

EVALUATION OF LEAD TOLERANT PLANT GROWTH PROMOTING RHIZOBACTERIA FOR PLANT GROWTH AND PHYTOREMEDIATION IN LEAD CONTAMINATION

Evaluación del efecto de rizobacterias promotoras del crecimiento tolerantes al plomo en el crecimiento vegetal y la fitorremediación en contaminación por plomo

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ABSTRACT

The present study was conducted to evaluate five pre-characterized lead tolerant plant growth promoting rhizobacteria: *Pseudomonas gessardii* strain BLP141 (S2), *Pseudomonas fluorescens* A50 (S5), *Pseudomonas syringae* pv. *syringae* B728a (S6), *Pseudomonas stutzeri* strain 28a24 (S8) and *Pseudomonas fluorescens* strain LMG 2189 (S10), for their plant growth promotion, phytoremediation potential and physiological response on *Helianthus annuus* L. as test crop, exposed to different lead concentrations (300, 600 and 900 mg/kg). Crop was harvested after 30 days of sowing and data regarding growth parameters (root shoot length, root shoot fresh and dry weights), physiological attributes (photosynthetic rate, transpiration rate and substomatal CO₂), and lead content in root and shoot of sunflower, were recorded. Results revealed that lead contamination decreased the growth and physiological attributes of plants at all levels of lead stress; however inoculation with lead tolerant rhizobacteria reversed the toxic effect of lead on the plants and improved the growth and physiological attributes of sunflower in lead contamination, as compared to plants grown in the un-inoculated lead contaminated treatments. Among the five isolates, S2, S5 and S10 promoted more growth, improving the physiological attributes and phytoremediation potential of plants at all levels of contamination as compared to plants grown in lead stress without inoculation.

Palabras clave: contaminación, metales pesados, rizobacterias promotoras del crecimiento, fisiología vegetal, girasol

RESUMEN

Se evaluaron cinco rizobacterias promotoras del crecimiento tolerantes al plomo pre-caracterizadas: *Pseudomonas gessardii* cepa BLP141 (S2), *Pseudomonas fluorescens* A50 (S5), *Pseudomonas syringae* pv. *syringae* B728a (S6), *Pseudomonas stutzeri* cepa 28a24 (S8) y *Pseudomonas fluorescens* cepa LMG 2189 (S10), con relación a su efecto en el crecimiento, potencial de fitorremediación y respuesta fisiológica en *Helianthus annuus* L. como cultivo de ensayo expuesto a diferentes concentraciones de plomo (300, 600 y 900 mg/kg). El cultivo se cosechó después de 30 días de sembrado y se registraron los

datos referentes a parámetros de crecimiento (longitud del brote y la raíz, peso fresco y seco del brote y la raíz), atributos fisiológicos (tasa fotosintética y de transpiración, CO₂ estomático) y contenido de plomo en raíces y brotes de girasol. Los resultados indican que la contaminación por plomo mermó tanto el desarrollo como los atributos fisiológicos de las plantas en todos los niveles de agresión por plomo; sin embargo, la inoculación con rizobacterias tolerantes al plomo revirtió los efectos tóxicos y mejoró tanto el crecimiento como los atributos fisiológicos del girasol contaminado por este metal, en comparación con plantas cultivadas sin este tratamiento. De las cinco cepas aisladas, S2, S5 y S10 promovieron mayor crecimiento y mejoraron en mayor medida los atributos fisiológicos y el potencial de fitorremediación de las plantas en todos los niveles de contaminación en comparación de plantas cultivadas en un ambiente de agresión por plomo sin inoculación.

