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PGPR ASSISTED BIOREMEDIATION OF HEAVY METALS AND NUTRIENT ACCUMULATION IN ZEA MAYS UNDER SALINE SODIC SOIL

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Abstract

An experiment was conducted to evaluate the effects of four plant growth promoting rhizobacteria; *Pseudomonas putida, Bacillus pumilus, Lysinibacillus sphaericus* and *Exiguobacterium aurantiacum* isolated from saline soil on the uptake, accumulation and translocation of essential nutrients and heavy metals in *Zea mays* L. grown in saline sodic field. The PGPR exhibited significant increases in Na, K, Ca, Fe and Zn concomitant with significant decreases in Cd and Ni contents were recorded in the rhizosphere soil of maize inoculated with *Pseudomonas putida.* The PGPR increased availability of Fe and Zn in the rhizosphere soil, their uptake in roots and its translocation to leaves and grain. *Bacillus pumilus* effectively decreased Cd, Ni and Pb accumulation in grains whereas, Ni, Pb, Cr and Cd accumulation were more effectively reduced in leaves and grains of *Pseudomonas putida* inoculated plants. *Pseudomonas putida* increased biological accumulation coefficient of Cr, biological concentration factor for Cr, Cd and Ni but increased translocation factor for Pb. Whereas, *Bacillus pumilus* enhanced TF for Cd and Pb. *Bacillus pumilus* inoculated plants had significantly lower Na and K but had higher Ca, Fe and Zn over C. *Pseudomonas putida* and *Bacillus pumilus* can be implicated for enhanced bioremediation of heavy metals and for increased Zn and Fe accumulation in leaves and grain.

Key words: PGPR, *Zea mays*, Phytoremeditors, Nutrient acquisiters, Heavy metals.

Introduction

Salinity and sodicity induces many secondary stresses, heavy metal toxicity is one of them. Hence, plant adaptation to heavy metals in saline soil is gaining increased attention (Kholodova *et al.,* 2010). In alkaline salt affected soil, the damaging effect on plants is more severe (Heshmatpur & Rad, 2012). It also has an inhibitory effect on plant growth, root development, photosynthetic activity and availability and accumulation of mineral nutrients (Garg & Bhandari, 2011; Sen *et al.*, 2013; Shereen *et al.,* 2020). Salinity induced osmotic stress render the availability of essential elements such as K, Ca, Fe and Zn, causing nutrient deficiency in plants. They have also negative impact on plant biomass i.e., excessive accumulation of Cd in soil reduces shoot to root growth in maize (Jiang *et al.,* 2020; Sager *et al.,* 2020; Wang *et al*., 2013). Toxicity of Pb cause reduction in germination, suppressed growth, reduce height and negatively affect shoot and root dry mass, disturb mineral nutrition and decreases the protein content in maize (Ghani, 2010; Hussain *et al*., 2013).

Rhizoremediation is an environmental friendly biological solution to remediate toxicity caused by heavy metal. It consists of soil microbiota when applied, stimulate some mechanism that clean up contaminated environment. Different mechanisms like bioaccumulation and biosorption is employed by PGPR to alleviate heavy metal toxicity (Ahemad, 2014; Ma *et al.*, 2011). They secrete low molecular weight siderophore chelators that form stable complexes with metals such as iron, cadmium, copper, lead and zinc (Schalk *et al.*, 2011). Furthermore, PGPR inoculation of plants increase uptake of several essential nutrients such as calcium, potassium, iron, copper and zinc required for improved growth of plants (Wani & Khan, 2010). There are several genera of *Pseudomonas* spp. and *Bacillus* spp. that have the ability to solubilize zinc and make it available necessary for growth of maize (Goteti *et al*., 2013).

Due to greater biomass production, metal bioaccumulation and translocation to aerial parts and high recovery (%), maize could be used in bioremediation process (Aliyu, 2014). The current study aimed to investigate the role of PGPR isolated from saline soil on the uptake and accumulation of essential nutrients and phytoremediation of heavy metals by maize grown in saline sodic soil.

Materials and Methods

Geographical and physiochemical characteristics of experimental area: The study area falls within the semi-arid zone, located between latitude of 31° 52′ N, longitude of 73° 20′ E, and elevation of 195.6 m above sea level. The texture of soil was sandy clay loam characterized by a salinity combined with sodicity. The physicochemical characteristic was pH = 8.3, EC_e = 4.6 dS/m, organic matter = 0.43%, SAR = 15.4, NO_3-N =16.48 mg/kg, available P and available K, 2.25 mg/kg and 63.7mg/kg respectively (Ullah & Bano, 2019).

