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Plant growth promoting bacteria enhances photosynthesis, nodulation and root system architecture in lentil under lead toxicity

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ABSTRACT

The usage of plant growth-promoting bacteria (PGPB) in mitigation of harmful effects of lead (Pb) toxicity in plants and bioremediation of it from soils is a sustainable, effective and low-cost strategy. The experiment was laid out to investigate the role of PGPB on morphological and physiological growth, root system architecture and nodulation of lentil under Pb stress. The experiment was conducted according to completely randomized factorial design with four replications at the laboratory of the Field Crops Department, Siirt University, Siirt in 2022. The four Pb levels and three bacterial inoculations were used in the experiment. Plant height, seedling fresh weight, root fresh weight, seedling dry weight, root dry weight, total chlorophyll content, taproot length, number of lateral roots, total root length and number of nodule varied between 15.7-25.9 cm, 0.123-0.235 g, 0.019-0.092 g, 0.0104-0.0326 g, 0.0076-0.0146 g, 27.9-47.2%, 8.9-19.2 cm, 4.00-14.67, 17.6-44.8 cm and 1.37-10.63, respectively. Bio-priming with PGPB containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity increased dry matter accumulation up to 56.1% and 22.9% in shoots and roots, respectively. Chlorophyll content enhanced up to 17.9% compared with control application. Besides, root system architecture and nodule formation were improved by bio-priming both under stress and non-stress conditions. Bio-priming with PGPB may be a sustainable solution to mitigate oxidative stress and promote plant growth and yield in lentil under Pb-contaminated soils.

Keywords: biological nitrogen fixation, Pb contamination, *Lens culinaris*, oxidative stress, seed priming

INTRODUCTION

Although lead (Pb) is a vulnerable heavy metal for the industry due to its various properties such as low melting temperature, high density, easy molding and acid resistance, it has destructive impacts on the health of all living cells (Mitra et al., 2020). Accumulation of Pb in soils caused contaminated agricultural lands, therefore, Pb has included in the food chain through the last quarter-century. Besides, Pb has low solubility and is classified as both carcinogenic and mutagenic (Diels et al., 2002). Due to this threatening phenomenon, studies on lead toxicity in agricultural soils have been increasing nowadays.

The Pb is considered the second harmful heavy metal after arsenic (Pourrut et al., 2011). Plants suffer from Pb toxicity in contaminated soils, thereby, it reduces nutrient uptake, photosynthesis, plant growth and development, water and ion balance, enzyme activity, membrane stability and permeability (Bharwana et al., 2013; Ashraf et al., 2015; Javed et al., 2018; Yin et al., 2018; Neethu et al., 2020) and induces lipid peroxidation, osmotic stress depending on the synthesis of reactive oxygen species (ROS) and ethylene (Verma and Dubey, 2003; Huihui et al., 2020).

Moreover, the higher concentration of Pb inhibits CO_2 fixation and photosynthesis rate via denaturing the structure of chloroplast and pigment-protein integration (Sharma and Dubey, 2005). Thus, Pb dynamics in soil and

remediation strategies such as physiological, chemical and biological methods have a pivotal role in agricultural production and food safety. Biological strategies, which are based on three basic methods including phytoremediation, microbe-induced remediation and remediation by organic materials, are eco-friendly and cost-effective ways for cleaning Pb contaminated soils (Zulfiqar et al., 2019). Out of them, microbe-induced remediation with plant growth-promoting bacteria (PGPB) is not only successful in remediation of heavy metals by precipitate, sequester, or changing the oxidation state of Pb (Kang et al., 2016), and also alleviate oxidative stress in the plant by 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Çığ et al., 2021; Murali et al., 2021).