INTRODUCTION

Lead (Pb) is one of the toxic heavy metals that have negative impacts on soil diversity, growth of plant and health of human beings through entering the food chain. Soils are the ultimately reserves for lead and serve as one of the chief possibilities for human exposure to this metal (Mangkoedihardjo and Surahmaida 2008). In Pakistan, due attention has not been given to the control and management of industrial wastes and other pollutants (Faryal et al. 2007). Water and soil polluted with heavy metals cause main environmental and human health issues that are still lacking affordable and effective technical solutions. Remediation techniques could be physical, chemical and biological approaches (Dermont et al. 2010). As compared to physical and chemical techniques, phytoremediation is a successful technique for the remediation of soils and water polluted with metals (Terry and Bañuelos 2000, Hadi and Bano 2009), with no side effects, more aesthetic benefits and more time-effective technology (Jadia and Fulekar 2009). It has been reported that sunflower is an efficient hyper-accumulator of heavy metals, especially lead and cadmium from soils. Due to more biomass production, fast growing, more canopy and taproot system, sunflower has the ability to remediate metal polluted soils (Sewalem et al. 2014). However, when ions of heavy metals are present in the environment in quantities that exceed the permissible limits, they are taken up by plant roots and reach the shoots, damaging metabolic processes and causing a reduction in growth (Bingham et al. 1986, Mourato et al. 2015). Most of the hyper-accumulators are not suitable for phytoremediation due to their slow growth and low biomass production in contaminated soils (Cheng 2003, Chibuike and Obiora 2014). The use of phytoremediation alone is time consuming, and success

with this technique depends upon accumulation of heavy metals, tolerance to them and biomass formation capacity of plants in a metal stress environment (Grčman et al. 2001, Chibuike and Obiora 2014). This problem could be solved by using phytoremediation associated with a metal-tolerant plant growth promoting rhizobacteria (PGPR) (Ma et al. 2011). Recently, microbe-assisted phytoremediation has emerged as a more successful approach for remediation of metal polluted soils (Koo and Kyung-Suk 2009), since PGPR have the capacity to enhance the growth of host plants through various mechanisms involving the production of specific compounds and increasing nutrient uptake. Several established studies also indicate that PGPR can reduce the toxicity of heavy metals and promote the growth of plants under stress by Ni, Pb or Zn (Jing et al. 2007). Furthermore, some rhizobacteria can excrete organic acids to enhance the bioavailability of heavy metals and certain varieties of bacteria (mainly PGPR) have been reported as phytoextraction assistants, such as *Pseudomonas* spp., *Bacillus* spp., *Mesorhizobium* sp., *Microbacterium* spp., *Rhizobium* spp., *Variovorax* sp., *Rhodococcus* sp., *Psychrobacter* spp., *Flabobacterium* sp., *Sinorhizobium* sp. and *Achromobacter* sp. (Koo and Kyung-Suk 2009). Metal-tolerant bacteria could endure metal stress via different mechanisms like exclusion of metal ions from the cell, intracellular metal ions accumulation and/or sequestration, metals biotransformation to less toxic forms and adsorption/desorption of metals (Mamaril et al. 1997). Mechanisms involved in transfer and mobilization of heavy metals by the rhizosphere microbes include acid and siderophore production, as well as phosphate solubilization (Khan et al. 2009). These metal-tolerant bacteria could enhance phytoremediation through the production of plant growth regulators like auxins, gibberellins and cytokinins (Bottini et al. 2004,

Egamberdiyeva 2007), and can produce a similar effect as exogenously applied growth regulators (Hadi et al. 2010). They may also facilitate the growth of plants by reducing the ethylene-mediated stress by synthesizing ACC deaminase (Belimov et al. 2001). The mechanisms used by *Pseudomonas* sp. to improve the phytoremediation process include the improvement of the biomass production, the release of phytohormones, and the production of siderophore and low molecular weight organic acids. The latter increase the bioavailability and mobility of metals in soil and the accumulation in aerial plant parts (Chen et al. 2014).

The aim of this study was to evaluate the potential effect of selected lead tolerant PGPR on the growth and physiology of *Helianthus annuus* L. as a test crop, to elucidate their phytoremediation potential in the presence of lead stress.