Experimental materials: Seeds of maize cv. "Islamabad Gold" was purchased from Crop Science Department, NARC Islamabad, Pakistan. Three bacteria *P.putida* (Acc no. KX580766), *L. sphaericus* (Acc no. KX580767), *B. pumilus* (Acc no. KX580768), isolated from the roots of weeds collected from Khewra salt range (pH = 8.2, ECe = 4.9 dS/m) and *E. aurantiacum* (Acc no. KX580769), isolated from oily sludge in Chakwal (pH=7.9, ECe = 2.7 dS/m) were used during the present investigation.

Experimental design: A randomized complete block design (RCBD) was applied in which each treatment was composed of three replicates. The size of plot / treatment was $4\times5m^2$ with $50\times100cm^2$ paths separating adjacent plots and blocks, respectively. Treatments were based on: C = Un-inoculated control, T_1 = inoculated with *P. putida,* T_2 = inoculated with *L. sphaericus,* T_3 = inoculated with *B. pumilus,* T_4 = inoculated with *E. aurantiacum.*

Growth conditions of the field: The average temperature of maize growing area was 27.5°C with 12.5 hr photoperiod, and humidity varying from 57 to 69 %. At 94 days after sowing (DAS), five plants were selected from each row of each plot randomly for analyses of physiological parameters**.**

Method of inoculation: Twenty four hour old bacterial cultures were inoculated in 100 ml LB broth and kept on shaker for 48hr. the cultures were centrifuged for 10 min at 10,000 rpm. Supernatant was discarded while pellets were suspended in dH_2O up to 1ml and the OD was measured at 660 nm and adjusted to 1. Sterilization of seeds was done with 10% chlorox and autoclaved dH_2O. Seeds (300) for each treatment were soaked in respected inocula for 2-3 hr prior to sowing. In addition, inocula of each bacterium (1L / bacterium) was added in the rhizosphere soil. Plant materials were harvested at 94 days after sowing, rhizosphere soil was collected for physicochemical analyses and plants were analyzed for nutrients and heavy metals.

Extraction method and soil analysis: Soltanpour & Schwab, (1977) method was used for analyzing metal contents of rhizosphere. About 1.97 g of 0.005 M diethylenetriamine pentacetate (DTPA) and 79 g of ammonium bicarbonate (NH_4HCO_3) to 800 ml of distilled water were added and mixed thoroughly to prepare extraction solution. Then it was diluted to 1L by adding dH_2, and pH was adjusted to 7.6 by using ammonium hydroxide. 10 ml extraction solution was mixed with 10 g air-dried soil and shaken for 15 min in a shaker at 180 cycles/min. It was filtered through Whatman No. 42 filter paper, and the filtrate was analyzed for the presence of heavy metals through an atomic absorption spectrophotometer (Shimadzu AA-700).

Analysis of plant metal tissues: Wet acid digestion method of Rashid, (1986) was used. About 1 g powdered plant materials were mixed with 10 ml Nitric- perchloric acid in flask and kept overnight in dark. Next day the flasks were kept fume hood at 150°C for 1hr at temperature which was raised gradually to 235°C. Then extract was filtered through Whatman No.42 filter paper and was diluted using 50 ml distilled water. Samples were analyzed through an atomic absorption spectrophotometer.

Biological concentration factor (BCF), translocation factor (TF) and biological accumulation coefficient (BAC): The BCF was calculated using equation (1) (Yoon *et al.*, 2006). TF was calculated using equation (2) (Cui *et al.*, 2007). BAC was calculated using equation (3) (Li *et al.*, 2007).

1. BCF = metal concentration of plant roots / metal concentration of soil
2. TF = heavy metals in plant shoot / heavy metals in plant root
3. BAC = heavy metal concentration in shoots / heavy metal concentration in soil

Statistical analysis

Analysis of variance (ANOVA) was carried out through statistics 8.1. Based on the least significant difference at $p = 0.05$, the values were separated (Steel and Tori, 1980). Data was presented with ± standard deviation.

Results

Effect of PGPR on Nutrient uptake: All the inoculation treatments significantly increased the accumulation of Na, K, Ca, Zn and Fe in the rhizosphere soil of a saline sodic field. Na accumulation was maximum in the rhizosphere of *P. putida* inoculated plants. The Ca content was significantly (27% and 23%) higher over control in *B. pumilus* and *E. aurantiacum* inoculation treatments (Table 1). Na and K content of rhizosphere soil was significantly greater than control in the rhizosphere of all inoculated plants except that of *B. pumilus*. Whereas, the Fe content was higher only in the rhizosphere soil of plants inoculated in the *P. putida*. The Zn content was higher in all the inoculated plants; maximum being in *P. putida* inoculation plants rhizosphere.