PGPB can be described as bacterial strains inducing nutrient and water uptake, gaining nitrogen and phosphorus to plants by biological nitrogen fixation and phosphate mineralization and promoting plant growth (Glick, 2020). Besides, PGPB increases stress tolerance due to mechanisms such as secretion of various phytohormones, vitamins and growth regulators, restriction of ethylene synthesis with ACC deaminase activity, decreasing of pathogen damage by the secret of antibiotic and fungicidal compounds (Ha-Tran et al., 2021; Mushtaq et al., 2021). PGPB strains have been used to mitigate Pb stress and bioremediate in many plant species including sunflower (Motesharezadeh and Savaghebi, 2011), pea (Shabaan et al., 2020), spinach (Zafar-ul-Hye et al., 2020), clover (Shah et al., 2020), tomato (Khanna et al., 2019) and mung bean (Arif et al., 2019). However, there is insufficient information about the impacts of Pb toxicity on lentil growth and the usage of PGPB in alleviating Pb stress. It is known that Pb toxicity has adverse effects on germination characteristics and inhibits seedling growth in lentil (Kıran and Şahin, 2005; Cokkizgin and Cokkizgin, 2010), but, impacts of Pb on development of root system architecture (RSA), nodulation, chlorophyll content, enzymatic and genetic responses are not clear. In addition, Jebara et al. (2015) stated that inoculation of *Lens culinaris* with bacterial strains that were isolated from root nodules of lentils constituted a symbiotic system useful for phytostabilization of Pb-contaminated soils and improved antioxidant defense systems in plants under Pb stress. However, it is not clear the influences of non-symbiotic PGPB strains on lentil growth, development, response to nodulation and plant physiology. Thus, this study aimed to investigate the improving role of PGPB strains on lentil growth, root characteristics and nodulation under Pb stress.

MATERIAL AND METHODS

Experimental materials

The red lentil cultivar (cv.Tigris), which was registered by GAP International Agricultural Research and Training Center (GAPUTAEM) by selection method in 2013, was used in the experiment. Tigris has high adaptation to region environmental conditions, and has early-maturing phenology (Aslan and Gayberi, 2017). Lead nitrate ($Pb(NO_3)_2$) was used in the experiment to constitute artificial Pb stress in target plants. Two PGPB strains (KF58C and KF58B), which were isolated from rural areas of Siirt province in 2020 and various superior traits such as nitrogen fixation, phosphate solubilizing, siderophore production and ACC deaminase enzyme activity were determined by laboratory tests, were used for bio-priming. The descriptive informations of strains were given in Table 1.

Multiplication of strains and bio-priming process of lentil seeds

The nutrient agar solution was prepared to take 20 g nutrient agar for each liter of distilled water and sterilized at 121 °C for 15 minutes by autoclave. Bacterial strains, which have been protected at -86 °C as the stock culture in the laboratory of Siirt University, were taken by sterile needle and sown on solidified feed-lots. Bacterial strains were incubated at 2±25 °C for 24 hours. The nutrient broth (Merck-VM775843711) was used for the liquid feed-lot. The just one colony was taken from nutrient agar medium, transferred into nutrient broth liquid feed-lot and incubated at 2±26 °C for 24 h and 120 rpm in the shaker. The bacteria concentrations were turbidimetrically arranged to ~10^8 CFU ml^{-} (Sonkurt and

Table 1. Descriptive informations and superior properties of PGPB strains

Code of strain	Specie name	Nitrogen fixation	Phosphorus solubilizing	Siderophore production	ACC deaminase activity
KF58C	*Microbacterium oxydans*	+	L	++	+
KF58B	*Brevibacterium frigoritolerans*	+	L	+++	++

(Bacterial strains were chosen due to their ACC deaminase activity and siderophore production abilities. The common trait of them is ACC deaminase enzyme activity since this enzyme is particularly has a major role in stress management due to restriction of ethylene synthesis during stress. Moreover, siderophore production provides advantages on nutrient uptake to plants under stress conditions. KF58B has higher siderophore production and ACC deaminase enzyme activity compared with KF58C and other properties nearly equal. +++: Very high, ++: High, +: Normal, L: Low)

Çığ, 2019). Before bacterial inoculation, lentil seeds were subjected to surface sterilization with 70% ethyl alcohol for 1 minute and 10% sodium hypochlorite (NaOCl) for 5 minutes. Seeds were primed with bacterial strains for 4 hours. After biopriming, seeds were dried to initial moisture content for 24 hours under dark conditions. Control seeds were subjected to the pure and sterile nutrient broth to eliminate the impacts of early water uptake on germination and seedling growth.

Experimental area and design

The experiment was laid out under controlled conditions in the laboratory of the Field Crops Department in the Faculty of Agriculture, Siirt University, Siirt, Turkey. The temperature levels were between 23-25 and 15-17 °C during day and night, respectively. The humidity level changed between 60-70% and the light/dark period was 14:10 h.