MATERIALS AND METHODS

Five pre-characterized lead tolerant PGPR, *Pseudomonas gessardii* strain BLP141 (S2), *Pseudomonas fluorescens* A50 (S5), *Pseudomonas syringae* pv. *syringae* B728a (S6), *Pseudomonas stutzeri* strain 28a24 (S8), and *Pseudomonas fluorescens* strain LMG 2189 (S10) were selected to analyze their potential on plant growth promoting and phytoremediation. Jars/small pots (400 mL) were employed for cultivation of sunflower plants, filled with sterilized sand and contaminated with lead chloride salt at three concentrations: 300, 600 and 900 mg/kg. Inoculum was prepared by growing the selected strains in 250 mL conical flask with 100 mL of LB broth incubated at 28 ± 2 °C in an orbital shaking incubator at 100 rpm for three days. To attain uniform cell density (10^8 - 10^9 CFU/mL), an optical density of 0.5, recorded at a wavelength of 535 nm, was obtained by dilution. Surface sterilization of sunflower seeds was done by momentarily dipping in ethanol (95 %) and for 3 min in HgCl₂ solution (0.2 %); subsequently six rinses with sterilized water were carried out. By using lead chloride salt, three levels (300, 600 and 900 mg/kg of sand) of lead stress were developed. Sunflower seeds were dipped in the broth of the respective culture of isolates for inoculation for 5 min. Then three inoculated sunflower seeds of each strain were kept in small pots contaminated with different levels of lead and without lead, except for the control, where only LB broth without inoculum dipped seeds was placed. Experiments consisted of 48 pots and after germination one plant per pot was maintained: 36

pots contained inoculated plants and 12 pots remained without inoculum. To meet the nutritional and water requirements of the seedlings, half strength Hoagland solution (Hoagland and Arnon 1950) was applied in the small pots whenever needed. The pots were arranged according to a completely randomized design (CRD) with factorial arrangement and three repeats. Data regarding root/shoot length, fresh and dry weights, and lead contents in plants were determined after 30 days of sowing. Physiological attributes such as photosynthetic and transpiration rates, and substomatal CO₂ were determined using a CIRUS-3 instrument before one day of harvesting. For lead determination, 50 mg of grinded shoots and roots of sunflower samples were placed in conical flasks; a 10 mL HNO₃:HClO₄ mixture in 3:1 ratio (on volume basis) was added to each flask and kept overnight. The next day, all flasks were heated on a hot plate and digested until the material became clear. Then the flasks were cooled and all materials were poured into 50 mL volumetric flasks; distilled water was added to each flask to increase volume up to 50 mL and contents filtered with filter paper. Lead was analyzed with an Atomic absorption spectrophotometer. Total biomass of plant was estimated by shoot dry weight plus root dry weight. For determination of lead removal, total dry weight of a plant was multiplied by its lead content. Lead removal efficiency (LRE) was determined by the following equation:

$$\text{LRE (\%)} = \frac{B1-B2}{B2} \times 100 \quad (1)$$

where B1 is the lead uptake/removal by inoculated plants through and B2 the lead uptake/removal by uninoculated plants.

For the measurement of Pb in sand after harvesting, 2 g of air-dried sand were placed in 50 mL flasks and digested in a mixture of HCl, HNO₃ and HClO₄. Residues were diluted with deionized water and analyzed with an atomic absorption spectrophotometer (Tuzen 2003).

The microbial population was determined by using the dilution plate technique with general purpose agar media (glucose peptone agar media). Media plates were inoculated with sand (obtained from the plants' rhizosphere) solution and incubated at 28 ± 2 °C for 72 h. Colony forming units (cfu/g of sand) from each sample were calculated through a colony counter. Two-way analysis of variance (ANOVA) was performed. Means were compared ($p < 0.05$) by applying Duncan's new multiple range test (DMRT) (Steel and Torrie 1984). Data were analyzed with statistical software (Statstix 8.1).

RESULTS

Lead contamination reduced the shoot length up to 26, 39 and 67 % at 300, 600 and 900 mg/kg lead stress, respectively, as compared to the control crop (which had neither inoculation nor heavy metal stress) (**Table I**). However, the shoot length was increased by inoculation with lead tolerant rhizobacterial isolates as compared to plants grown in metal stress without inoculation. Isolates S2 and S5 showed the most promising results, enhancing the shoot length up to 21 and 18 %, respectively, at 900 mg/kg lead stress, as compared to plants grown at the same stress without inoculation.

