2) treatment. Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 3).
Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 6: Effect of applied amendments on translocation factor of Pb from rice shoot to paddy

Treatment	Normal Soil	Salt-Affected Soil	Mean
T_1 = Control	0.050 k	0.057 k	0.054 I
T_2 = 100 mg Pb kg^{-1} soil	0.238 ab	0.244 a	0.241 A
T_3 = 100 mg Pb kg^{-1} soil + 2.5 g gyp kg^{-1} soil	0.157 h (-34.0)	0.162 k (-33.6)	0.160 F (-33.8)
T_4 = 100 mg Pb kg^{-1} soil + 5 g gyp kg^{-1} soil	0.120 i (-49.4)	0.124 i (-49.3)	0.122 G (-49.3)
T_5 = 100 mg Pb kg^{-1} soil + 7.5 g gyp kg^{-1} soil	0.061 k (-74.3)	0.090 j (-63.1)	0.076 H (-68.7)
T_6 = 100 mg Pb kg^{-1} soil + 1.5 g RP kg^{-1} soil	0.215 cd (-9.6)	0.223 bc (-8.7)	0.219 B (-9.2)
T_7 = 100 mg Pb kg^{-1} soil + 3 g RP kg^{-1} soil	0.198 ef (-16.8)	0.202 de (-17.2)	0.200 C (-17.0)
T_8 = 100 mg Pb kg^{-1} soil + 4.5 g RP kg^{-1} soil	0.191 ef (-19.7)	0.196 ef (-19.6)	0.194 CD (-19.7)
T_9 = 100 mg Pb kg^{-1} soil + 115 mg DAP kg^{-1} soil	0.184 fg (-22.6)	0.193 ef (-20.9)	0.189 DE (-21.8)
T_{10} = 100 mg Pb kg^{-1} soil + 130 mg DAP kg^{-1} soil	0.179 g (-24.9)	0.188 fg (-22.9)	0.183 E (-23.9)
LSD	0.02		0.0009
Mean	0.159 B	0.168 A	
LSD	0.0002		

Values in parenthesis are percent increase (+) or decrease (-) over that 100 mg Pb kg^{-1} soil (T_2) treatment. Means sharing dissimilar letter in a row or in a column are statistically significant ($p \leq 0.05$, n = 3).
Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 7: Mean squares of various rice traits as influenced by soil applied amendments in normal and salt-affected Pb-spiked soil conditions.

SOV	df	Total chlorophyll contents (TCC)	Pb uptake by rice straw	Pb uptake by rice paddy	Translocation factor (TF) of Pb from rice shoot to paddy
Soils	1	295.0**	0.01**	0.002**	0.001**
Treatments	9	234.1**	0.21**	0.006**	0.022**
Soils× Treatments	9	81.4**	0.02**	0.0005**	0.00008**
Error	40	0.8	0.001	0.0002	0.00002

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

Discussion

Chlorophyll content is frequently determined in crop plants owing to evaluate the effect of abiotic and biotic stresses, as deviations in pigment content are interconnected to visual symptoms of plant disorders and photosynthetic efficiency (Purnama et al., 2015). In the present study, the total chlorophyll contents (TCC) of rice was decreased by both Pb and salinity stress (Table 3). The reduction in TCC of rice was more pronounced in salt-affected soil than normal Pb-spiked soil. The application of amendments significantly ($p \leq 0.05$, Table 7) countered the toxic effects of Pb on TCC of rice both in normal and salt-affected soils. The application of gypsum was more effective followed by DAP in alleviating the harmful effects of Pb and salinity on TCC.

Previously, the Pb-influenced decrease in chlorophyll contents of rice (Li et al., 2012) and wheat (Bhatti et al., 2013) were also reported. Sharma and Dubey (2005) described more degradation of chlorophyll owing to increased chlorophyllase activity in Pb-treated plants. Ernst (1998) recognized that the inhibition of chlorophyll synthesis persuaded by Pb stress was often manifested as chlorosis. Similarly, Ewais (1997) reported that Pb decreased growth and chlorophyll contents in three weed *Chenopodium ambrosioides*, *Digitaria sanguinolis* and *Cyperus difformis*. The Pb inhibited synthesis of chlorophyll by affecting the uptake of essential nutrients by plants (Iqbal et al., 2017a). The Pb induced reduction in TCC can be due to the inhibition of the enzymes activities responsible for the synthesis of chlorophyll. The Pb stress hinders the growth of the plant and even causes the death of plant by disturbing the uptake of Mg and Fe, and thereby decreasing the photosynthesis via degradation of chlorophyll (Pourrut et al., 2011).

A significant effect of salinity/sodicity on chlorophyll concentration in tested rice cultivars being higher decrease in salt sensitive cultivars than tolerant once were reported by Khan and Abdullah (2003). Ali et al. (2004) reported that the synthesis of photosynthetic pigments was severely affected by soil salinity. The decrease in chlorophyll concentrations was attributed to the inhibitory effects of the accumulated ions of different salts on the synthesis of the diverse chlorophyll elements. In chloroplast, the salts disturb the ability of the forces interconnected the multifarious pigment protein liquid. Ali et al. (2004) described that membrane-bound chloroplast stability is reliant on its stability which cause a reduction in chlorophyll contents in higher salinity due to occasional endure integral. In the current study, the existence of salinity further intensified the harmful effects of Pb stress on chlorophyll contents of rice.