The experiment was conducted according to completely randomized factorial design with four replications and repeated two times. The four Pb levels (P0: Control, P1: 1 mM, P2: 2 mM and P3: 3 mM $Pb(NO_3)_2$) and three bacterial inoculations (Control: No inoculation, B1: Bio-priming with KF58C and B2: Bio-priming with KF58B) were used in the experiment. The 3 kg capacity pots were used and filled with a homogenous mixture of peat:soil at a ratio of 1:2 w/w. The soil was excavated from the A-horizon of arable land of Siirt University and sieved before mixing with peat. The pots were filled with air-dried soil-peat mixture. A pot was saturated with tap water up to 100% of field capacity by the method of Amiri et al. (2017). After determining the volume of required water for %100 field capacity, the required water amount was calculated for 80% moisture levels. All pots were adjusted to 80% of field capacity at the beginning of the study.

Surface sterilization was done to control seeds by 10% of sodium hypochlorite (NaOCl) for 5 minutes and seeds were washed three times with distilled water then dried by filter paper to initial water content for one hour at room temperature. Eight seeds were sown in each pot and they were thinned to 4 plants one week after stand establishment, therefore, each replication was constituted from four plants. A pot experiment was laid out by artificial contamination of $Pb(NO_3)_2$ solutions including 1, 2 and 3 mM. The stock solution was prepared using $Pb(NO_3)_2$ (Merck, Modderfontein, South Africa). A basal dose of N, P_2O_5 and K_2O, were applied at the rate of 25, 50 and 50 kg per hectare in the form of urea, triple superphosphate (TSP), and potassium sulphate (SOP), respectively (Mahmood et al., 2010). The pots were irrigated once a week according to 80% of field capacity with Pb-containing solutions from tinning time to at the end of five weeks. Control plants were irrigated with tap water.

Harvest and experimental observations

Plants were harvested at the end of five weeks. Before harvesting, chlorophyll content (TCC) of upper lentil leaves was determined by SPAD meter (SPAD-502 Konica Minolta Sensing, Inc., Japan). Three readings were done from upper young and fresh leaves of each plant from each pot, thereby, the mean of twelve readings represented the pot TCC value (Zakeri ve ark., 2015).

Plant height (PH) of each plant was measured. Four plants from each pot were uprooted and washed carefully under running tap water to remove the peat-soil mixture. Plants were dried between two layers of filter papers to remove surface water and separated into root and shoot, and immediately weight to determine root fresh weight (RFW) and shoot fresh weight (SFW). The number of nodules (NN) was manually counted. Scanning and image analysis software was used to determine taproot length (TL), the number of lateral roots (NRL) and total root length (TRL). Root samples were placed on a flat and white surface and scanned by the hand-scanner (ISCAN, handheld scanner) in color at 600 dpi resolution. The characteristics of the scanned samples were found in the Image J program by Ceritoglu et al. (2020). After the scanning process, root and shoot samples were separately oven-dried at 68 °C up to constant weight and then weighed to determine the shoot dry weight (SDW) and root dry weight (RDW).

Statistical analysis

The normality of the data was tested using the Shapiro-Wilks test. The data conformed to the assumption of normality. Results were calculated with analysis of variance according to the completely randomized factorial design and Fisher's Least Significant Difference (LSD) test was applied to find the reason for possible differences by R software v.3.5.2 (R Core Team, 2018).

RESULTS

The experiment was laid out to investigate the response of lentil to different levels of Pb toxicity at the seedling stage and evaluate the mitigative impacts of bio-priming with PGPB strains. All Pb levels, particularly 2 and 3 mM, had destructive effects on seedling growth, chlorophyll content and nodule formation. Bio-priming applications with both PGPB strains were effective in the alleviation of Pb-stress. The strains (KF58C and KF58B) are nearly equal in terms of nitrogen fixation and phosphate solubilizing capacity, however, KF58B is superior as siderophore production and ACC deaminase activity compared with KF58C. Thus, although both strains reduced plant stress, KF58B exhibited higher performance in the alleviation of plant stress and preserved the plant from the detrimental effects of Pb-toxicity.