The *P. putida* and *B. pumilus* inoculated plants had significantly (46% and 37%) higher Na accumulation in roots as compared to uninoculated control plants grown in saline soil. Whereas, the *L. sphaericus* and *E. aurantiacum* inoculations showed decreased Na accumulation. Ca content was significantly higher (53%) over control in *E. aurantiacum* inoculated plants. K (65%) and Zn (28%) contents were higher in the roots of *P. putida* inoculated plants over control. However, the roots of all inoculated plants showed decrease in Fe content over untreated plants (Table 2). The stem had accumulated lower Na, Ca and Fe contents as compared to that in roots. The *P. putida* had significantly higher Na (48%), Ca (20%), K (18%) and Zn (47%) accumulation in stem as compared to uninoculated control plants. Fe accumulation was significantly higher (110%) over control in *E. aurantiacum* inoculated plants (Table 2).

The leaves had accumulated higher % of K, Ca, Na and lower % Fe and Zn contents compared to that in the stem. The *P. putida* had significantly higher K (96%), Ca (65%), Na (64%), Fe (54%) and Zn (41%) accumulation in leaves compared to control plants. PGPR inoculation invariably increased Fe and Zn contents in grain and leaves. Plants inoculated with *P. putida* exhibited maximum accumulation of Na (61%), K (58%), Zn (48%), Ca (43%) and Fe (42%) contents in grains over control plants, similar trend was followed by *L. sphaericus* and *B. pumilus* inoculation treatments (Table 3).

Table 1. Effect of PGPR on macro-micronutrients (mg/Kg) content in rhizosphere soil of maize grown in saline sodic soil having sodium absorption ratio 19.4. The seeds were inoculated with broth culture of the PGPR prior to sowing and measurements were made 94 days after sowing.

Treatment	Na	Ca	K	Fe	Z n
C	250^{d} (± 2.64)	155^{d} (± 1.24)	36.9^{c} (± 1.83)	10.4^{c} (± 1.09)	21.1^{c} (± 1.75)
T_1	450^{a} (± 1.38)	178^{c} (± 1.75)	63.6^{a} (± 2.63)	14.7^{a} (± 0.84)	27.4^{a} (± 1.62)
T_2	391^{b} (± 2.11)	168^{c} (± 2.05)	51.8^{b} (± 2.13)	11.7^{b} (± 0.27)	25.9^{a} (± 1.83)
T_3	210^{e} (± 2.25)	203^{a} (± 2.86)	28.7^{c} (± 1.74)	12.2^{b} (± 1.36)	23.7^{b} (± 1.39)
T_4	378^{c} (± 1.97)	196^{b} (± 1.63)	58.4^{a} (± 1.61)	9.07^{c} (± 1.05)	24.1^{b} (± 1.51)
LSD	1.39	2.31	0.93	1.37	3.26

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

Table 2. Effect of PGPR on macro-micronutrients (mg/Kg) in root and stem of maize grown in saline sodic soil having the SAR 19.4. Measurements were made 94 days after sowing.

Treatment	Na	Ca	K	Fe	Z n
			Maize root		
C	312^{c} (±0.96)	329^{c} (±2.15)	29.4^{d} (±1.42)	5.85^{a} (±0.28)	19.9^{c} (±1.17)
T_1	499^{a} (±1.63)	342^{c} (±1.92)	57.8^{a} (±1.65)	4.94^{b} (±0.16)	26.4^{a} (±1.68)
T_2	261^{d} (±1.35)	254^{d} (±1.13)	49.5^{ab} (±1.11)	3.64^{b} (±1.03)	21.5^{b} (±1.43)
T_3	453^{b} (±1.82)	404^{b} (±1.69)	18.7^{d} (±0.69)	6.76^{a} (±0.28)	25.4^{a} (±1.12)
T_4	197^{e} (±0.52)	558^{a} (±2.11)	34.9^{c} (±1.43)	3.84^{b} (±0.49)	23.4^{ab} (±1.83)
LSD	4.37	3.11	2.97	2.53	5.39
			Maize stem		
C	164^{d} (±1.28)	235^{b} (±1.44)	29.1^{b} (±1.39)	2.69^{c} (±0.37)	18.3^{d} (±0.72)
T_1	268^{a} (±2.36)	289^{a} (±2.39)	34.9^{a} (±1.77)	3.94^{b} (±0.85)	29.8^{a} (±1.28)
T_2	230^{c} (±1.93)	158^{d} (±1.84)	33.8^{a} (±1.91)	4.81^{b} (±1.26)	23.4^{bc} (±2.35)
T_3	244^{b} (±1.23)	167^{c} (±1.93)	16.2^{c} (±0.83)	4.94^{b} (±079)	26.6^{b} (±1.95)
T_4	252^{ab} (±0.87)	181^{c} (±1.73)	14.8^{c} (±0.28)	7.31^{a} (±0.62)	27.4^{b} (±1.21)
LSD	2.74	5.41	2.68	1.88	4.79

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

Table 3. Effect of PGPR on macro-micronutrients (mg/Kg) of leaves and grain of maize grown in saline sodic soil having the SAR 19.4. Measurements for leaves metal were made 94 days after sowing, while for grains measurements were made at harvesting.

