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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.

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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

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