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Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil

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HIGHLIGHTS

- Lead tolerant PGPR promoted the growth of sunflower plants under lead stress.
- Lead tolerant PGPR induce stress tolerance in plants.
- Physiology and antioxidant activities are improved by PGPR.
- Lead tolerant PGPR improved lead uptake in sunflower plants.
- By using this strategy plant and soil health is improved.

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ABSTRACT

Present study was conducted to evaluate the effect of lead tolerant plant growth promoting rhizobacteria (LTPGPR) on growth, physiology, yield, antioxidant activities and lead uptake in sunflower in soil contaminated with lead under pot conditions. Three pre-characterized LTPGP strains (S2 (*Pseudomonas gessardii* strain BLP141), S5 (*Pseudomonas fluorescens* A506) and S10 (*Pseudomonas fluorescens* strain LMG 2189)) were used to inoculate sunflower growing in soil contaminated with different levels (300, 600 and 900 mg kg^{-1}) of lead by using lead nitrate salt as source of lead. Treatments were arranged according to completely randomized design with factorial arrangements. At harvesting, data regarding growth attributes (root shoot length, root shoot fresh and dry weights), yield per plant, physiological attributes (Chlorophyll 'a', 'b' and carotenoids content), antioxidant activities (Ascorbate peroxidase, catalase, superoxide dismutase and glutathione reductase), proline and malanodialdehyde content, and lead content in root, shoot and achenes of sunflower were recorded. Data were analysed by standard statistical procedures. Results showed that lead contamination reduced the plants growth, physiology and yield at all levels of lead stress. But application of LTPGPR in soil contaminated with lead improved plant growth, physiology, yield, and antioxidant activities, proline, and reduced the malanodialdehyde content (that is reduced by the application of different strains in lead contamination) of sunflower as compared to plants grown in soil without inoculation. Inoculation also promoted the uptake of lead in root, shoots and reduced the uptake of lead in achenes of plants as compared to plants in lead contamination without inoculation.

1. Introduction

Heavy metal contamination of soil, water and air has caused serious hazards to agriculture and environment due to rapid industrialization and urbanization. The interest has been increased to develop strategies to reduce the toxicity of metals which are posing threats to our environment. Lead (Pb) contamination has been increased in soil, water and air due to various anthropogenic activities. The main sources of lead contamination in soil could be weathering of lead containing rocks, use of leaded gasoline in motor vehicles, shedding paint chips, waste disposal and use of

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sewage water for irrigation (Pendias and Pendias, 1992; Ehsan et al., 2016). According to criteria proposed by the World Health Organization (WHO) for safe Pb limits, in different locations of Pakistan the Pb concentration in soil and vegetation is above the critical toxic level (Waseem et al., 2014).

Phytoremediation is new and very successful approach for the remediation of metals polluted soils and water. In this technique, the plant survival and metals uptake capabilities are very important for effective remediation of soils (Macek et al., 2000). Hyper-accumulators have capability to extract considerable amounts of pollutants from shallow soil surfaces and water (Garbisu and Alkorta, 2003; Atma et al., 2017). But even hyper-accumulator plants in metals stress grow slowly due to toxicity of metals and attain very low biomass (Huang et al., 1997; Zubair et al., 2016). So, phytoremediation of metals contaminated soils could take years for restoration of soils to their standard healthy state. The success rate is variable, depending upon the concentration of metals and soil type of particular site.

Efficacy of phytoremediation can be enhanced by the assistance of metal tolerant plant growth promoting bacteria (PGPB) which increase plant growth by different direct and indirect mechanisms by counteracting the toxic effects of metals (Asghar et al., 2013). Researchers have identified numerous beneficial plant growth promoting rhizobacteria that are closely associated with plants and can improve plant growth by production of growth regulators like auxin, cytokinin, and gibberellin or enzymatic lowering of plant ethylene level (El-Tarabily, 2008). Of particular interest, here is reduction of stress induced ethylene production in plant which at high concentration has inhibitory effect on plant growth especially when plant is growing in stress conditions (Mayak et al., 2004). Recently positive role of auxins on metal uptake and plant growth in metal stress conditions has been documented (Fassler et al., 2010). The seeds/roots inoculation of hyper-accumulators in metals stress conditions may facilitate root growth possibly by lowering the elevated level of stress induced ethylene. These bacteria may also be selected for enhancing plant growth by providing the plants with plant growth regulators especially auxin and ultimately could improve the efficiency of phytoremediation by hyper-accumulators (Fassler et al., 2010).

The objective of this study was to evaluate the effect of lead tolerant rhizobacteria on growth, yield, physiology, antioxidant activities, yield and lead uptake in sunflower in lead contaminated soil.

2. Materials and methods

A pot experiment was conducted to evaluate the effect of pre-characterized lead tolerant plant growth promoting rhizobacteria [S2 (*Pseudomonas gessardii* strain BLP141, Accession No. KJ547711.1), S5 (*Pseudomonas fluorescens* A506, Accession No. CP003041.1) and S10 (*Pseudomonas fluorescens* strain LMG 2189, Accession No. GU198103.1) (Isolated in Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan)] on growth, physiology, yield, antioxidant activities and lead uptake in sunflower in soil amended with lead.

2.1. Inoculum preparation and seed inoculation

For preparation of inoculum above mentioned strains were grown in 250 mL conical flask individually having 100 mL LB broth and incubated at $28 \pm 2^{\circ}C$ in the orbital shaking incubator (100 rpm) for three days. For 10^8–10^9 CFU mL^{-1}, an optical density 0.5 was recorded at a wavelength of 535 nm. Seeds of sunflower (*Helianthus annus* var Hisun-33) were disinfected by dipping in ethanol (95%) and in $HgCl_2$ (0.2%) solution for 3 min. Then seeds were inoculated by mixing with peat based slurry having 3 day old inoculum of respective strains and sugar solution (10%) whereas, the seeds for control were mixed with peat containing sterilized broth and solution of sugar. Inoculated seeds were air dried under shade for 6–8 h.