Treatment	Na	Ca	K	Fe	Z n
			Maize leaves		
C	28.3^{c} (±1.73)	116^{d} (±2.18)	11.7^{c} (±1.53)	4.55^{c} (±0.73)	22.7^{c} (±0.79)
T_1	54.7^{a} (±1.51)	229^{a} (±2.27)	33.7^{a} (±1.14)	7.98^{a} (±1.94)	34.3^{a} (±0.42)
T_2	21.7^{c} (±0.69)	194^{b} (±1.79)	24.1^{b} (±1.93)	5.98^{b} (±0.27)	24.1^{bc} (±0.89)
T_3	42.9^{b} (±0.82)	170^{c} (±0.93)	18.8^{b} (±0.37)	5.33^{b} (±1.52)	29.5^{b} (±0.53)
T_4	39.6^{b} (±1.65)	106^{d} (±1.29)	19.9^{b} (±0.20)	5.98^{b} (±1.15)	26.2^{b} (±0.25)
LSD	1.75	1.21	1.62	1.87	5.73
			Maize grains		
C	22.4^{bc} (±0.79)	075^{c} (±1.38)	7.73^{b} (±0.81)	5.81^{b} (±0.36)	24.4^{c} (±0.27)
T_1	41.9^{a} (±1.49)	117^{a} (±1.75)	14.1^{a} (±1.62)	8.93^{a} (±0.55)	39.3^{a} (±0.48)
T_2	34.6^{a} (±1.13)	105^{b} (±1.98)	11.4^{a} (±0.35)	6.71^{a} (±0.26)	28.6^{b} (±1.13)
T_3	25.5^{b} (±0.58)	103^{b} (±0.67)	9.03^{ab} (±1.44)	4.34^{b} (±0.73)	31.5^{b} (±0.11)
T_4	29.8^{b} (±1.36)	105^{b} (±1.68)	7.49^{b} (±0.72)	6.13^{ab} (±0.42)	27.9^{b} (±0.69)
LSD	1.35	2.29	0.99	1.42	1.09

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = P. putida, T_2 = L. sphaericus, T_3 = B. pumilus, T_4 = E. aurantiacum. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

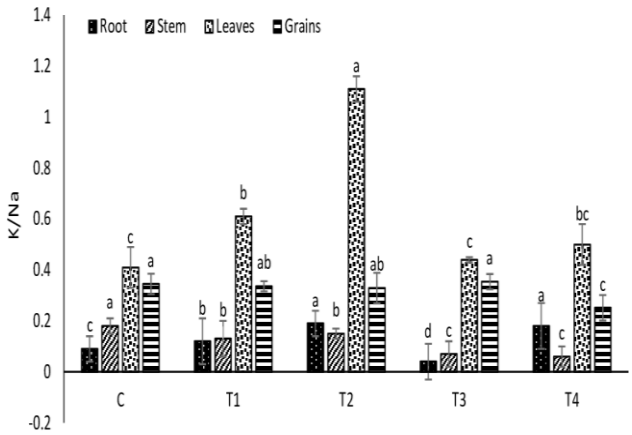

Fig. 1. Effect of PGPR on K/Na ratio in root, stem, leaves and grain of maize grown under saline sodic soil having sodium absorption ration 19.4. C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*.

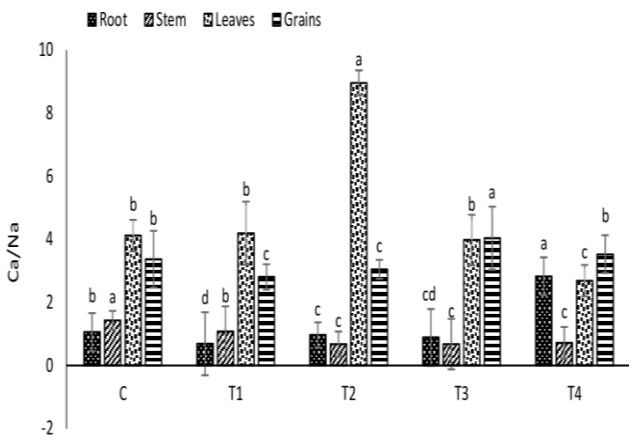

Fig. 2. Effect of PGPR on Ca/Na ratio in root, stem, leaves and grain of maize grown under saline sodic soil having sodium absorption ration 19.4 C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*.

