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Efficiency of heavy metals-tolerant plant growth promoting bacteria for alleviating heavy metals toxicity on sorghum

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ABSTRACT

A green-house experiment was conducted to investigate the efficiency of three heavy metal-tolerant plant growth promoting bacterial strains (HMT-PGPB) (*Alcaligenes faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 to alleviate the heavy metal's toxic effects on sorghum plant (*Sorghum bicolor*, L.) in addition to their ability to enhance plant growth. The oxidative enzymes, photosynthetic pigments, growth characteristics, heavy metals uptake and heavy metals translocation factor (TF) were estimated in sorghum cultivated in soil contaminated with heavy metals under green-house conditions. Results showed that the application of these HTM-PGPB strains as biofertilizer of sorghum help plant to ignore the toxic effects of heavy metals and enhance growth characteristics.

1. Introduction

The application of municipal wastewater or industrial wastes as fertilizers and liming agents in agriculture is a separate issue. Application of this type of wastes requires constant monitoring of the amount and proportion of harmful factors, including heavy metals. Toxicity in soil, water and plant systems was depend on the supply of the metal waste, soil, and groundwater chemistry at the location (Rakshit et al., 2017). Plant associated microorganisms, especially plant growth promoting rhizobacteria (PGPR), are known to play a vital role in promoting plant growth and also in remediating soils from organic and metal pollutants by various mechanisms (Rajkumar et al., 2012). There are many literature reviews dealing with the role of PGPR in mobilization, phytoextraction, and phytoremediation of heavy metals from soil (Sessitsch et al., 2013). By various mechanisms such as solubilizing metal minerals, acidifying the rhizosphere environment, enhancing root surface area for heavy metal uptake, and increasing the release of root exudates, these PGPR efficiently enhance the mobilization of heavy metals. Moreover, they have many mechanisms for alleviation the toxicity of heavy metals such as phytohormones (IAA&GA_3) production (Merdy et al., 2009) salicylic acid exerts which has positive roles in maintain and enhancing heavy metals tolerance (Yan et al., 2011) proline accumulation (Szekely et al., 2008) exopolysaccharides production (Iqbal et al., 2002) biosurfactants (Rufino et al., 2008) and siderophores production (Rajkumar et al., 2010). Although plants require certain heavy metals for their growth and excessive amounts of these metals can become toxic to plants (Shahid et al., 2014). The ability of plants to accumulate essential metals equally enables them to acquire other non-essential metals. As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly (Blaylock and Huang, 2000). In addition to inhibition of enzymatic functioning and disruption of nucleic acid structure, toxic heavy metals interfere with the uptake, distribution of essential nutrients, and the displacement of essential metals from their normal binding sites on biological molecules (e.g., As and Cd compete with Pb and Zn, respectively) in plant, causing deficiencies and nutrient imbalance (Sharma and Archana, 2016). Heavy metals pollution is a menace to our environment as they are foremost contaminating agents of our food supply, especially vegetables (Kacholi and Sahu, 2018). Additionally, heavy metals can induce oxidative stress by overproduction of reactive oxygen species (ROS), which can destroy cell's inherent defense system and can cause cell damage or death (Sana et al., 2017).

In this request, a green-house experiment was designed to investigate the efficiency of three heavy metal-tolerant plant growth promoting bacterial strains (HMT-PGPB) (*Alcaligenes faecalis*

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Table 1
Mechanical and chemical analyses of the experimental soil.

Parameters	Unit	Values	Parameters	Unit	Values	Heavy metals		
A. Mechanical analysis			B. Chemical analysis					
Coarse sand	(%)	3.91	Organic matter	(%)	1.52	Cd^{2+}	mg/l	ND
Fine sand	(%)	24.04	$CaCO_3$	(%)	0.55	Cu^{2+}		ND
Silt	(%)	25.22	Total nitrogen	(%)	0.23	Zn^{2+}		0.04
Clay	(%)	44.14	Total phosphorus	(%)	0.12	Pb^{2+}		0.16
Textural class	(%)	Clayey loam	Total potassium	(%)	0.27			
			pH		8.2			

MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 to alleviate the heavy metal's toxic effects on sorghum plant *(Sorghum bicolor*, L.) in addition to their ability to enhance plant growth.