It was observed that the shoot fresh and dry weights significantly ($p < 0.05$) decreased by lead

stress as compared to plants grown in normal conditions without lead contamination and inoculation (control) (**Table I**). However, inoculation with lead tolerant rhizobacterial isolates reverted the toxic effect of lead on plants and improved the shoot fresh and dry weights in lead stress. It was noticed that among the isolates, more increment was shown by S2, which promoted increases of 31 and 44 % at 900 mg/kg in shoot fresh and dry weights, respectively, as compared to plants grown at the same stress without inoculation.

Root length was significantly ($p < 0.05$) decreased by lead contamination as compared to control. Reduction in root length was up to 26, 35 and 52 % in lead contamination of 300, 600 and 900 mg/kg, respectively, as compared to plants grown in normal condi-

TABLE I. EFFECT OF LEAD TOLERANT PGPR ON SHOOT ATTRIBUTES (LENGTH, FRESH WEIGHT, AND DRY WEIGHT) OF SUNFLOWER PLANTS EXPOSED TO LEAD

Pb (mg/kg)	Treatment	SL (cm)	SFW (mg)	SDW (mg)
0	No inoculation	20.86 d	1980 c	850 e
	S2	27.08 a	2760 a	1200 a
	S5	26.65 ab	2560 ab	1116 c
	S6	24.09 c	2380 b	1038 d
	S8	25.53 bc	2700 ab	1174 b
	S10	26.60 ab	2700 ab	1177 b
300	No inoculation	16.59 hi	1490 d-i	620 j
	S2	18.66 e	1700 cd	743 f
	S5	18.26 eg	1630 c-f	710 g
	S6	17.5 e-h	1570 d-g	680 h
	S8	18.06 ef	1640 c-f	716 g
	S10	18.18 ef	1650 c-e	721 g
600	No inoculation	15.00 jl	1280 e-j	530 m
	S2	17.067 fi	1500 d-h	656 i
	S5	16.66 gi	1430 d-i	623 j
	S6	15.99 ik	1370 d-i	597 k
	S8	16.46 hg	1440 d-i	630 j
	S10	16.58 hi	1450 d-i	634 j
900	No inoculation	12.47 m	970 j	390 o
	S2	15.06 jl	1270 f-j	56 l
	S5	14.66 kl	1180 h-j	514 l
	S6	13.99 l	1120 ij	489 n
	S8	14.46 kl	1190 h-j	521 m
	S10	14.58 kl	1230 g-j	530 m

PGPR: plant growth promoting rhizobacteria, SL: shoot length, SFW: shoot fresh weight, SDW: shoot dry weight, S2: *Pseudomonas gessardii* strain BLP141 (), S5: *Pseudomonas fluorescens* A50, S6: *Pseudomonas syringae* pv. *syringae* B728a, S8: *Pseudomonas stutzeri* strain 28a24, S10: *Pseudomonas fluorescens* strain LMG 2189

Means sharing the same letters are statistically non-significant with the Duncan's new multiple range test at $p < 0.05$

tions with no contamination was applied (**Table II**). However, lead tolerant rhizobacterial isolates increased the root length at all levels of lead stress as compared to plants grown in lead stress without inoculation. Among the five isolates, S5 and S10 showed better results and promoted the root length up to 32 and 26 % at 900 mg/kg lead contamination, respectively, as compared to plants grown at the same stress without inoculation.

A significant ($p < 0.05$) reduction in root fresh and dry weight was recorded by the exposure of sunflower plants to lead stress. Root fresh and dry weights reduced up to 120 and 107 % in lead contamination of 900 mg/kg, respectively, as compared to control without metal stress and inoculation (**Table II**). Lead tolerant rhizobacterial isolates significantly ($p < 0.05$) increased root fresh and dry

weights at all levels of lead contamination as compared to plants grown in stress without inoculation. It was observed that among the five isolates, S10 remained at the top and improved the root fresh and dry weights up to 45 and 27 %, respectively, as compared to plants grown at same stress without inoculation.