2.2. Experimental setup

The earthen pots were filled with 10 kg soil with sandy clay loam texture having pH 7.64, organic matter 0.63%, EC 1.29 dS m^{-1}, saturation percentage 38.6%, extractable potassium 125.6 mg kg^{-1}, available phosphorous 7.5 mg kg^{-1} and while lead was not detectable in that soil. Before filling of pot, this soil was polluted by lead using lead nitrate ($PbNO_3$) salt as source of lead and finally, three levels of lead (300, 600, and 900 mg kg^{-1}) were developed. The soil was permitted to equilibrate for two weeks after lead contamination. Treatments were arranged according to completely randomized design with factorial arrangements replicated thrice. After two weeks of germination thinning was done to retain one seedling in a pot. Recommended doses ($N = 145$ kg ha^{-1}, $P = 60$ kg ha^{-1} and $K = 55$ kg ha^{-1}) of NPK fertilizer was applied in the form of Urea, DAP (Di-ammonium phosphate) and Murate of Potash. Water was applied whenever needed.

2.3. Determination of growth parameters and yield

At harvesting (105 days after sowing), plant growth attributes such as shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight and yield were recorded. All parts of plant oven dried at 70 °C until constant weight and then weighed.

2.4. Determination of chlorophyll pigments (chlorophyll a, b and carotenoids)

For determination of chlorophyll pigments (chlorophyll *a*, *b* and carotenoids), 0.5 g of leaf sample was homogenized with acetone 80% (v/v) and then filtrate through filter paper. Absorbance of filtrate was measured by spectrophotometer at 663, 645 and 480 nm for chlorophyll *a*, *b* and carotenoids, respectively (Arnon, 1949).

2.5. Determination of ascorbate peroxidase, catalase and malanodialdehyde (MDA)

Ascorbate peroxidase activity was determined using a modified method of Nakano and Asada (1981) where it was determined by following reduction in absorbance at 290 nm due to decrease in ascorbate through H_2O_2 (Elavarthi and Martin, 2010). Catalase activity was measured according to Aebi (1984) where H_2O_2 decomposition is followed spectrophotometrically at 240 nm. One enzyme activity unit is equal to 1 μmol H_2O_2 decomposed per minute (Elavarthi and Martin, 2010). Malanodialdehyde (MDA) content was determined from the difference (A532 − A600) in absorbance by using Beer and Lambert's equation and expressed in terms of nmol g^{-1} (Jambunathan, 2010).

2.6. Determination of superoxide dismutase (SOD), glutathione reductase (GR) and proline

Superoxide dismutase (SOD) was determined as method described by (Elavarthi and Martin, 2010) where SOD activity is determined by recording the decrease in superoxidenitro blue tetrazolium complex absorbance by the enzyme. Glutathione

reductase (GR) activity was determined by enhance in absorbance at 412 nm due to decrease of (5, 5′-dithiobis (2-nitrobenzoic acid)) (DTNB) to 2-nitro-5-thiobenzoic acid (TNB). Three mL of reaction mixture having 30 μL sample extract (extracted in 50 mM, pH 7.8 potassium phosphate buffer with 2 mM EDTA), NADPH (0.1 mM), DTNB (0.75 mM) and 1 mM oxidized glutathione. The activity was determined in terms of nmol NADPH mg^{-1} protein min^{-1}at 25 ± 2 °C (Smith et al., 1988). For proline determination 1 g leaf sample was homogeniged with 3% sulphosalyclic acid and then filtered through filter paper. After the addition of glacial acetic acid and acid ninhydrin mixture was heated for 1 h at 100 °C in water bath and by using ice bath reaction was stopped. Through toluene mixture was extracted and the absorbance was taken at 520 nm. Proline concentration was measured by using standard curve and was expressed as μmol g^{-1} (Bates et al., 1973).

2.7. Determination of Pb in root, shoot and achenes of sunflower

For determination of Pb in root, shoot and achenes of sunflower, these samples were washed with deionized water, then oven dried at 70 °C for 24 h and grinded to powdered form. Then 0.5 g of grinded shoot, root and achenes of sunflower samples were placed in conical flasks and 10 mL HNO_3:$HClO_4$ mixture in 3:1 ratio (on volume basis) was added in the each flask and kept for overnight. Next day, all flasks were heated on hot plate and digested up to the material become clear. Then flasks were cooled and all the materials was poured to the 50 mL volumetric flasks and distill water was added to each flask to make volume up to 50 mL and filtrate by using filter paper. Known strengths standard were used for assurance for quality control. Standard materials used were lead sulphate and lead chloride. Lead (Pb) was analyzed with the help of Atomic Absorption Spectrophotometer.

2.8. Statistical analysis

Two way analysis of variance (ANOVA) was performed and means were compared by standard statistical procedure. Data were analyzed by statistical software (Statstix 8.1).