2. Materials and methods

2.1. Pots and experimental soil

This experiment was carried out at Faculty of Agriculture, Benha University, Egypt during summer 2017 in plastic pots (35 cm diameter and 30 cm depth) containing clay loam soil (10 kg/pot). Mechanical and chemical analyses of the experimental soil are shown in Table 1.

2.2. Heavy metal solutions preparation and soil treatment

Four heavy metal salts, copper sulphate ($CuSO_4{\cdot}5H_2O$); cadmium chloride ($CdCl_2$); zinc sulphate ($ZnSO_4{\cdot}7H_2O$) and lead acetate ($Pb(CH_3COO)_2{\cdot}3H_2O$) at three concentrations from each metal were used as follows: (100, 200 and 400 mg/l); (10, 20 and 40 mg/l), (250,500 and 1000 mg/l), (200,400 and 800 mg/l), respectively. After that, these solutions were added to soil for one week before cultivation (Setkit et al., 2014).

2.3. Preparation of HMT-PGPB strains inocula

The inocula of *Alcaligenes faecalis* MG257493.1 at 9×10^8 CFU/ml (El-Alkshar et al., 2018), *Alcaligenes faecalis* MG966440.1 and for *Bacillus cereus* MG257494.1 at 9×10^8 and 8×10^7 CFU/ml, respectively (El-Meihy et al., 2018) were prepared in Mueller-Hinton broth medium (Oxoid, UK) which consists of (g/l): 2.0 beef Extract, 17.5 acid Hydrolysate of casein, 1.5 starch, final pH 7.3 ± 0.1 at 30 ± 2 °C for 48 h. Equal dose from each cell suspension were mixed and used as mixture inoculum.

2.4. Experimental design

The treatments were distributed randomly placed in a greenhouse for 9 weeks using randomized complete block design (RCBD) as shown in Table 2. Three replicates of each treatment were used.

Table 2
Distribution of treatments.

A) Control	• Without any amendments • With bacterial inoculation only
B) With heavy metals only	• Cu^{+2} (100-200-400) mg/kg • Cd^{+2} (10-20-40) mg/kg • Zn^{+2} (250-500-1000) mg/kg • Pb^{+2} (200-400-1000) mg/kg • Mixture of Cu^{+2} (200) + Cd^{+2} (20) + Zn^{+2} (500) + Pb^{+2} (400) mg/kg
C) With heavy metals and bacterial inoculation	• The same treatments in group (B) were repeated with bacterial inoculation

2.5. Cultivation process

Seeds of sorghum (*Sorghum vulgare* L.) Giza 1 was obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were soaked in mixture of all strains with Arabic Gum (20%) for 60 min. before sowing. Fifteen seeds were sown in each pot and ten vigorous seedlings were retained after germination. Chemical fertilizers (NPK) were used as recommended with (30 kg N_2 as ammonium sulfate, 150 kg P_2O_5 as calcium superphosphate and 50 kg K_2O as potassium sulfate and irrigated according to the recommendations of the Ministry of Agriculture, Egypt.

2.6. Determinations

2.6.1. Assessment of plant oxidative enzymes

Peroxidase and polyphenol oxidase activities were determined according to the methods described by Allam and Hollis (1972) and Matta and Dimond (1963), respectively.

2.6.2. Photosynthetic pigments

Photosynthetic pigments (chlorophyll *A* & *B* and carotenoids) were determined spectrophotometrically according to Nornal (1982) and calculated as mg/g fresh weight of leaves after 6 weeks from cultivation.