Results showed that reduction in physiological attributes (photosynthetic rate, transpiration rate and substomatal CO_2) was observed due to lead contamination (**Table III**). However, improvement in the physiological attributes of sunflower in lead contamination was observed by the application of lead tolerant bacteria as compared to plants grown in lead contamination without inoculation. Data showed that the maximum increment (12 %) in photosynthetic rate was shown by S10 at 900 mg/kg lead contamination, as compared to plants grown at the

TABLE II. EFFECT OF LEAD TOLERANT PGPR ON ROOT ATTRIBUTES (ROOT LENGTH, ROOT FRESH WEIGHT AND ROOT DRY WEIGHT) OF SUNFLOWER PLANTS EXPOSED TO LEAD

Pb (mg/kg)	Treatment	RL (cm)	RFW (mg)	RDW (mg)
0	No inoculation	15.86 c	640 d	310 d
	S2	20.53 ab	827 b	420 ab
	S5	22.083 a	872 a	400 bc
	S6	19.09 b	769 c	370 c
	S8	21.603 a	870 a	420 ab
	S10	21.657 a	889 a	430 a
300	No inoculation	11.73 d-h	430 j-l	230 g-i
	S2	13.06 de	526 ef	250 e-g
	S5	13.66 d	380 mn	260 e
	S6	12.59 df	507 fg	240 e-h
	S8	13.18 de	531 ef	250 ef
	S10	13.26 de	534 ef	260 ef
600	No inoculation	10.23 jk	550 e	190 kl
	S2	11.46 e-j	462 h-j	220 h-j
	S5	12.06 d-g	470 hi	230 e-i
	S6	10.99 f-k	443 i-k	210 i-k
	S8	11.58 e-i	466 hi	230 g-i
	S10	11.66 d-h	486 gh	230 g-i
900	No inoculation	7.69 l	290 o	150 m
	S2	9.467 kl	381 mn	190 kl
	S5	10.17 jk	400 lm	180 l
	S6	8.993 l	360 n	170 l
	S8	9.583 i-l	386 mn	180 kl
	S10	9.74 h-k	420 kl	190 j-l

PGPR: plant growth promoting rhizobacteria, RL: root length, RFW: root fresh weight, RDW: root dry weight, S2: *Pseudomonas gessardii* strain BLP141 (), S5: *Pseudomonas fluorescens* A50, S6: *Pseudomonas syringae* pv. *syringae* B728a, S8: *Pseudomonas stutzeri* strain 28a24, S10: *Pseudomonas fluorescens* strain LMG 2189 Means sharing the same letters are statistically non-significant with the Duncan's new multiple range test at $p < 0.05$

same stress without inoculation. Maximum improvement in transpiration rate (9 %) was shown by S2 at 900 mg/kg lead stress, as compared to plants grown at the same stress without inoculation. Results show a 13 % increment in substomatal CO₂ stimulated by S5 at 900 mg/kg lead contamination as compared to plants grown at the same stress without inoculation.

Lead concentration in the root of inoculated sunflower plants in lead contamination significantly ($p < 0.05$) increased as compared to plants grown in lead stress without inoculation (**Table IV**). Isolates S10 and S2 showed maximum improvement in lead concentration in root of plants in lead stress and increased the lead content in root up to 18 and 17 %, respectively, at highest level of lead as compared to the same level of uninoculated lead treatment. Isolate S8 showed minimum increment in lead content in

root and increased only 9 % lead content in root at 900 mg/kg (of sand) lead stress as compared to plants grown in uninoculated same lead stress.

Inoculation with lead tolerant bacteria improved the lead concentration in shoot of sunflower plants at all levels of lead contamination as compared to plant grown in metal stress without inoculation (**Table IV**). Among the isolates, S5 and S10 improved maximum metal content in shoot up to 21 and 20 %, respectively, at highest level of lead as compared to same level of un-inoculated lead. Isolates S6 and S8 showed least effective performance and enhanced minimum metal content in shoot of sunflower in lead contamination as compared to plants grown in lead contamination without inoculation. Data (**Table IV**) also showed that maximum lead removal efficiency was observed by isolates S10 and S5 at 900 mg/kg

TABLE III. EFFECT OF LEAD TOLERANT PGPR ON PHYSIOLOGICAL ATTRIBUTES (PHOTOSYNTHETIC RATE, TRANSPIRATION RATE AND SUBSTOMATAL CO₂) OF SUNFLOWER PLANTS EXPOSED TO LEAD