3. Results

Shoot length of sunflower in lead contamination significantly decreased as compared to plants grown in un-inoculated control without lead stress. However, improvement in shoot length at all levels of lead contamination was observed by the application of lead tolerant plant growth promoting rhizobacteria in metal contaminated soil (Table 1). It was observed that inoculation with lead tolerant rhizobacteria (S2) promoted the shoot length up to (15.27%) at 900 mg kg^{-1} lead contamination as compared to plants grown at same level of metal stress without inoculation. Data (Table 1) showed that reduction in shoot fresh weight was observed by lead contamination as compared to un-inoculated control without contamination. Results showed that more severe reduction in shoot fresh weight was observed by lead stress at 900 mg kg^{-1} lead. Lead contamination at rate of 900 mg kg^{-1} reduced the shoot fresh weight up to (32%) as compared to plants grown in un-inoculated control without stress. However, application of lead tolerant rhizobacteria (S5) promoted the shoot fresh weight at all levels of lead stress and increased the shoot fresh weight up to (16%) at highest concentration of lead as compared to plants grown at same level of lead without inoculation. Lead contamination significantly decreased the shoot dry weight as compared to control (un-inoculated without contamination) (Table 1). Reduction in shoot dry weight enhanced with increasing concentration of lead. Maximum decrease in shoot dry weight was observed at 900 mg kg^{-1} spiked soil as compared to control. It was observed that (36%) reduction in shoot dry weight was observed in soil contaminated with lead at rate of 900 mg kg^{-1} as compared to un-inoculated treatment without contamination. However, inoculation with lead tolerant rhizobacteria improved the shoot dry weight at all levels of metal stress. Lead tolerant rhizobacteria (S10) enhanced the shoot dry weight up to (23%) at 900 mg kg^{-1} lead stress as compared to plant grown at 900 mg kg^{-1} lead concentration without inoculation.

Lead concentration at rate of 900 mg kg^{-1} significantly reduced the root length up to (35%) as compared to plants grown in un-inoculated soil without lead contamination (Table 2). Results showed that lead tolerant rhizobacteria improved the root length in soil contaminated with lead. Application of lead tolerant rhizobacteria (S2) increased the root length up to (28%) at highest concentration of lead as compared to plants grown at same level of lead without inoculation. Data regarding (Table 2) showed that root fresh weight decreased by increasing the lead contamination. It was observed that lead contamination at rate of 900 mg kg^{-1} reduced the root fresh weight up to (55%) as compared to control. However, application of lead tolerant rhizobacteria showed the positive effect

Table 1
Effect of lead tolerant plant growth promoting rhizobacteria on shoot length (SL), shoot fresh weight (SFW) and shoot dry weight (SDW) of sunflower in lead contamination under pot conditions.

Treatment		SL (cm)	SFW (g)	SDW (g)
Pb (mg kg^{-1})	Inoculation			
0	No inoculation	106.67 ± 1.52 bc	79.60 ± 1.13 bc	26.31 ± 0.27 bc
	S2	112.00 ± 2.00 a	83.58 ± 1.49 ab	27.86 ± 0.50 a
	S5	110.67 ± 3.05 ab	84.55 ± 2.25 a	28.18 ± 0.75 a
	S10	108.67 ± 3.21 ac	81.09 ± 2.40 ab	27.03 ± 0.80 ab
300	No inoculation	96.67 ± 3.20 fg	72.14 ± 2.40 de	23.84 ± 0.64 e
	S2	100.00 ± 2.00 ef	76.15 ± 4.60 c	25.38 ± 1.53 de
	S5	105.33 ± 1.51 cd	75.62 ± 1.55 cd	20.93 ± 0.84 g
	S10	101.33 ± 2.08 de	76.75 ± 2.50 c	25.21 ± 0.52 cd
600	No inoculation	87.00 ± 2.00 ij	64.93 ± 1.49 f	25.58 ± 0.83 bc
	S2	87.67 ± 2.51 i	70.10 ± 2.61 e	23.30 ± 0.87 ef
	S5	91.00 ± 2.01 hi	69.90 ± 2.62 e	23.37 ± 0.87 e
	S10	93.67 ± 3.51 gh	65.42 ± 1.88 f	21.81 ± 0.63 fg
900	No inoculation	72.33 ± 3.05 m	53.98 ± 2.28 h	16.94 ± 1.83 i
	S2	83.00 ± 2.02 jk	61.94 ± 1.49 fg	20.65 ± 0.50 gh
	S5	80.33 ± 2.50 kl	62.57 ± 3.18 f	19.40 ± 0.75 h
	S10	78.00 ± 3.01 l	58.21 ± 2.23 g	20.86 ± 1.06 gh

Means sharing the same letters are statistically non-significant at $p < 0.05$. Data are average of three replicates ± standard deviation (SD).

Table 2
Effect of lead tolerant plant growth promoting rhizobacteria on root length (RL), root fresh weight (RFW) and root dry weight (RDW) of sunflower in lead contamination under pot conditions.

Treatment		RL (cm)	RFW (g)	RDW (g)
Pb (mg kg^{-1})	Inoculation			
0	No inoculation	23.98 ± 0.72 cd	13.98 ± 0.73 bc	08.61 ± 0.66 bc
	S2	26.74 ± 1.12 a	15.86 ± 0.50 a	09.91 ± 0.31 a
	S5	26.18 ± 0.75 ab	16.24 ± 0.82 a	10.12 ± 0.47 a
	S10	25.03 ± 0.80 bc	15.03 ± 0.80 ab	09.39 ± 0.50 ab
300	No inoculation	21.84 ± 0.64 fg	11.46 ± 1.28 d	07.07 ± 0.62 d
	S2	23.21 ± 0.52 df	13.38 ± 1.53 c	08.37 ± 0.96 c
	S5	23.58 ± 0.83 ce	13.21 ± 0.52 c	08.25 ± 0.32 c
	S10	23.93 ± 0.66 cd	13.62 ± 0.78 c	09.16 ± 0.85 ac
600	No inoculation	18.26 ± 0.78 j	09.60 ± 0.43 e	05.91 ± 0.17 e
	S2	22.30 ± 1.01 eg	11.30 ± 0.87 d	07.11 ± 0.55 d
	S5	21.37 ± 0.87 gh	11.70 ± 0.60 d	07.06 ± 0.54 d
	S10	19.81 ± 0.63 i	09.81 ± 0.63 e	06.13 ± 0.39 de
900	No inoculation	15.61 ± 0.91 k	06.27 ± 0.98 g	03.56 ± 0.52 g
	S2	19.98 ± 1.89 hi	08.65 ± 0.50 ef	05.40 ± 0.31 ef
	S5	18.86 ± 1.06 ij	09.52 ± 0.74 e	06.20 ± 1.24 de
	S10	17.40 ± 0.74 j	07.40 ± 0.75 fg	04.63 ± 0.47 f