2.6.3. Growth characteristics

Plant height, number of leaves, fresh and dry weight of root and shoot systems were determined in each treatment by choosing five randomly plants at vegetative stage (after 6 weeks from cultivation).

2.6.4. Heavy metals uptake by sorghum

Heavy metal contents were determined after 6 weeks from cultivation in plant using atomic absorption as reported by Reichman and Parker (2007).

2.6.5. Heavy metals translocation factor (TF)

Heavy metals translocations from roots to shoots were measured by calculating the TF, as follow:

$$TF = C_{shoot}/C_{root}$$

Where: C_{shoot} and C_{root}, represent the heavy-metal concentrations in the plant shoots (mg/kg) and roots (mg/kg), respectively.

As recommended by Fayiga and Ma (2006), if TF values > 1 indicate that the heavy metal was effectively translocated from the roots to the shoots.

2.7. Statistical analysis

Obtained data were analyzed according to Snedecor and Cochran (1980). The differences among the means of different treatments were tested using the Duncan's multiple range tests (Duncan's, 1955). Statistical analysis was done using the Costate package program, version 6.311 (cohort software, USA).

Table 3
Effect of different heavy metals concentrations on oxidative enzymes in sorghum.

Treatments		PO (Abs. at 425 nm/g FW/ 15 min.)		PPO (Abs. at 420 nm/g FW/ 30 min.)	
		Inoculation with HMT-PGPB			
		Without	With	Without	With
Control		4.048[a]	4.550[a]	1.034[g]	1.293[h]
Cu^{2+} (mg/kg)	100	4.133[a]	4.280[c]	1.792[b]	2.014[b]
	200	3.751[a]	3.775[k]	1.145[e]	1.168[j]
	400	3.076[b]	3.483[m]	0.571[k]	1.079[l]
Cd^{2+} (mg/kg)	10	4.113[a]	4.327[b]	1.910[a]	1.912[c]
	20	3.978[a]	4.252[d]	0.833[i]	0.977[m]
	40	3.693[a]	4.038[e]	0.830[i]	0.933[n]
Pb^{2+} (mg/kg)	200	4.102[a]	3.828[i]	0.910[h]	1.761[d]
	400	3.979[a]	3.818[j]	0.830[i]	1.531[f]
	800	3.956[a]	3.763[l]	0.806[j]	1.173[i]
Zn^{2+} (mg/kg)	250	4.154[a]	3.998[f]	1.473[c]	1.566[e]
	500	4.000[a]	3.954[h]	1.315[d]	1.492[g]
	1000	3.948[a]	3.245[n]	1.317[d]	1.081[k]
Mixture (mg/kg)		3.859[a]	3.987[g]	1.091[f]	2.583[a]

[a, b, c] Means with different superscript in the same column are significantly different at ($P < 0.05$). PO: peroxidase, PPO: polyphenol oxidase.

3. Results and discussion

3.1. Effect of inoculation with HMT-PGPB on oxidative enzymes in sorghum leaves

Data in Table 3 indicated that the application of heavy metals in soil cause significant decrease in the estimated enzymes (peroxidase and polyphenol oxidase) either in inoculated plants with HMT-PGPB or in uninoculated ones. In this respect, Azuma et al. (2010) indicated that superoxide dismutase, polyphenol oxidase and peroxidase were the main oxidative enzymes which decreased under environmental stress conditions. Also, data indicated that the control plants either inoculated or uninoculated gave higher values of the two enzymes although they don't exposure to any heavy metals. This may be due to that the production of these enzymes in plants was a normal defense way to any biotic or abiotic stress conditions. Reactive oxygen species (ROS) which formed in higher plants under various abiotic stresses could increase the efficiency of plants against ROS by enhancing anti-oxidative enzymes including catalase, peroxidase, hydrogen peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase (Chookhampeng, 2011).