Pb (mg kg ⁻¹)	Treatment	A (μmol/m/s)	E (mmol /m/s)	Ci (μmol/mol)
0	No inoculation	13.30 d	5.10 e	810 c
	S2	13.48 c	5.70 a	837 b
	S5	13.98 ab	5.29 d	880 a
	S6	13.68 b	5.33 d	870 b
	S8	13.62 c	5.40 c	840 b
	S10	14.08 a	5.61 b	865 a
300	No inoculation	10.68 k	5.65 k	620 f
	S2	11.08 hi	6.00 f	669 de
	S5	11.68 f	5.95 h	680 d
	S6	11.18 gh	5.89 h	655 e
	S8	11.28 g	5.85 h	651 e
	S10	11.88 e	5.95 g	660 e
600	No inoculation	9.68 l	5.45 l	540 j
	S2	10.68 k	5.80 i	568 gh
	S5	11.02 h-j	5.65 k	580 g
	S6	10.94 ij	5.67 k	555 h-j
	S8	10.88 j	5.63 k	547 ij
	S10	12.18 gh	5.75 j	560 hi
900	No inoculation	8.88 o	4.80 q	460 m
	S2	9.58 m	5.20 m	518 k
	S5	9.92 l	4.95 p	520 k
	S6	9.28 n	5.10 n	510 k
	S8	9.38 n	5.05 o	505 k
	S10	9.98 l	5.13 n	480 l

PGPR: plant growth promoting rhizobacteria, A: photosynthetic rate, E: transpiration rate, Ci: substomatal CO₂, S2: *Pseudomonas gessardii* strain BLP141 (), S5: *Pseudomonas fluorescens* A50, S6: *Pseudomonas syringae* pv. *syringae* B728a, S8: *Pseudomonas stutzeri* strain 28a24, S10: *Pseudomonas fluorescens* strain LMG 2189

Means sharing the same letters are statistically non-significant with the Duncan's new multiple range test at $p < 0.05$

(of sand) lead stress as compared to other isolates at same lead concentration. Results regarding microbial population in rhizosphere and lead content (**Table V**) in sand after harvesting revealed that S2, S5 and S10 isolates performed better in lead contamination as compared to control treatment without inoculation and other isolates in lead stress. Isolates S10 and S5 showed maximum lead removal capacity among the isolates in lead stress and treatments having lead stress without inoculation.

Figure 1 shows that lead contamination reduced the total biomass of plants as compared to plants grown in control without inoculation and contamination. However, application of lead tolerant bacteria reversed the toxic effect of lead on plants and

enhanced total biomass of plants at all levels of lead contamination. Among all isolates, isolates S2, S5 and S10 showed most promising results at all levels of lead as compared to other isolates.

DISCUSSION

The present study was conducted to evaluate lead tolerant PGPR for plant growth promotion and phytoremediation potential exposed to lead stress by using sunflower as test crop. Our results showed a reduction in the growth and physiology of sunflower in lead contamination as compared to plants grown in uninoculated treatment without contamination.

TABLE IV. EFFECT OF LEAD TOLERANT PGPR ON THE PHYTOREMEDIATION POTENTIAL (LEAD IN ROOT AND SHOOT) OF SUNFLOWER PLANTS AND LEAD REMOVAL EFFICIENCY OF STRAINS

Pb (mg/kg)	Treatment	Pb (mg/kg)		Lead removal efficiency of strains (%)
		Root	Shoot	
0	No inoculation	ND	ND	ND
	S2	ND	ND	ND
	S5	ND	ND	ND
	S6	ND	ND	ND
	S8	ND	ND	ND
	S10	ND	ND	ND
300	No inoculation	146 l	104 k	ND
	S2	180 i	124 j	21.60
	S5	175 j	142 g	26.80
	S6	172 j	129 ij	20.40
	S8	167 k	132 gi	19.60
	S10	185 h	137 gh	28.80
600	No inoculation	205 g	162 f	ND
	S2	232 e	182 e	12.80
	S5	231 e	190 d	14.70
	S6	224 f	181 e	10.35
	S8	225 f	188 d	12.53
	S10	233 e	189 d	14.98
900	No inoculation	304 d	261 c	ND
	S2	358 a	287 b	14.15
	S5	333 bc	317 a	15.04
	S6	335 b	292 b	10.97
	S8	330 c	290 b	09.73
	S10	360 a	315 a	19.46