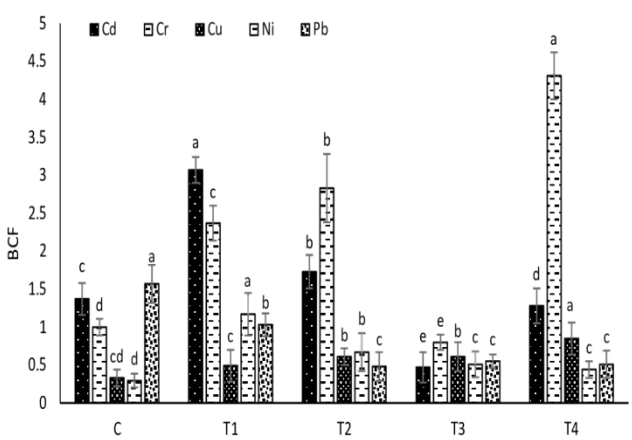

Fig. 3. Biological concentration factor (BCF) of different heavy metals in maize, grown under saline sodic soil having sodium absorption ration 19.4. C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*.

Effect of PGPR on K/Na ratio and Ca/Na ratio: Generally, the K/Na ratio was higher in roots of inoculated plants except that of *B. pumilus* (Figs. 1 and 2). The leaves of *P. putida* and *L. sphaericus* exhibited K/Na ratio significantly greater than C. As compared to control (uninoculated, salt stressed) no significant effects of *P. putida* and *B. pumilus* inoculations were recorded in Ca/Na ratio of the leaves and grains of the plants. Whereas *L. sphaericus* inoculated plants showed several fold (3x) increase in Ca/Na ratio of leaves as compared to control.

Heavy metal uptake: There was significant decrease in the Cd accumulation in the rhizosphere soil, maximum decrease was due to *P. putida* and *E. aurantiacum* inoculated plants (Table 4). The rhizosphere soil of *B. pumilus* inoculated plants showed least decrease in Cd content. The Cr content of the rhizosphere soil also showed significant decrease over C. except that of *B. pumilus*, *P. putida* inoculated plant rhizosphere showed no significant difference in Cr content over C. The Ni content was also lower than C; the least Ni was detected in the rhizosphere of *P. putida* inoculated plants. The rhizosphere soil of all inoculated plants showed significantly higher Pb content. The maximum being in *E. aurantiacum* and the least was recorded in *P. putida* inoculated plant rhizosphere.

The accumulation of Cr, Cu and Ni were higher in the roots of all inoculated treatments, whereas, Cd and Pb show decrease as compared to control. *P. putida* showed maximum significant increase in Ni accumulation in the roots over control. Inoculation with *B. pumilus* and *E. aurantiacum* had significant decreases on Cd accumulation in the roots. Pb accumulation was decreased in all the inoculated treatments over control (Table 5).

Inoculation with *E. aurantiacum* showed siginificant increases in Cd and Ni as compared to control. Cr accumulation was significantly higher in *P. putida*. All the inoculation treatments exhibited decrease in Pb accumulation in the stem. The *P. putida* inoculated plants do not show any significant difference with the C.

In leaves inoculation with *E. aurantiacum* showed significant increases in Cr and Ni accumulation over control (Table 6). Cd accumulation was significantly higher in *B. pumilus*. All the inoculation treatments decreased the Cd, Cr, Ni and Pb in the leaves. The Cd content did not different significantly with the control. *P. putida* being most effective. The inoculated treatments showed significant decrease in Cd, Cu and Ni accumulation in the grains. The Cr and Pb showed no significant effect of inoculation except *B. pumilus* and *E. aurantiacum* inoculations which showed the least decrease in Pb accumulation in grain over control plants.

Biological concentration factor (BCF), biological accumulation coefficient (BAC), translocation factor (TF): The BCF was significantly higher for Cd, Cr and Ni in *P. putida* inoculation followed by inoculation with *L. sphaericus* whereas, *B. pumilus* inoculation had slightly higher BCF only for Cu. *E. aurantiacum* had significantly higher BCF for Cr. (Fig. 3). As compared to control *L. sphaericus*, and *E. aurantiacum* showed higher BAC for Cr and Cd respectively whereas, *P. putida* showed higher BAC for Cr (Fig. 4).

B. pumilus exhibited highly significant increase in TF for Cd. The TF for Pb was significantly higher in all inoculation treatments over control. *B. pumilus* exhibited highly significant increase in TF for Cd (Fig. 5).

Table 4. Effects of PGPR on heavy metals (mg/Kg) of rhizosphere soil of maize grown in saline sodic soil having the SAR 19.4. The seeds were inoculated with broth culture of the PGPR prior to sowing and measurements were made 94 days after sowing.