Moreover, the application of heavy metals in mixture form causes significant decrease in the estimated enzymes than their individual form. This may be because the multiplier effect of these heavy metals on plant. In addition, exposure of plants to heavy metals leads to deactivate the normal balance of cells and causes increments in the production of ROS such as the hydrogen peroxide, superoxide radicals and hydroxyl radicals and formation of radical scavenging compounds i.e. ascorbate and glutathione (Celik and Atak, 2012). ROS generation increases lipid peroxidation, protein degradation and nucleic acid damages. This can accumulate abscisic acid, which responsible for wilting.

Peroxidase and polyphenol oxidase activities in sorghum plants were decreased with the increasing of heavy metals concentrations. This was true in plants cultivated under all heavy metals at all concentrations. It was clear that whether direct or indirect, plants exposed to high levels of heavy metals result in reduction or even complete cessation of all metabolic activities. Also, Aydinalpi and Marinova (2003) reported that heavy metals act as inhibitors of many biochemical processes, such as enzyme and hormone production. These results may be due to the direct consequences of heavy metals on sorghum by inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Jadia and Fulekar, 2009). Oxidative stress is related to formation of ROS) and cytotoxic compounds like methylglyoxal (MG) and perturbs the equilibrium of ionic homeostasis within the plant cells (Hossain et al., 2012; Sytar et al., 2013).

Additionally, the application of heavy metals in mixture form cause significant decrease in the estimated enzymes than their individual form. This may be because the multiplier effect of these heavy metals on plant. In addition, exposure of plants to heavy metals leads to deactivate the normal balance of cells and causes increments in the production of ROS such as the hydrogen peroxide, superoxide radicals and hydroxyl radicals and formation of radical scavenging compounds i.e. ascorbate and glutathione (Celik and Atak, 2012). ROS generation increases lipid peroxidation, protein degradation and nucleic acid damages. This can accumulate abscisic acid, which responsible for wilting.

Otherwise, the inoculation of plants with HMT-PGPB and cultivated in soil amended with heavy metals either individually or in mixture cause an increase of the two estimated enzymes. This trend of results was approved by Bano et al. (2012), who reported that to alleviate adverse effects of ROS, plants had antioxidant defense systems through production of enzymes like superoxide dismutase, peroxidases, polyphenol oxidase, phenylalanine ammonia lyase and catalase. Also, lead was the most significant toxic metal for the uninoculated sorghum plants and decrease the production of the oxidative enzymes at all concentrations compared to the inoculated ones. Though, there was correlation between stress tolerance and increasing activity of the antioxidant system in vegetable crops (Mittova et al., 2002).

Generally, when plants grown under stress conditions and inoculated with PGPR this cause an increase in two oxidative enzymes peroxidase and polyphenol oxidase at the beginning of plant life to protect plants against ROS resulted from stress and break free and conjugated phenols which formed under these conditions. But, after that these two enzymes were decreased and the enzyme phenylalanine ammonia lyase was increased because this enzyme works on the products of the two previous enzymes. Then, binds these products with proline and produce hydroxyl proline compound, which migrates from its synthesized places to the cell walls to enter the installation of lignin, which helps to strengthen cell walls to reduce transpiration process and thus reduces the stress suffered by the plant (Celik and Atak, 2012).

3.2. Effect of inoculation with HMT-PGPB on photosynthetic pigments in sorghum leaves

Data presented in Table 4 indicated that photosynthetic pigments increased in inoculated plants than uninoculated ones. This due to the beneficial effects of HMT-PGPB for alleviation the toxic effects of heavy metals. In this respect, Popova et al. (2012) demonstrated the relationship between total chlorophyll content in heavy metal exposed and bacterial treated plants. He proved that because the ability of the bacteria to metabolize heavy metal, the chlorophyll content was affected and cause increase in photosynthetic activity followed by increase in plant growth.