Means sharing the same letters are statistically non-significant at $p < 0.05$. Data are average of three replicates ± standard deviation (SD).

on root fresh weight at all levels of contamination and promoted the root fresh weight up to (52%) at 900 mg kg^{-1} lead as compared to plants grown at same level of lead without inoculation. Root dry weight negatively affected by lead contamination (Table 2). Negative effect of lead on root dry weight increases by increasing the lead contamination. Root dry weight decreased up to (59%) at 900 mg kg^{-1} metal stress as compared to un-inoculated control without contamination. However, inoculation with lead tolerant rhizobacteria reduced the toxic of lead on root dry weight at all levels of lead. Improvement (74%) in root dry weight was observed by use of lead tolerant rhizobacteria (S5) in soil contaminated with lead at 900 mg kg^{-1} as compared to plants grown at same level of lead without inoculation. Results regarding (Fig. 1) revealed that lead stress showed the toxic effect on yield per plant. Toxic effect of lead increased with increase in concentration of lead. Yield per plant decreased up to (53%) by lead contamination at rate of 900 mg kg^{-1} as compared to control. However application of lead tolerant bacteria reversed the toxic effect of lead on plants and improved the yield per plant at all levels of lead. It was observed that (45%) increment in yield per plant was obtained by inoculation with lead tolerant bacteria in soil contaminated with lead at the rate of 900 mg kg^{-1} as compared to plants grown at same concentration of lead without inoculation.

Soil contaminated with lead reduced the chlorophyll 'a', 'b' and carotenoids content as compared to plants grown in un-inoculated soil without contamination (Table 3). Chlorophyll 'a' content

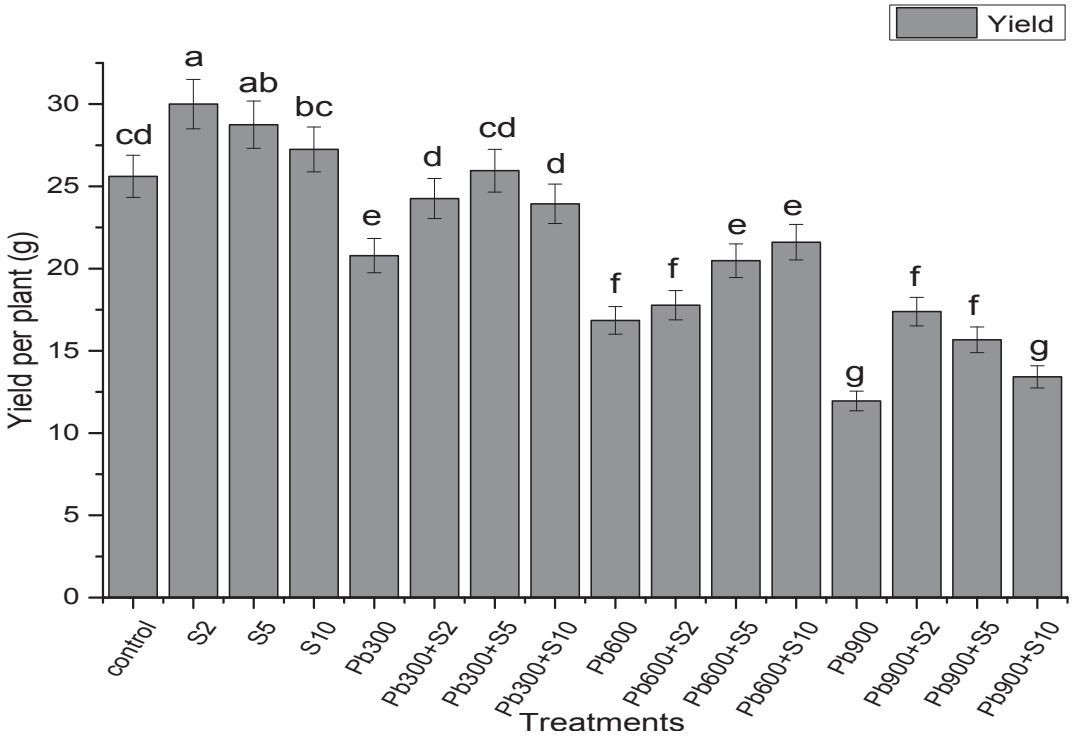

Fig. 1. Effect of lead tolerant plant growth promoting rhizobacteria on yield of sunflower in lead contaminated soil under pot condition.

Table 3
Effect of lead tolerant plant growth promoting rhizobacteria on chlorophyll 'a', 'b' and carotenoids content of sunflower in lead contaminated soil under pot conditions.