Bio-priming application and Pb-toxicity caused statistically significant differences ($P<0.01$ or $P<0.05$) in all investigated properties. Interaction of bio-priming and Pb-toxicity led to observing statistically significant differences in RFW and TL, but not the others. The PH, SFW, RFW, SDW and RDW varied between 15.7-25.9 cm, 0.123-0.235 g, 0.019-0.092 g, 0.0104-0.0326 g and 0.0076-0.0146 g, respectively. The highest PH was obtained from KF58B-primed seeds with 21.9, while the lowest PH was observed in control seeds. Increasing Pb levels significantly reduced the PH. The highest SFW was observed in bio-primed seeds with KF58B while the lowest one was obtained by KF58C-primed seeds. In terms of Pb levels, the highest SFW was determined in the control group, it decreased with rising Pb levels, but, all of them were in the same statistical group. Control and KF58B-primed seeds were nearly equal and higher than KF58B-primed seeds in terms of RFW. The 2 mM Pb and higher Pb concentrations drastically affected the RFW and the lowest RFW was seen in the 3 mM Pb level with 0.44 g. In terms of dry matter accumulation in shoots and roots, bio-priming with KF58B promoted biomass and dry matter accumulation compared with control and KF58C. The KF58B-primed seeds stimulated dry matter accumulation up to 56.1% and 22.9% in shoots and roots compared with control seeds, respectively. Similar to other properties, Pb toxicity inhibited dry matter accumulation in all treatments. Dry matter accumulation decreased up to 93.6% and 45.8% in shoots and roots depending on rising Pb toxicity, respectively. Thus, shoots were more drastically affected by Pb toxicity compared with roots (Table 2).

The TCC, TL, NRL, TRL and NN changed between 27.9-47.2%, 8.9-19.2 cm, 4.00-14.67, 17.6-44.8 cm and 1.37-10.63, respectively. The TCC increased with bio-priming applications compared with control. Pb toxicity reduced the TCC, which was the lowest (32.6%) in 3 mM Pb whereas it was the highest (46.1%) in control. The TCC decreased up to 41.4% depending on Pb toxicity, and it increased up to 17.9% with bio-priming compared with

Table 2. Seedling growth and dry matter accumulation in lentil plants depending on bio-priming applications under different Pb levels

Observation	Bacteria	Lead levels				
		0 mM	1 mM	2 mM	3 mM	Mean
PH (cm)	Control	21.9	19.3	18.2	15.7	18.7B
	KF58C	25.0	22.3	20.0	17.3	21.1AB
	KF58B	25.9	22.8	20.8	18.1	21.9A
	Mean	24.3A	21.4AB	19.7BC	17.0C	
SFW (g)	Control	0.215	0.149	0.137	0.123	0.156B
	KF58C	0.228	0.166	0.156	0.153	0.176AB
	KF58B	0.235	0.208	0.188	0.181	0.203A
	Mean	0.226A	0.174B	0.160B	0.152B	
RFW (g)	Control	0.062cd	0.078a-c	0.086ab	0.039e	0.066A
	KF58C	0.072b-d	0.088ab	0.060d	0.019f	0.060B
	KF58B	0.090a	0.092a	0.011f	0.075a-d	0.067A
	Mean	0.075B	0.086A	0.052C	0.044D	
SDW (g)	Control	0.0274	0.0185	0.0131	0.0104	0.0173C
	KF58C	0.0305	0.0253	0.0194	0.0163	0.0229B
	KF58B	0.0326	0.0287	0.0262	0.0202	0.0270A
	Mean	0.0302A	0.0242B	0.0196BC	0.0156C	
RDW (g)	Control	0.0130	0.0127	0.0104	0.0076	0.0109B
	KF58C	0.0144	0.0134	0.0117	0.0098	0.0123AB
	KF58B	0.0146	0.014	0.0136	0.0116	0.0134A
	Mean	0.0140A	0.0134A	0.0119AB	0.0096B	
		PH	SFW	RFW	SDW	RDW
LSD (Bacteria)		3.08*	0.032**	0.006*	0.0039**	0.0022*
LSD (Lead)		3.94**	0.041**	0.008**	0.0050**	0.0018**
LSD (BxL)		8.94ns	0.093ns	0.018**	0.0114ns	0.0065ns
MS		24.7	0.0035	0.0020	0.00013	0.000012
CV (%)		14.6	17.7	9.4	17.2	18.0