Generally, chlorophyll *A* and carotenoids contents in uninoculated sorghum leaves were significantly decreased with the increasing of heavy metals concentrations. This is because the hazard effect of high concentrations of heavy metals which cause weakness of plants and their vital activities. Also, the absence of HMT-PGPB will cause increase in the accumulation of heavy metals inside the plants. This was true with all heavy metals and in accordance with Popova et al. (2012) who proved that the application of heavy metals individually or in mixture cause decrease in photosynthetic pigments.

Also, Celik and Atak (2012) reported that the produced ROS might

Table 4
Effect of heavy metals on photosynthetic pigments in sorghum leaves.

Treatments		Chlo.A		Chlo. B		Carotenoids	
		Inoculation with HMT-PGPB					
		Without	With	Without	With	Without	With
Control		0.9^{h}	2.1^{b}	0.5^{e}	1.2^{bc}	1.3^{f}	3.1^{ab}
Cu^{2+} (mg/kg)	100	1.9^{b}	0.7^{i}	1.1^{a}	1.3^{b}	3.1^{ab}	3.2^{a}
	200	1.4^{d}	2.1^{b}	0.7^{cd}	1.2^{bc}	2.6^{bc}	2.9^{cd}
	400	1.3^{de}	1.9^{c}	0.6^{de}	0.5^{i}	2.1^{cde}	2.6^{e}
Cd^{2+} (mg/kg)	10	1.4^{d}	1.4^{g}	0.9^{b}	1.3^{b}	2.6^{bc}	3.2^{a}
	20	1.3^{de}	2.9^{a}	1.2^{a}	1.1^{cd}	2.2^{cd}	2.8^{d}
	40	1.3^{de}	1.7^{de}	1.2^{a}	0.6^{hi}	2.0^{de}	2.3^{f}
Pb^{2+} (mg/kg)	200	2.2^{a}	1.5^{gf}	1.2^{a}	1.0^{de}	3.4^{a}	3.1^{ab}
	400	1.7^{c}	1.2^{h}	0.8^{bc}	1.6^{a}	2.6^{bc}	2.9^{cd}
	800	1.1^{fg}	1.4^{g}	0.6^{de}	0.7^{gh}	1.3^{f}	2.5^{e}
Zn^{2+} (mg/kg)	250	1.8^{bc}	1.8^{cd}	1.2^{a}	0.9^{ef}	3.2^{a}	3.1^{ab}
	500	1.2^{ef}	1.6^{ef}	0.7^{cd}	0.8^{gf}	1.8^{def}	2.1^{f}
	1000	1.0^{gh}	1.1^{h}	0.9^{b}	0.6^{hi}	1.6^{ef}	1.9^{g}
Mixture (mg/kg)		1.3^{de}	2.2^{b}	0.5^{e}	0.9^{ef}	1.9^{de}	3.0^{bc}

[a, b, c] Means with different superscript in the same column are significantly different at ($P < 0.05$).

cause photoinhibitory and photooxidative damage in chloroplasts. Thus, chlorophyll content could be considered as a good reflective to plant response to environmental stress because under adverse conditions chlorophyll (a) and (b) and total chlorophyll contents were decreased.

Although zinc at 1000 mg/kg soil was the most toxic effect on chlorophyll (a) in uninoculated plants leaves, this effect was significantly decreased by the inoculation with HMT-PGPB. This may be due to all used bacteria were zinc-tolerant bacteria and able to alleviate its toxic effects on plants. Moreover, the lowest significant chlorophyll (a) values was observed in inoculated plant leaves and cultivated in soil amended with copper at 100 mg/kg soil. These results were confirmed by Hrynkiewicz and Baum (2011) who reported that the use of beneficial microbes might enhance plant's tolerance to adverse environmental stresses, which including heavy metals. This might be resulted from the activities of the four HMT-PGPB strains against stresses through production of EPS, biosurfactants, proline, salicylic acid and sidrophores, chelating various metal ions and promoting the growth of plant.