Treatment		Chlorophyll 'a' (μg g^{-1} FM)	Chlorophyll 'b' (μg g^{-1} FM)	Carotenoids (μg g^{-1} FM)
Pb (mg kg^{-1})	Inoculation			
0	No inoculation	17.08 ± 0.38 bc	08.47 ± 0.24 ce	10.68 ± 0.75 cd
	S2	19.75 ± 0.90 a	10.50 ± 0.98 a	12.84 ± 0.57 ab
	S5	20.34 ± 1.36 a	09.87 ± 0.45 ab	13.55 ± 0.38 a
	S10	18.24 ± 1.45 ab	09.27 ± 0.47 bc	11.86 ± 0.94 bc
300	No inoculation	12.12 ± 1.72 d	05.10 ± 0.45 hi	07.10 ± 0.31 g
	S2	15.26 ± 2.78 c	07.81 ± 0.75 de	09.92 ± 1.81 d
	S5	15.62 ± 1.51 c	08.63 ± 0.51 cd	10.82 ± 0.46 cd
	S10	14.94 ± 1.20 c	07.47 ± 0.47 ef	09.71 ± 0.61 de
600	No inoculation	08.52 ± 0.96 ef	03.07 ± 0.28 k	03.67 ± 0.22 ij
	S2	11.48 ± 1.58 d	06.47 ± 1.24 fg	07.46 ± 1.02 fg
	S5	11.60 ± 1.58 d	05.74 ± 0.79 gh	08.54 ± 0.24ef
	S10	10.11 ± 0.41 de	04.39 ± 0.57 ij	05.70 ± 0.74 h
900	No inoculation	06.33 ± 1.17 g	01.83 ± 033 l	02.51 ± 0.41 j
	S2	06.67 ± 0.90 f	04.19 ± 0.73 ij	04.34 ± 0.57 i
	S5	07.05 ± 1.95 f	3.34 ± 0.45 jk	04.58 ± 1.25 hi
	S10	06.75 ± 0.90 f	2.88 ± 0.66 kl	04.20 ± 0.68 i

Means sharing the same letters are statistically non-significant at $p < 0.05$. Data are average of three replicates ± standard deviation (SD).

decreased up to (75%), chlorophyll 'b' reduced up to (78%) and carotenoids content decreased up to (76%) at 900 mg kg^{-1} lead contamination as compared to plant grown in soil without un-inoculated and un-contaminated control. It was observed that inoculation with lead tolerant bacteria reduced the toxic effect of lead on chlorophyll 'a', 'b' and carotenoids content and improved the chlorophyll 'a', 'b' and carotenoids content at all levels of lead contamination as compared to plants grown in soil contaminated with lead without inoculation. This showed the positive effect of lead tolerant bacteria on chlorophyll 'a', 'b' and carotenoids content of sunflower in contaminated soil.

Improvement in ascorbate peroxidase and catalase content of sunflower in lead contaminated soil was observed by the application of lead tolerant bacteria while reduction in malanodialdehyde content was obtained by inoculation in lead contamination (Table 4). Ascorbate peroxidae content increased up to (12%) at 900 mg kg^{-1} heavy metal contamination by lead tolerant bacteria (S5) as compared to soil contaminated with lead at rate of 900 mg kg^{-1} without inoculation. Data showed that (26%) increment in catalase was observed by inoculation with lead tolerant bacteria (S10) at 900 mg kg^{-1} lead as compared to same concentration of lead without inoculation. Malanodialdehyde content was reduced up to (36%) by lead tolerant bacteria (S5) at highest concentration of lead as compared to same un-inoculated lead level.

Inoculation with lead tolerant bacteria promoted the superoxide dismutase, glutathione reductase and proline content of sunflower in lead contamination (Table 5). Results showed that (26%) increment was observed in superoxide dismutase by lead tolerant bacteria (S5) at highest concentration of lead as compared to plants grown at un-inoculated same concentration of lead. Glutathione reductase increased (24%) by inoculation with lead tolerant bacteria (S10) at 900 mg kg^{-1} metal stress as compared to same level of lead without inoculation. Lead tolerant bacteria (S5) promoted the proline content up to (22%) at 900 mg kg^{-1} lead as compared to plants grown at 900 mg kg^{-1} lead stress without inoculation. Data (Figs. 2–4) showed significant effect of inoculation on lead concentration in root, shoot and achenes of sunflower in lead contaminated soil. It was observed that inoculation with lead tolerant bacteria increased the lead concentration in root at all levels of lead as compared to plant grown in lead contamination without inoculation (Fig. 2). Inoculation with lead tolerant bacteria

Table 4
Effect of lead tolerant plant growth promoting rhizobacteria on ascorbate peroxidase (APX), catalase and MDA content of sunflower in lead contaminated soil under pot condition.

Treatment		MDA (nmol g^{-1} FW)	APX (μmol H_2O_2 mg^{-1} protein min^{-1})	Catalase (μmol H_2O_2 mg^{-1} protein min^{-1})
Pb (mg kg^{-1})	Inoculation			
0	No inoculation	12.33 ± 0.66 ij	23.33 ± 1.22 j	661.67 ± 15.00 j
	S2	10.66 ± 0.15 j	25.00 ± 0.52 ij	840.00 ± 23.45 hi
	S5	10.33 ± 0.38 jk	28.00 ± 0.60 hi	750.00 ± 12.24 ij
	S10	08.33 ± 0.19 k	26.33 ± 0.15 ij	790.00 ± 25.80 i
300	No inoculation	19.52 ± 0.52 ef	28.33 ± 0.67 hi	805.00 ± 30.60 i
	S2	14.66 ± 1.04 h	33.67 ± 0.52 fg	1000.00 ± 18.23 fg
	S5	15.96 ± 0.94 gh	33.33 ± 0.40 fg	940.00 ± 11.15gh
	S10	13.61 ± 0.66 hi	31.33 ± 1.67 gh	1010.00 ± 07.84 fg
600	No inoculation	27.66 ± 0.34 c	37.33 ± 0.57 ef	1103.30 ± 09.27ef
	S2	24.66 ± 0.49 d	39.67 ± 2.13 de	1270.00 ± 13.45cd
	S5	21.55 ± 0.50 e	44.00 ± 0.66 cd	1320.00 ± 08.95 c
	S10	18.66 ± 1.34 f	42.33 ± 0.43 cd	1190.00 ± 22.50de
900	No inoculation	39.66 ± 0.41 a	45.67 ± 0.47 c	1345.00 ± 19.30 c
	S2	33.00 ± 1.00 b	54.67 ± 0.79 ab	1530.00 ± 14.30 b
	S5	25.33 ± 0.66 d	56.33 ± 0.76 a	1640.00 ± 10.43 ab
	S10	28.00 ± 0.60 c	51.00 ± 0.95 b	1690.0 ± 21.00 a

Means sharing the same letters are statistically non-significant at $p < 0.05$. Data are average of three replicates ± standard deviation (SD).