(PH: Plant height, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight, MS: Mean of squares, CV: Coefficient of variation, **: $P<0.01$, *: $P<0.05$, ns: No significant difference)

Table 3. Differences in total chlorophyll content, root system characteristics and nodule formation depending on bio-priming applications under different Pb toxicity

Observation	Bacteria	Lead levels				
		Control	1 mM	2 mM	3 mM	Mean
TCC (%)	Control	43.8	38.7	32.5	27.9	35.7B
	KF58C	47.2	43.2	38.1	33.4	40.5A
	KF58B	47.2	44.5	40.0	36.6	42.1A
	Mean	46.1A	42.1B	36.9C	32.6D	
TL (cm)	Control	18.1ab	15.0cd	13.5de	8.9f	13.8B
	KF58C	16.6bc	14.4d	14.3d	9.2f	13.2B
	KF58B	19.2a	18.3ab	14.7d	12.5e	16.6A
	Mean	17.9A	15.9B	13.4C	10.9D	
NLR	Control	11.43	7.83	5.50	4.00	7.19B
	KF58C	12.83	10.00	7.13	5.77	8.93AB
	KF58B	14.67	11.00	8.17	7.33	10.29A
	Mean	12.98A	9.61B	6.93C	5.70C	
TRL (cm)	Control	36.0	28.8	23.2	17.6	26.4B
	KF58C	40.1	31.1	21.3	19.6	28.0AB
	KF58B	44.8	40.1	35.6	31.8	38.1A
	Mean	40.3A	33.3AB	26.7AB	23.0B	
NN	Control	7.70	5.00	1.93	1.37	4.00B
	KF58C	8.67	6.27	5.37	2.07	5.59AB
	KF58B	10.63	8.33	6.07	4.43	7.37A
	Mean	9.00A	6.53AB	4.46BC	2.62C	
		TC	TL	NLR	TRL	NN
LSD (Bacteria)		1.75**	0.91**	0.179**	11.4*	1.98**
LSD (Lead)		2.22**	1.05**	2.286**	14.5*	2.53**
LSD (BxL)		5.05ns	1.81*	5.184ns	33.0ns	5.74ns
MS		94.2	27.4	27.4	227.8	21.4
CV (%)		4.3	7.4	19.8	16.1	14.2

(TCC: Total chlorophyll content, TL: Taproot length, NRL: Number of lateral roots, TLR: Total root length, NN: Number of nodules, MS: Mean of squares, CV: Coefficient of variation, **: $P<0.01$, *: $P<0.05$, ns: No significant difference)

control plants. Bio-priming promoted the RSA properties containing the TL, NRL and TRL compared with control seeds under different Pb levels. The highest TL, NRL and TRL were determined as 16.6 cm, 10.29 and 38.1 cm in KF58B-primed seeds, respectively. The lowest TL, NRL and TRL were observed as 13.8 cm, 7.19 and 26.4 cm in control seeds, respectively. The TL, NRL and TRL decreased up to 64.2%, 127.7% and 75.2% depending on Pb toxicity, respectively. Bio-priming with KF58B exhibited higher performance on nodule formation compared with KF58C and the control group. The highest NN was obtained from KF58B with 7.37 while the lowest one was observed in control with 4.00. Pb toxicity reduced the NN up to 243.5% compared with non-stress conditions (Table 3).

DISCUSSION

The experiment revealed that a higher concentration of Pb (>1 mM) caused significant growth inhibitions on different morphological and physiological processes such as total biomass, nodulation, dry matter accumulation and the RSA in above- and underground parts of lentil seedlings. In addition, bio-priming with PGPB containing siderophore production and ACC deaminase activity had a pivotal role in the mitigation of Pb stress. The results are in agreement with experiments in which inoculation of seeds with Pb-resistant bacterias helps plants in the alleviation of Pb stress in sunflower (Saleem et al., 2018), pea (Shabaan et al., 2021), wheat (Janmohammadi et al., 2013), rice (Pal and Sengupta, 2016), grass pea (Abdelkerim et al., 2018), tomato (Burd et al., 2000) and chili (Pal et al., 2018).