Regarding the response of chlorophyll (b) to presence of heavy metals in soil, data indicated that Pb^{2+} at 800 mg/kg soil was the most significant toxic metal for the uninoculated plants. While, Cu^{2+} at (400 mg/kg) soil was the most toxic one in inoculated plants. Additionally, cadmium significantly effect on chlorophyll (b) content than either chlorophyll (a) or carotenoids. This trend was in harmony with Popova et al. (2012) who reported that chlorophyll content decreased only in the variant treated with 25 μM Cd. The application of heavy metals individually or in mixture cause decrease in photosynthetic pigments.

3.3. Effect of inoculation with HMT-PGPB on growth characteristics of sorghum plants

Data in Table 5 displayed the effect of heavy metals and/or inoculation with HMT-PGPB on sorghum growth characteristics (plant height, shoot and root fresh weight, shoot and root dry weight). Generally, the application of heavy metals in soil decreased all estimated growth characteristics either in inoculated or uninoculated plants. Plants exposed to heavy metals showed noticeably a stunted growth compared to that cultivated in the control soil (free of heavy metals). This trend of results was true with all four heavy metals and could be because the excessive accumulation of these metals can be toxic to most plants. These results were confirmed by Burd et al. (2000), who reported that heavy metals ions when present at an elevated level in the environment, are adsorbed by roots and translocated to different plant parts, leading to impaired metabolism and reduced growth.

In soil free of heavy metals, the inoculated plants gave higher records of all estimated parameters than uninoculated ones. This may be due to that HMT-PGPB have an exceptional ability to promote plant growth by various mechanisms, viz. production of plant growth regulators (IAA, GA_3) which confirmed in previous studies. In heavy metals contaminated soils, it was clear that cadmium at all concentrations was more toxic metal on most estimated parameters. This because that Cd inhibits root and shoot growth, affects nutrient uptake and homeostasis, and frequently is accumulated by plants (Belimov et al., 2003).

The inoculation of sorghum plants with HTM-PGPB cause a significant increase in plant height in presence of Pb^{2+} at all concentrations compared with uninoculated plants. The highest significant plant height (98 cm) was recorded in plants grown in soil amended with Pb^{2+} at 200 mg/kg soil and inoculated with HMT-PGPB. This may be because HMT-PGPB can produce plant promoting substances in metal-stressed environments which confirmed in this study and confirmed with (Wani et al., 2007).

The synthesized IAA molecules by PGP heavy metal tolerant bacteria are secreted and transported into plant cells. These auxins have dual roles, one is to participate in plant cell growth and the other is to promote ACC synthase activity to increase the ethylene titer. Stress induces an increase in ACC levels and, therefore, emulates the action of IAA molecules. Increased ACC molecules then diffuse from plants and are imported into PGPR cells where they are subjected to the action of ACC deaminase. Because of this, microbes and plants are more tolerant to stress-induced growth inhibition that is mediated by ethylene (Tak et al., 2013). Although, the lowest significant leaves number was observed in uninoculated plants which grown in soil amended with zinc at 1000 mg/l, higher number was recorded in inoculated plants grown under the same conditions. Several plants associated bacteria have been reported to accelerate phytoremediation in metal contaminated soils by promoting plant growth and health, and they play a significant role in accelerating phytoremediation (Dary et al., 2010). Moreover, fresh shoots and roots weight were also affected with presence of heavy metals. Although the lowest significant fresh weight was recorded in uninoculated plants which grown in soil amended with copper at 400 mg/kg soil, the highest significant values were observed in inoculated plants which grown in soil amended with copper at 100 mg/kg soil. This may be due to two reasons; the first is the high concentration of copper which led to decrease in plant growth included root system. However, the second reason is that the presence of HTM-PGPB that able to alleviate the toxic effect of heavy metals on plants.