Table 5
Effect of lead tolerant plant growth promoting rhizobacteria on superoxide dismutase (SOD), glutathione reductase (GR) and proline content of sunflower in lead contaminated soil under pot condition.

Treatment		SOD (unit mg^{-1} protein)	GR (nmol NADPH mg^{-1} protein min^{-1})	Proline (umol g^{-1} FW)
Pb (mg kg^{-1})	Inoculation			
0	No inoculation	447.78 ± 12.50 j	200.80 ± 06.00 h	1.94 ± 0.15 h
	S2	500.00 ± 08.12 ij	254.55 ± 08.70 fg	2.27 ± 0.30 gh
	S5	560.00 ± 17.30 hi	227.20 ± 05.20gh	2.55 ± 0.23 fg
	S10	526.60 ± 13.70 ij	239.30 ± 04.12 g	2.39 ± 0.40 g
300	No inoculation	520.00 ± 07.00 hi	259.00 ± 04.75 fg	2.61 ± 01.00 fg
	S2	673.33 ± 22.20 fg	284.80 ± 10.15 ef	3.06 ± 0.47 e
	S5	666.60 ± 09.75 fg	303.00 ± 04.60 e	2.85 ± 0.67 ef
	S10	626.60 ± 16.10 gh	306.00 ± 07.50 e	3.03 ± 0.18 e
600	No inoculation	718.80 ± 06.80 ef	320.30 ± 03.22 e	3.17 ± 0.70 e
	S2	880.00 ± 20.33 c	360.60 ± 08.70 d	3.85 ± 0.83 cd
	S5	793.30 ± 11.20 de	400.00 ± 05.22 c	4.00 ± 0.25 c
	S10	846.60 ± 10.50 cd	384.80 ± 11.45 cd	3.61 ± 0.33 d
900	No inoculation	896.67 ± 13.50 c	414.20 ± 09.10c	4.21 ± 1.10 c
	S2	1093.30 ± 12.70 ab	496.90 ± 08.70 ab	4.64 ± 0.78 b
	S5	1126.60 ± 27.40 a	463.60 ± 04.10 b	5.12 ± 0.45 a
	S10	1020.00 ± 12.80 b	512.12 ± 05.35 a	4.97 ± 0.67 ab

Means sharing the same letters are statistically non-significant at $p < 0.05$. Data are average of three replicates ± standard deviation (SD).

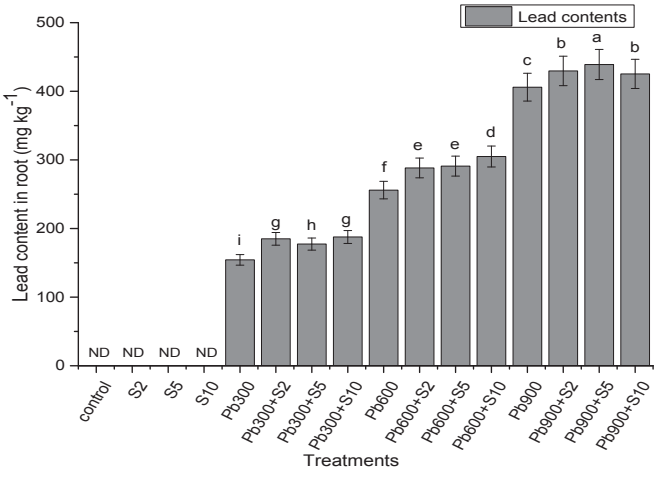

Fig. 2. Effect of lead tolerant plant growth promoting rhizobacteria on lead content in root of sunflower in lead contaminated soil under pot condition.

(S5) improved the lead content in root up to (8%) at 900 mg kg^{-1} lead stress as compared to plants in same stress without inoculation. Lead concentration in shoot improved by inoculation with lead tolerant bacteria at all levels of lead contamination (Fig. 3). It was noticed that application of lead tolerant bacteria (S10) increased the lead content in shoot up to (9%) at 900 mg kg^{-1} lead concentration as compared to plants in same content of metal without bioaugmentation. Lead concentration in seeds/achenes of sunflower plant by inoculation with lead tolerant bacteria in lead contamination significantly decreased as compared to plant grown in lead stress without inoculation (Fig. 4). At 900 mg kg^{-1} metal stress lead tolerant bacteria (S5) decreased the lead content in achenes up to (22%) at highest level of lead as compared to same level of un-inoculated lead stress.

4. Discussion

Present study was conducted to evaluate the effect of lead tolerant plant growth promoting rhizobacteria on growth, physiology, yield, antioxidant activities and lead uptake in sunflower in lead amended soil. Our results showed reduction in growth, physiology and yield of sunflower in lead amended soil as compared to plants grown in un-inoculated treatment without contamination. Reduction in the growth, physiology and yield increases by increasing the lead content in soil. These findings are in agreement with work of (Hussain et al., 2006, 2013; McComb et al., 2012; Kaur, 2014). Reduction in growth and yield by lead stress might be due to lead reduced the uptake of nutrients, caused interference in respiration, decreased the photosynthetic activity of plants and disturb the cell membrane permeability (Sharma and Dube, 2005). Lead also caused in interference in activities of enzymes that involved in the photosynthetic Calvin cycle, sugar and nitrogen metabolism (Verma and Dubey, 2003), also caused structural damage, reduction of biochemical and physiological activities and growth inhibition (Jayasri and Suthindhiran, 2017). Higher concentration of Pb may also caused production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (Verma and Dubey, 2003; Souguir et al., 2011). The ROS can cause oxidative stress, damage cell membrane and breakage DNA strand, and ultimately reduced the plant growth and yield (Verma and Dubey, 2003).