In present trial, the improvement in TCC by the application of amendments was due to decrease in Pb uptake and translocation in rice. Since the uptake of Pb in rice was highly reduced by the applied gyp in both normal and salt-affected Pb-spiked soils, hence the detrimental effects of Pb on TCC were ameliorated. The application of DAP also resulted in reduced Pb uptake and translocation in rice plants, resultantly the toxic effects of Pb on TCC were also decreased in normal and salt-affected Pb-spiked soils. However, RP was proved less effective in improving physiological functions such as TCC of rice plants.

The Pb uptake by rice shoots (Table 4) and paddy (Table 5) and translocation (Table 6) of Pb in rice plants grown in Pb-spiked soils was significantly ($p \leq 0.05$, Table 7) higher than non-spiked (normal and salt-affected) soils. The application of gypsum was more useful to reduce Pb uptake and translocation. The DAP was ranked second effective treatment and its application resulted in decreased the uptake and translocation of Pb from rice shoot to paddy in both normal and salt-affected Pb-spiked soils. The Pb uptake in rice straw and paddy, and translocation from shoot to paddy was not effectively reduced by RP. The chemical immobilization of Pb in soil, with the addition of amendments, reduced the mobility and phyto-availability of Pb, which resulted in reduced uptake of Pb by rice straw and paddy. The formation of less soluble anglesite mineral, i.e., $PbSO_4$, with the addition of gypsum in Pb-spiked soil had been earlier reported (Illera et al., 2004; Garrido et al., 2005; Iqbal et al., 2017b). The use of gypsum under reduced conditions was expected to transform Pb into less soluble PbS (Illera et al., 2004; Hashimoto et al., 2011). Similarly, application of phosphate amendments was also proved very effective for chemical immobilization of Pb (Basta and McGowen, 2004). The DAP increased the potential for the formation of Pb-pyromorphite which reduce phyto-availability of Pb (Khan and Jones, 2008). Chen et al. (2006) found that Pb uptake by wheat was considerably restrained by $Ca(H_2P0_4)$ fertilizer and there was a- significant negative correlation between the level of P and the uptake of Pb. The translocation of Pb from root to shoot was also reduced because P application reduced the soluble and exchangeable fractions of Pb in soil. Therefore, the application of gyp, DAP and RP can provide an efficient way to decrease Pb availability, uptake and translocation in plants grown on metal spiked soils.

In the present experiment, application of gypsum reduced the uptake of Pb by rice plants in Pb-spiked normal and salt-affected soil. The other beneficial effects of gypsum can be due to the provision of soluble Ca in soil solution which reduced the uptake of Pb (Antosiewicz, 2005). The concentration of Pb in the shoot was enhanced with a decrease in Ca in growth medium, which indicate increased root to shoot transport with decreased level of Ca. The enhanced uptake and translocation of Pb were presumed to be the result of Ca-dependent Pb transport system in plasma membrane (Sunkar et al., 2000). It was assumed that the Ca pathway was used by Pb ions to cross the membranes owing to the great affinity of Pb to Ca binding sites in biotic configurations (Vijverberg et al., 1994). The diverse Ca-channels were also found porous to a range of monovalent and divalent cations. Thus, at a greater concentration, Ca favorably engaged these sites and restricted the invasion of other cations (White, 2000; Sanders et al., 2002). According to Ernst (1998), Ca immobilized Pb in the plant roots by forming Pb precipitates in the cell walls. The form and size of Pb holding precipitates were found to be dependent on the Ca level in the medium, and large deposits were formed at high Ca (Antosiewicz, 2005). Calcium might also play role in cell signaling and Ca-mediated signaling that led to a partial block of metal uptake (Antosiewicz and Hennig, 2004). The contribution of Ca in the signal transduction succeeding a numeral abiotic stimulus was also recognized (Sanders et al., 2002). Calcium might also involve in the signal transduction against heavy metal stress and thereby reduce metal uptake. In salt-affected soils, the beneficial effects of Ca in reducing Pb uptake due to the role of Ca, to maintain cell membrane integrity, thereby reduced the uptake of Na and Pb. Soluble Ca reduce the binding of Na to the cell wall and plasma membrane (Rengel, 1992) and improved the integrity and functions of plasma membrane (Lauchli, 1990). Therefore, the application of gypsum was superior because it not only reduced Pb bioavailability by immobilizing it but also reduced the Pb transport across the cell membrane.

Conclusion

The chlorophyll contents of rice were significantly ($p \leq 0.05$) decreased both in normal and salt-affected Pb-spiked soils than in non-spiked soils. In salt-affected Pb-spiked soil, decrease in chlorophyll contents, and increase in uptake and translocation of Pb were more prominent than that found in normal Pb-spiked soil. The decrease in chlorophyll contents of rice was counteracted by the application of gyp, RP and DAP with their variable amounts. With increasing amounts of applied amendments, the Pb uptake and translocation in rice were decreased gradually. Thus, application of 7.5 g gyp kg^{-1} soil was proved the most efficient in improving chlorophyll contents in rice as well as reducing Pb uptake and translocation in rice grown in both normal and salt-affected Pb-spiked soils. However, the present results need to be confirmed in field trial and economic feasibility must be worked out.

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