These results were confirmed by Aydinalpi and Marinova (2003) who proved that the presence of high heavy metal concentrations in soil may cause detrimental effects on both soil microbes and plants. Therefore, improvement of the interactions between plants and beneficial rhizosphere microbes can enhance biomass production and tolerance of the plants to heavy metals and are considered to be an important component of phytoremediation technology (Glick, 2003). Also, Clemens and Ma (2016) reported that although the toxic effect of heavy metals on plants, it has been known that plants possess several defense strategies to avoid or tolerate heavy metals intoxication but beyond certain limits these mechanisms fail and survival of plant.

Respecting the effect of heavy metals mixture on sorghum growth, it was clear that all estimated parameters were lower in uninoculated plants that inoculated plants. This could be because the toxic effect of heavy metals and role of HMT-PGPB which possess many strategies (salicylic acid, proline, exopolysaccharide, biosurfactant and sidrophores) to alleviate the toxic effect of heavy metals as previously confirmed (Table 3) and as many researchers demonstrated. They indicted that SA pretreatment alleviates lead-induced membrane damage

Table 5
Effect of heavy metals in presence or absence of HM-tolerant PGPB on growth characteristics of sorghum.

Treatments		Plant Height (cm)		No. of of leaves		Shoot fresh weight (g/plant)		Root fresh weight (g/plant)		Shoot dry weight (g/plant)		Root dry weight (g/plant)	
		Inoculation with HMT-PGPB											
		Without	With	Without	With	Without	With	Without	With	Without	With	Without	With
Control		94.0^{a}	96.0^{b}	8.0^{ab}	9.0^{a}	58.1^{b}	46.6^{i}	9.3^{b}	6.3^{l}	8.7^{b}	10.3^{de}	1.9^{b}	3.2^{cde}
Cu^{2+} (mg/kg)	100	92.0^{b}	97.0^{ab}	7.0^{bc}	7.0^{bc}	51.5^{c}	77.2^{j}	7.6^{d}	13.4^{i}	6.2^{d}	8.6^{g}	1.2^{e}	3.6^{cd}
	200	83.0^{d}	93.0^{c}	6.0^{cd}	6.0^{c}	42.2^{d}	66.2^{j}	4.7^{g}	6.9^{i}	6.1^{d}	6.7^{i}	1.2^{e}	3.2^{de}
	400	40.0^{e}	73.5^{i}	6.0^{cd}	6.0^{c}	36.9^{i}	52.7^{c}	3.3^{j}	5.7^{c}	5.2^{e}	5.9^{j}	1.1^{fg}	2.1^{g}
Cd^{2+} (mg/kg)	10	76.5^{f}	87.0^{e}	7.0^{bc}	7.0^{bc}	37.2^{h}	80.6^{a}	6.8^{e}	7.1^{a}	5.7^{d}	9.5^{f}	1.6^{c}	3.7^{c}
	20	70.0^{h}	80.0^{h}	6.0^{cd}	6.0^{c}	36.7^{j}	55.0^{e}	5.6^{f}	3.9^{e}	5.2^{e}	7.6^{h}	1.2^{ef}	2.8^{ef}
	40	50.0^{k}	68.0^{k}	6.0^{cd}	6.0^{c}	29.2^{l}	51.5^{g}	4.3^{h}	3.8^{g}	4.5^{c}	4.5^{k}	1.0^{g}	2.1^{g}
Pb^{2+} (mg/kg)	200	83.0^{d}	98.0^{a}	9.0^{a}	8.0^{ab}	57.3^{c}	72.7^{f}	8.5^{c}	12.9^{f}	9.4^{a}	12.3^{b}	2.1^{a}	4.2^{b}
	400	86.0^{c}	90.0^{d}	7.0^{bc}	7.0^{bc}	41.1^{f}	58.3^{b}	6.7^{e}	6.1^{h}	5.0^{ef}	12.0^{c}	1.9^{b}	3.6^{cd}
	800	64.0^{i}	85.0^{f}	6.0^{cd}	6.0^{c}	36.4^{k}	49.2^{h}	3.6^{i}	4.2^{b}	4.2^{gh}	10.5^{d}	1.5^{c}	1.7^{g}
Zn^{2+} (mg/kg)	250	79.0^{e}	94.0^{c}	8.0^{ab}	8.0^{ab}	63.6^{a}	68.3^{d}	13.2^{a}	10.6^{d}	8.2^{b}	13.9^{a}	1.5^{cd}	5.2^{a}
	500	73.0^{g}	83.0^{g}	6.0^{cd}	8.0^{ab}	29.9^{g}	38.6^{l}	8.5^{c}	8.8^{j}	7.4^{c}	11.2^{c}	1.4^{d}	2.0^{g}
	1000	56.0^{j}	81.0^{h}	5.0^{de}	6.0^{c}	25.6^{e}	28.2^{m}	7.5^{d}	8.5^{k}	4.6^{fg}	9.9^{e}	1.2^{e}	1.7^{g}
Mixture (mg/kg)		63.0^{i}	71.0^{j}	4.0^{e}	8.0^{ab}	25.3^{m}	38.9^{k}	4.7^{g}	7.9^{j}	3.8^{h}	8.9^{g}	1.2^{g}	2.7^{f}