Physiological attributes (Chlorophyll 'a', 'b' and carotenoids content) are reduced by lead contamination. This reduction in physiological attributes might be due to lead avert the inclusion of iron (Fe) molecule into phytoporphyrin ring of chlorophyll and reduced the synthesis of chlorophyll (Nyitrai et al., 2002; Jaleel et al., 2009). Lead reduces the production of chlorophyll molecule either by decreasing the activity of chlorophyllase or minimizing the uptake of Mg and Fe by plants (Sharma and Dube, 2005). Lead also degraded the chlorophyll molecule (Dogan and Colak, 2009).

In our study lead contamination increased the activities of antioxidants (Ascorbate peroxidase, catalase, superoxide dismutase and glutathione reductase) and proline in plants as compared to plants grown in soil without inoculation and un-contamination. In order to survive with heavy metals toxicity, or to keep heavy metals level in physiological range, plants induce a mechanism to control the uptake and metals detoxification. To alleviate the harmful effect of ROS, plants promote the activities of antioxidants that protect the plants against oxidative stress produced by lead concentration (Janmohammadi et al., 2013). Malanodialdehyde (MDA) content increased in lead contamination that is signals of oxidative stress (Janmohammadi et al., 2013).

However, inoculation with lead tolerant rhizobacteria in lead contaminated soil upturned the poisonous effect of lead and improved the growth, physiology, and yield of sunflower in lead

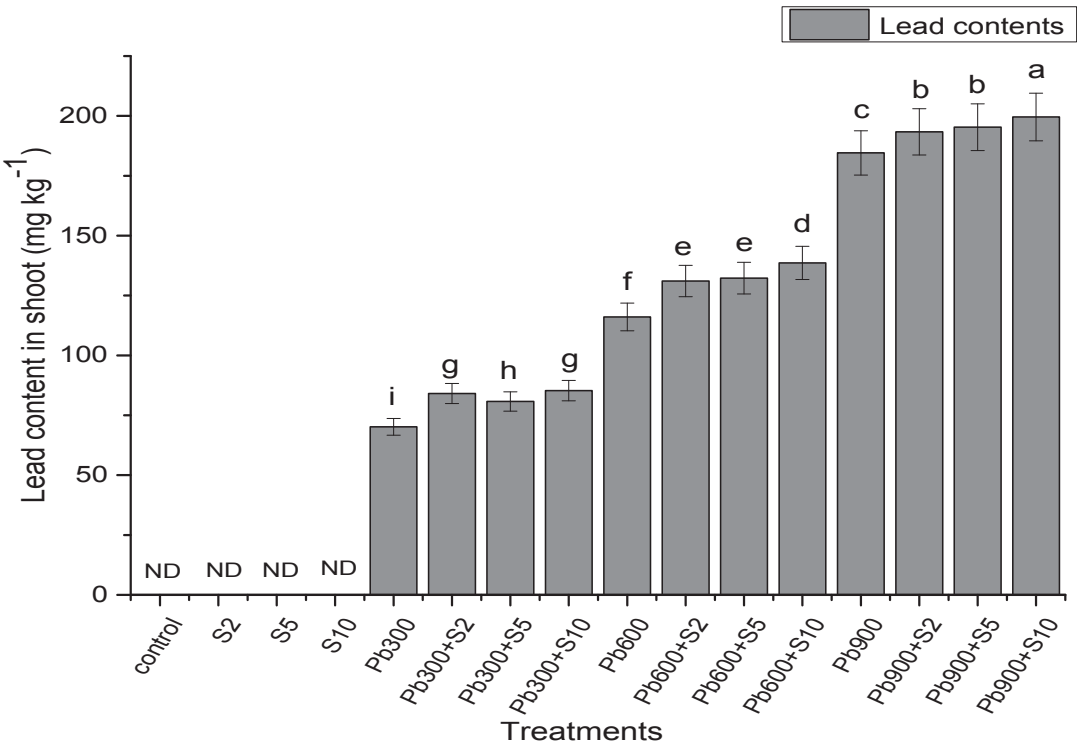

Fig. 3. Effect of lead tolerant plant growth promoting rhizobacteria on lead content in shoot of sunflower in lead contaminated soil under pot condition.