Treatment	Cd	Cr	Cu	Ni	Pb
C	1.13^{a} (±0.02)	0.12^{b} (±0.07)	23.5^{b} (±1.6)	1.35^{a} (±0.03)	3.85^{d} (±1.71)
T_1	0.34^{c} (±0.09)	0.14^{b} (±0.05)	30.3^{a} (±2.03)	0.88^{d} (±0.04)	4.68^{c} (±1.09)
T_2	0.66^{b} (±0.04)	0.01^{d} (±0.09)	22.6^{b} (±1.07)	1.03^{c} (±0.21)	6.18^{b} (±1.61)
T_3	0.74^{b} (±0.17)	0.32^{a} (±0.01)	27.9^{a} (±1.12)	1.17^{b} (±0.04)	6.57^{b} (±1.32)
T_4	0.34^{c} (±0.01)	0.06^{c} (±0.06)	27.6^{a} (±2.08)	1.11^{b} (±0.21)	8.13^{a} (±1.47)
LSD	0.48	0.79	1.43	1.06	2.31

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

Table 5. Effects of PGPR on heavy metals (mg/Kg) of root and stem of maize grown in saline sodic soil having the SAR 19.4. Measurements were made 94 days after sowing.

Treatment	Cd	Cr	Cu	Ni	Pb
			Maize roots		
C	1.55^{a} (±0.07)	0.12^{c} (±0.01)	08.5^{d} (±0.53)	0.40^{cd} (±0.10)	6.05^{a} (±0.61)
T_1	1.32^{b} (±0.05)	0.33^{ab} (±0.07)	14.7^{bc} (±0.37)	1.03^{a} (±0.01)	4.81^{b} (±0.37)
T_2	1.14^{bc} (±0.07)	0.41^{a} (±0.02)	13.9^{bc} (±0.09)	0.69^{b} (±0.01)	2.99^{c} (±0.21)
T_3	0.35^{de} (±0.08)	0.29^{b} (±0.05)	17.3^{ab} (±0.18)	0.59^{bc} (±0.01)	3.64^{c} (±0.11)
T_4	0.55^{d} (±0.04)	0.28^{b} (±0.01)	23.7^{a} (±0.21)	0.49^{c} (±0.09)	4.16^{b} (±0.06)
LSD	1.57	2.23	1.18	0.96	1.54
			Maize stem		
C	1.76^{b} (±0.12)	0.16^{c} (±0.01)	23.9^{bc} (±0.41)	0.79^{b} (±0.06)	8.19^{a} (±1.73)
T_1	0.51^{c} (±0.31)	0.41^{a} (±0.05)	24.9^{b} (±0.91)	0.72^{b} (±0.07)	8.26^{a} (±0.94)
T_2	1.55^{b} (±0.18)	0.26^{b} (±0.08)	33.8^{a} (±1.43)	0.53^{c} (±0.92)	6.89^{b} (±0.41)
T_3	1.57^{b} (±0.07)	0.16^{c} (±0.04)	25.7^{b} (±0.76)	0.64^{bc} (±0.87)	6.76^{b} (±1.07)
T_4	2.13^{a} (±0.05)	0.28^{b} (±0.07)	21.3^{c} (±1.09)	0.91^{a} (±0.38)	5.66^{bc} (±1.09)
LSD	0.97	0.28	4.17	3.27	1.33

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

Table 6. Effects of PGPR on heavy metals (mg/Kg) of leaves and grains of maize grown in saline sodic soil having the SAR 19.4. Measurements for leaves metal were made 94 days after sowing, while for grains measurements were made at harvesting.

Treatment	Cd	Cr	Cu	Ni	Pb
			Maize leaves		
C	0.77^{b} (±0.04)	0.54^{b} (±0.09)	15.2^{d} (±0.03)	0.88^{b} (±0.03)	5.85^{a} (±0.06)
T_1	0.72^{b} (±0.05)	0.37^{c} (±0.05)	60.7^{a} (±0.18)	0.39^{c} (±0.07)	0.98^{d} (±0.05)
T_2	0.61^{c} (±0.03)	0.59^{b} (±0.08)	20.5^{c} (±0.21)	0.23^{d} (±0.05)	1.37^{c} (±0.09)
T_3	0.89^{a} (±0.04)	0.34^{c} (±0.03)	25.7^{b} (±0.17)	0.16^{e} (±0.02)	1.43^{c} (±0.03)
T_4	0.55^{d} (±0.07)	1.13^{a} (±0.04)	21.8^{c} (±0.11)	1.62^{a} (±0.08)	1.82^{b} (±0.07)
LSD	1.27	0.52	0.28	1.09	0.43
			Maize grains		
C	1.21^{a} (±0.09)	1. 28^{c} (±0.02)	18.6^{a} (±0.21)	1.21^{a} (±0.05)	3.84^{a} (±0.04)
T_1	0.28^{c} (±0.05)	1.28^{c} (±0.06)	07.8^{c} (±0.19)	0.66^{b} (±0.03)	3.74^{a} (±0.09)
T_2	0.33^{b} (±0.08)	1.22^{d} (±0.05)	10.4^{b} (±0.09)	0.31^{e} (±0.08)	3.45^{ab} (±0.01)
T_3	0.07^{d} (±0.02)	1.42^{b} (±0.03)	4.42^{d} (±0.08)	0.44^{d} (±0.02)	2.67^{c} (±0.05)
T_4	0.34^{b} (±0.07)	1.64^{a} (±0.09)	6.24^{c} (±0.13)	0.76^{bc} (±0.09)	2.93^{c} (±0.03)
LSD	2.67	3.16	1.65	0.99	1.74