PGPR: plant growth promoting rhizobacteria, ND: non detectable, S2: *Pseudomonas gessardii* strain BLP141 (), S5: *Pseudomonas fluorescens* A50, S6: *Pseudomonas syringae* pv. *syringae* B728a, S8: *Pseudomonas stutzeri* strain 28a24, S10: *Pseudomonas fluorescens* strain LMG 2189

Means sharing the same letters are statistically non-significant with the Duncan's new multiple range test at $p < 0.05$

TABLE V. MICROBIAL POPULATION IN RHIZOSPHERE, LEAD REMOVAL CAPACITY OF STRAINS AND LEAD IN SAND AFTER HARVESTING

Pb (mg/kg)	Treatment	Pb in sand after harvesting (mg/kg)	Pb removal capacity (mg/kg)	Microbial population in rhizosphere (CFU/g)
0	No inoculation	ND	ND	Negligible
	S2	ND	ND	3.45×10^5
	S5	ND	ND	4.12×10^5
	S6	ND	ND	2.30×10^5
	S8	ND	ND	3.60×10^5
	S10	ND	ND	4.12×10^5
300	No inoculation	109 g	250 j	Negligible
	S2	55 hi	304 hi	3.33×10^5
	S5	42 ij	317 hi	3.90×10^5
	S6	58 hi	301 i	3.23×10^5
	S8	60 h	299 i	3.39×10^5
	S10	37 j	322 h	3.67×10^5
600	No inoculation	292 d	367 g	Negligible
	S2	245 ef	414 f	3.45×10^5
	S5	238 ef	421 f	4.12×10^5
	S6	254 e	405 f	2.30×10^5
	S8	246 ef	413 f	3.60×10^5
	S10	237 f	422 f	4.70×10^5
900	No inoculation	394 a	565 e	Negligible
	S2	314 c	645 bc	4.50×10^5
	S5	309 c	650 b	4.40×10^5
	S6	332 b	627 cd	4.36×10^5
	S8	339 b	620 d	2.13×10^5
	S10	284 d	675 a	4.67×10^5

PGPR: plant growth promoting rhizobacteria, ND: non detectable, S2: *Pseudomonas gessardii* strain BLP141 (), S5: *Pseudomonas fluorescens* A50, S6: *Pseudomonas syringae* pv. *syringae* B728a, S8: *Pseudomonas stutzeri* strain 28a24, S10: *Pseudomonas fluorescens* strain LMG 2189

Means sharing the same letters are statistically non-significant with the Duncan's new multiple range test at $p < 0.05$

These findings are similar to those of Hussain et al. (2006, 2013) and McComb et al. (2012). It is known that the reduction in plant growth by lead

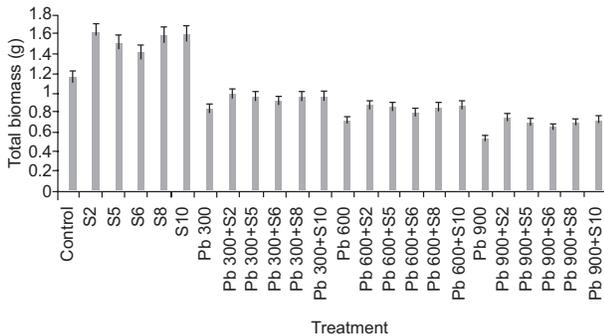


Fig. 1. Effect of lead tolerant plant growth promoting rhizobacteria on total biomass of sunflower plants exposed to lead

contamination might be due to its interference with the uptake of nutrients, respiration, photosynthetic rate and cell membrane permeability (Sharma and Dubey 2005). Lead can also disrupt the activity of enzymes in various metabolic pathways such as those involved in the photosynthetic Calvin cycle, and the metabolism of nitrogen and sugar (Verma and Dubey 2003).