Pb has destructive effects on plants from germination to maturity due to antagonistic impacts on enzymes such as α-amylase, protease, δ-aminolevulinate, ribulose 1,5-bisphosphate and glutamine synthetase (Lee et al., 1976; Prassad and Prassad, 1987; Sengar et al., 2009). Visual non-specific symptoms of lead toxicity in plants are inhibition of root growth by affecting the RSA, blackening of the root surface, stunted aboveground growth, chlorosis and leaf fall (Dogan et al., 2009). Reduction of seedling growth may be due to inhibition of nutrient uptake by lead toxicity, thereby, it caused the decrease of respiration rates and photosynthetic activity of leaves (Sharma and Dube, 2005; Hadi and Aziz, 2015). Also, Verma and Dubey (2003) indicated that Pb toxicity disturbs enzymatic activity involved in the Calvin cycle, nitrogen and sugar metabolism. Moreover, Pb toxicity leads to structural cell wall damage, reduces water and nutrient uptake due to disruption of physiological and biochemical processes, causes ion homeostasis, thus, oxidative stress revealed by lipid peroxidation and increasing the synthesis of the ROS (Souguir et al., 2011; Bharwana et al., 2013; Jayasri and Suthindhiran, 2017; Javed et al., 2018). Due to this reason, biopriming of seeds with PGPB containing high ACC deaminase activity (KF58B) mitigated the Pb stress, promoted shoot and root growth, increased the TCC and NN, and improved the RSA (Table 2 and 3).

Nodule formation and biological nitrogen fixation performance of legumes are significantly affected by photosynthesis since nitrogen fixation is a complex process and dependent on photosynthesis products. Thus, increasing Pb concentration significantly restricted the nodule formation while PGPB containing high ACC deaminase activity promoted nodule formation by reducing oxidative stress and inducing photosynthesis and nutrient flux to the roots. Saleem et al. (2018) pointed out that inoculation of sunflower seeds with Pb resistant PGPB strains improved antioxidant enzyme activities such as catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase and osmolyte content like proline in plants under high Pb contamination compared with non-inoculated seeds. Janmohammadi et al. (2013) achieved similar results in wheat. Although bio-priming with PGPB enhanced the seedling growth and photosynthetic activity under both stress and non-stress conditions, PGPB containing higher siderophore and ACC deaminase activity exhibited superior performance on stress management in our experiment, and these observations are in agreement with previous studies.

Although Pb hinders the growth of all plant organs, the root is more influenced since it is exposed to more lead accumulation since endodermis functions form a barrier

across the radial transport of lead, thereby, it inhibits the movement of Pb through the xylem (Seregin and Ivanov, 1997). Accumulated lead in underground parts of plants leads to restraining root elongation and number of lateral roots per unit area reduces total root surface area and causes localized swellings which are indicators due to roots that failed to emerge throughout the rhizodermis (Kopittke et al., 2007). Low nutrient flux from leaves to roots via photosynthesis also hinders root formation and elongation, thereby, it affects root system characteristics. It is considered that bio-priming with PGPB especially containing ACC deaminase activity enhanced the RSA and NN due to both reducing plant stress and also having synergistic impacts on *Rhizobium* activity. Alemneh et al. (2020) stated that PGPBs containing ACC deaminase activity and IAA production promote nodulation, improve the competitiveness of *Rhizobium* for nodule formation, increase nitrogen fixation due to upregulating the expression of genes associated with legume–rhizobia symbioses, and delay nodule senescence.

CONCLUSION

Rising Pb concentration has destructive impacts on the morphological and physiological growth of lentil during the seedling stage. In a nutshell, lead toxicity reduced biomass and dry matter accumulation, restricted photosynthesis in leaves and nodulation on roots, and disturbed the root system architecture. However, PGPB containing ACC deaminase activity promoted plant growth and development by reducing oxidative stress and enhanced root characteristics and nodulation. Thus, bio-priming with PGPB may be a sustainable and effective solution to mitigate oxidative stress and promote plant growth and yield in lentil under lead-contaminated soils. Besides, the synergistic interplay of PGPB and rhizobia in lentil is required to investigate as molecular and genetic pathways under lead-contaminated field conditions.

AUTHOR CONTRIBUTIONS

ME, FÇ and MC designed, run the experiments, collected data, and wrote the Ms draft. FC made statistical analysis, gave feedback and edited the Ms draft. All authors read and approved the final manuscript.

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