C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05)

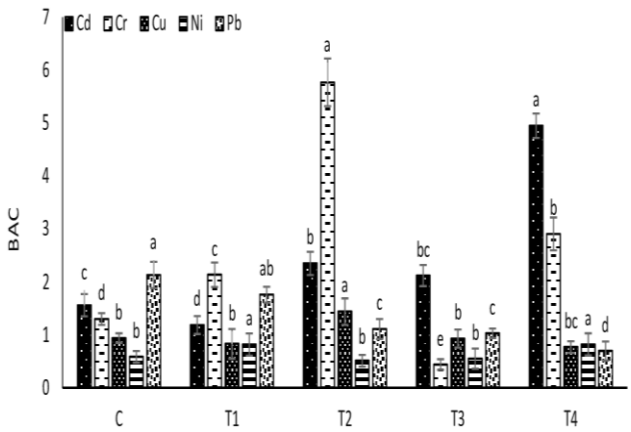

Fig. 4. Biological accumulation coefficient (BAC) of different heavy metals in maize, grown under saline sodic soil having sodium absorption ration 19.4 C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*.

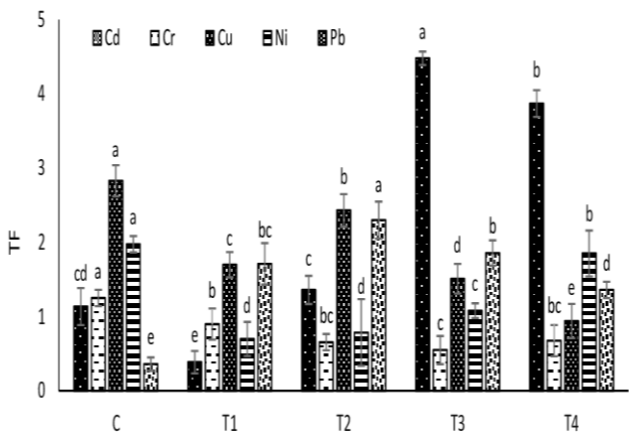

Fig. 5. Translocation factor (TF) of different heavy metals in maize, grown under saline sodic soil having sodium absorption ration 19.4. C = Uninoculated plants grown in saline sodic soil of the field, T_1 = *P. putida*, T_2 = *L. sphaericus*, T_3 = *B. pumilus*, T_4 = *E. aurantiacum*.

Discussion

Results have shown that maize inoculated with *P. putida* and *Bacillus* spp. significantly increased the nutrient content of Na, Ca, K, Fe and Zn in different parts of maize. The higher uptake of essential nutrients compared to control plants(uninoculated grown in saline sodic soil) could be justified by the fact that the unavailable forms of these nutrients in the saline sodic soil were solubilized and made available in the root region by the PGPR applied (Saravanan *et al*., 2011). Plants inoculated with *P. putida* usually have higher Na, K, and Zn content in root, stem, leaves and grains than that of control plants. Increased uptake of Na, K and Zn have been reported in maize plants following inoculation with *Pseudomonas* sp. (Goteti *et al.*, 2013). Maintenance of higher K/Na ratio in roots and leaves of PGPR inoculated plants demonstrate that PGPR induced tolerance in plants against salt stress (Rojas-Tapias *et al*., 2012). Ion homeostasis is a key factor for plant survival which is achieved by maximum concentration of K, Ca and lower concentration of Na in roots, shoots and leaves under saline condition (Aleman *et al*., 2011; Hasegawa, 2013; Munns & Tester, 2008). The high Ca/Na and K/Na ratio in the leaves of *L. sphaericus* treated plant demonstrate the salt tolerance strategy of the PGPR used as bioinoculant.

The rhizosphere of untreated uninoculated plants grown in saline soil revealed accumulation of Cd, Cu, Ni and Pb. Most of the Pb was taken up by the roots and translocated to the stem and the leaves. Uninoculated plant roots had taken up higher Cd from rhizosphere soil and translocated it to stem from where only 50% was translocated to the leaves. Maize is a moderate hyperaccumulator which accumulate high concentrations of Cd in roots compared to stem and leaves (Anjum *et al.*, 2015). About 50% Ni from soil was taken up by roots of uninoculated plants grown in saline sodic soil but very little Ni was translocated to stem, leaves and grain.