Data regarding a reduction in physiological attributes (photosynthetic and transpiration rate, substomatal CO₂) by lead contamination might be due to lead, which prevents the incorporation of Fe in the phyto porphyrin ring of the chlorophyll molecule, causing a reduction in chlorophyll synthesis/content (Shakya et al. 2008). Lead decreases the synthesis of chlorophyll either by reducing the chlorophyllase activity or by decreasing the uptake of essential elements like Mg and Fe by plants (Sharma and Dubey

2005). Lead also causes degradation of the chlorophyll molecule (Dogan and Colak 2009).

Inoculation with lead tolerant bacteria in lead contamination reversed the toxic effect of lead and improved the growth and physiology of sunflower as compared to uninoculated plant grown in lead stress. This improvement with lead tolerant PGPR might be due to phosphate solubilization (Yasmin and Bano 2011, Peña and Reyes 2007), siderophore production (Glick et al. 1999, Meyer 2000), phytohormones production (Asghar et al. 2004, Humphry et al. 2007), and induced systemic resistance in plants against phytotoxicity of metals (Mishra et al. 2006), which may result in plant growth promotion through the improvement of total biomass production. PGPR may also improve the uptake and availability of nutrients by recycling of organic wastes (Asghar et al. 2006). Kumar et al (2009) reported that PGPR *Enterobacter aerogenes* and *Rahnella aquatilis* decreased Ni and Cr toxicity in *Brassica juncea* (Indian mustard) and improved plant growth under pot culture experiments.

Improvement of physiological parameters in plants exposed to lead contamination by inoculation with lead tolerant PGPR might be due to the promotion of Fe uptake by these bacteria in plants, which could have enhanced the chlorophyll content (Burd et al. 2000) and improved the leaf area, which could ultimately improve the photosynthetic rate and other physiological attributes of plants (Glick et al. 1999).

Our research showed that inoculation with lead tolerant bacteria improved the lead content in roots and shoots of sunflower as compared to plants grown in stress without inoculation. These results are similar to the work of Braud et al. (2009), Ma et al. (2011), and Kamran et al. (2015). Data regarding improvement in lead concentration in roots and shoots by inoculation of lead tolerant PGPR might be due to the capability of lead tolerant bacteria to reduce the pH of soil, which may facilitate metals uptake by converting these elements into a soluble and available form (Abou-Shanab et al. 2006). Bacteria could have produced organic acids, degrading enzymes, iron chelators and siderophores, thus reducing the toxic effect of metals on plants and increasing the uptake of heavy metals (Yousaf et al. 2010). By changing the solubility and availability of pollutants and the production of organic acids, chelates, and redox enhance, plant growth promoting rhizobacteria enhance the uptake of heavy metals in plants, which might also be due to the rhizospheric microbial population in inoculated treatments that

mobilize metals and enhance the uptake in plants. For this reason, in the inoculated treatment there is a low amount of lead in sand after harvesting, as compared to lead in contaminated treatments without inoculation. The study of Krishna et al (2012) also suggested that heavy metal levels can be minimized by using bacteria with the ability to produce plant growth promoting trends. Soil microbes associated with plant roots are also helpful in the phytoextraction of heavy metals through the degradation of pollutants (Liao et al. 2006).

This research showed that the evaluation of lead tolerant PGPR on the basis of their plant growth promotion activities, as well as their physiology and phytoremediation potential, is a viable approach to improve plant growth and physiology to remediate heavy metal contamination. Plant growth, physiology and phytoremediation potential can be improved by the synergistic use of plants and microbes.

CONCLUSION

It might be concluded from this study that lead contamination adversely affected the plant growth and physiology of sunflower. However, the application of lead tolerant PGPR reversed the toxic effect of lead on plant growth and physiology. The study showed that *Pseudomonas gessardii* strain BLP141, *Pseudomonas fluorescens* A506, and *Pseudomonas fluorescens* strain LMG 2189 have a phytoremediation potential, as well as the ability to induce stress tolerance in plants growing in lead stress, and to improve the growth and physiology (photosynthetic rate, transpiration rate and substomatal CO₂), in plants under various levels of lead contamination.

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