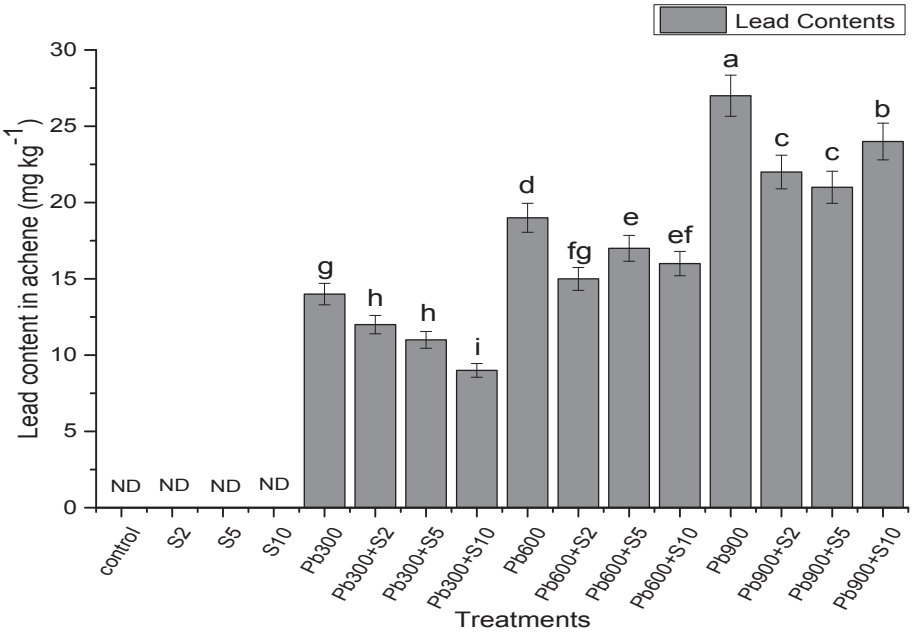

Fig. 4. Effect of lead tolerant plant growth promoting rhizobacteria on lead content in achene of sunflower in lead contaminated soil under pot condition.

amended soil as compared to un-inoculated plant grown in soil contaminated with lead. Several researches demonstrated that plant growth promoting rhizobacteria increased the plants growth, physiology and yield under metals toxicity (Jacobson et al., 1994; Glick et al., 1998; Gupta et al., 2002). Improvement in growth and yield in lead contamination by lead tolerant plant growth promoting bacteria might be due to solubilization of phosphate (Yasmin and Bano, 2011; Gupta et al., 2002; Pena and Reyes, 2007), siderophores creation (Glick et al., 1999; Meyer, 2000), phytohormones production (Asghar et al., 2004; Humphry et al., 2007), caused systemic resistance in plants against toxicity of metals (Mishra et al., 2006) which might caused in plant growth improvement. It has been observed that plant growth promoting rhizobacteria may also promote availability and uptake of nutrients by organic waste recycling (Asghar et al., 2006). These bacteria also facilitate the growth of plants by reducing the ethylene-mediated stress in plants by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Gamalero and Glick, 2015). Kumar

et al. (2009) stated that the PGPR (*Enterobacter aerogenes* and *Rahnella aquatilis*) decreased the heavy metals toxicity in *Brassica juncea* (Indian mustard) and increased plant growth under pot experiment. Improvement in physiological attributes (Chlorophyll 'a', 'b' and carotenoids content) of plants in lead contamination by inoculation with lead tolerant plant growth promoting bacteria might be due to lead tolerant plant growth promoting rhizobacteria increased the uptake of Fe in plants which could have promoted the chlorophyll production (Burd et al., 2000), enhanced the leaf area of plants that ultimately improved the photosynthetic activities and other physiological parameters of plants (Glick et al., 1999).

In our results, lead tolerant plant growth promoting rhizobacteria improved the ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase and proline contents in plants in lead contamination as compared to plants grown in lead contamination without inoculation. These findings can be correlated with work of Janmohammadi et al. (2013), they reported that inoculation with plant growth promoting rhizobacteria promoted the ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase and proline contents in plants in lead contamination as compared to un-inoculated plants grown in lead contamination due to enhancing the activity of antioxidant enzymes. Our results revealed that inoculation with lead tolerant bacteria reduced the MDA content in plants that might be due to stimulatory effect of rhizobacteria on defensive plant mechanism (Janmohammadi et al., 2013).

Our research showed that inoculation with lead tolerant bacteria improved the lead content in root and shoot of sunflower as compared to plants grown in contaminated soil without inoculation. These results are correlated with work of (Braud et al., 2009; Kamran et al., 2015). Improvement in lead concentration in root and shoot by lead tolereant PGPR in lead contamination might be due to the capacity of lead tolerant rhizobacteria to decrease the pH of soil that played important role in solubilization and uptake of metals (Abou-Shanab et al., 2006). Bacteria could have produced the organic acids, chelates, caused redox changes and increased the uptake of heavy metals (Liao et al., 2006; Yousaf et al., 2010). By changing the availability and solubility of lead and redox changes, PGPR enhanced the heavy metal uptake in plant. These results can be correlated with work of Krishna et al. (2012). Plant roots excrete the organic acids and protons that can also acidify the soil, decreased the heavy metals adsorbtion on soil and increased the mobility of the metals. These metal tolerant bacteria could also enhanced phytoremediation by production of plant growth regulators i.e. Auxins, Gibberellins and Cytokinins (Kong and Bernard, 2017) Furthermore, some rhizobacteria can excrete organic acids to enhance the bioavailability of heavy metals. Lead tolerant plant growth promoting rhizobacteria increased the production of phytohormones, nutritive status of soil through nutrient solubilization and nitrogen fixation, and reduced the ethylene production in plants, ultimately plant growth and biomass is increased in heavy metal stress, due to improvement in growth and biomass of plants, lead uptake in plants also increased (Burd et al., 1998; Glick, 2004).

In present study, inoculation with lead tolerant bacteria reduced the concentration of lead in seeds/achenes of plants in heavy metal contaminated soil as compared to un-inoculated plants grown in lead contaminated soils. This reduction in lead in achenes might be due to lead tolerant bacteria immobilized lead in root/shoot by negatively charged particles, caused precipitation of lead and increased sequestration of lead in shoot that reduced the translocation of lead into seeds/achenes of plants (Wani and Khan, 2010).

5. Conclusion

It is concluded from this research that lead contamination adversely affected the growth, physiology and yield of sunflower plants. However, inoculation with LTPGP strains promoted the growth, physiology, antioxidant activities, yield and phytoremediation potential of sunflower plants in lead stressed conditions. This study reveals that LTPGPR induce stress tolerance in plants in lead contamination and also increase lead content in plant in lead contamination. By using this strategy soil will be cleaned from lead.

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