PGPR treatments decreased Cd and Cr accumulation (except *B. pumilus* which had Cr content higher than the control) but significantly increased Pb accumulation in the rhizosphere soil. maximum being in *E. aurantiacum*. The *B. pumilus* and *E. aurantiacum* very efficiently decreased the Cd accumulation in roots. Although *B. pumilus* had higher Cd in soil than *E. aurantiacum* but less was translocated to roots, whereas, *P. putida* and *L. sphaericus* had higher Cd retained in the root. *P. putida* inoculation showed greater than 3-fold decrease in Cd accumulation in stem over untreated salt stressed plant. *P. putida* is the most tolerant candidate to Cd toxicity in soil (Yong *et al*, 2014), and showed higher Cd retained by roots and leaves, but decreased 2x less in grain.

E. aurantiacum had higher Cr in leaves as well in grains. *P. putida* and *L. sphaericus* have accumulated greater amount of Cr over the control (untreated salt stressed). Expect *P. putida* which had higher Cu in the rhizosphere soil all other treatments have no significant effects. Heavy metal tolerant bacteria may increase the Cu availability in soil, possibly through the excretion of organic acids (Gube, 2016; Seymen *et al*., 2015; Ullah *et al.*, 2015), which is then translocated into the roots of inoculated maize in a significant amount (Sheng *et al*., 2012).

Malekzadeh *et al.*, (2016) reported that inoculation of maize plants with Ni-resistant PGPR significantly increased Ni accumulation in plants roots without diminishing their biomass as compared to uninoculated plants.

Ni translocation from stem to leaves was much lower in PGPR treatments except *E. aurantiacum* which showed significantly higher Ni accumulation over control. The translocation of Pb from stem to leaves was reduced both in the untreated plants and PGPR treated plants. PGPR further enhanced the decrease in the accumulation of Pb in leaves. The least accumulation was recorded in *P. putida.* Plant roots initiate the synthesis and deposition of callose which acts as a barrier against Pb penetration in roots (Samardakiewicz *et al.*, 2012). Furthermore, inoculation of corn plants with *Bacillus* sp. and *Pseudomonas* sp. significantly (p =0.05) decreases the uptake of Pb (Mohamed & Almaroai, 2017).

Noteworthy, the maize growing in untreated salt stressed plants showed significantly higher accumulation of Cd, Ni and Cu in grains possibly being translocated from leaves. PGPR significantly decreased Cd, Cu and Ni

accumulation in grains. As reflected in the Pb content of grain which did not differ significantly with the control, whereas in leaves significantly less Pb was accumulated in this treatment.

The least TF of Ni in *P. putida* inoculated plants may be attributed to nonsignificant increase of BCF of Ni though BAC was significantly greater than C (salt stressed, inoculated).

Noteworthy, in *B. pumilus* inoculated plants the BCF and BAC of all the heavy metals was lower than that of C. But in roots of *B. pumilus* inoculated plants, the TF was significantly higher for Cd. That was reflected in leaves where maximum increase in Cd accumulation was recorded over C whereas, in grains the Cd accumulation was minimum compared to all other treatments.

The BCF was significantly higher than C in *E. aurantiacum* inoculated plants but only the TF for Cd and Pb was greater than C though the BAC was higher for Cd and Cr both but not for Pb. Both leaves and grain exhibited higher accumulation of Cr than that of C.

TF for Ni in *B. pumilus* was lower than C that was also reflected in less Ni content of leaves and grains, but the leaf had Ni accumulation greater than C, as the BAC was higher. *P. putida* inoculation had not lowered Cd accumulation in leaves and Cr accumulation in grains but decreased Cr, Ni and Pb in leaves and Cd, Ni and Cu in grains significantly over C. Both *P. putida* and *B. pumilus* inoculated plants had reduced the Cr, Ni and Pb in leaves but *P. putida* was more efficient.

Conclusion

It is inferred that different PGPR adopt different strategy for accumulation and translocation of various types of heavy metals in different parts of the plant. The leaves and grains, the edible part of the plants consumed as food for cattle and human being had retained very low concentration of heavy metals due to PGPR application. The PGPR increased the availability of K and Ca, enhanced Fe and Zn in the rhizosphere soil PGPR enhanced the uptake by roots and its translocation to leaves and grain. *B. pumilus* effectively decreased Cd, Ni and Pb accumulation in grain, whereas, Ni, Pb, Cr and Cd accumulation can be more effectively reduced in leaves as well as in grain by *P. putida.* It is concluded that *P. putida* and *B. pumilus* can be implicated for enhanced bioremediation of heavy metals and for the increased Zn and Fe accumulation in leaves and grain.

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