$^{a, b, c}$ Means with different superscript in the same column are significantly different at ($P < 0.05$).

Table 6
Heavy metals uptake (mg/g plant) by sorghum plant as by using one of tested bacterial strains affected.

Treatments			Shoot		Root	
			Inoculation with HMT-PGPB			
			Without	With	Without	With
Control			0.00	0.00	0.00	0.00
Cu^{2+} (mg/kg)		100	13.5^{k}	3.2^{k}	10.6^{h}	1.40^{h}
		200	23.2^{j}	5.7^{i}	13.6^{e}	2.70^{f}
		400	27.6^{i}	7.7^{f}	13.9^{d}	3.20^{e}
Cd^{2+} (mg/kg)		10	6.7^{p}	0.7^{m}	3.7^{m}	0.24^{j}
		20	9.4^{n}	2.5^{l}	2.3^{o}	1.37^{h}
		40	9.7^{m}	3.6^{j}	4.8^{k}	1.39^{h}
Pb^{2+} (mg/kg)		200	41.4^{g}	9.4^{d}	9.4^{i}	3.50^{d}
		400	51.7^{f}	11.6^{c}	12.4^{f}	4.20^{b}
		800	56.6^{e}	13.4^{a}	17.5^{a}	6.50^{a}
Zn^{2+} (mg/kg)		250	34.6^{h}	6.8^{h}	8.7^{j}	1.24^{i}
		500	59.4^{d}	9.4^{d}	10.7^{h}	2.10^{g}
		1000	65.4^{b}	12.3^{b}	14.6^{c}	3.80^{c}
Mixture (mg/kg)	Cu^{2+}	200	13.2^{l}	0.2^{n}	4.3^{l}	0.19^{j}
	Cd^{2+}	20	7.5^{o}	0.8^{m}	2.7^{n}	1.44^{h}
	Pb^{2+}	400	60.9^{c}	7.4^{g}	11.1^{g}	3.10^{e}
	Zn^{2+}	500	80.7^{a}	8.5^{e}	15.6^{b}	2.70^{f}

$^{a, b, c}$ Means with different superscript in the same column are significantly different at ($P < 0.05$).

in rice, and cadmium toxicity in barley (Metwally et al., 2003) and maize plants (Krantev et al., 2008). From another view, it was clear that the uninoculated plants which cultivated in soil contaminated with the examined heavy metals either individually or in mixture exhibited some tolerance to the metals effect. This may be due to the presence of native microorganisms in soil or to the self-defense of plants to heavy metals stress as Tak et al. (2013) suggested, who said that plant stress generated by metal-contaminated soils can be countered by enhancing plant defense responses. Responses can be enhanced by alleviating the stress mediated impact on plants by enzymatic hydrolysis of ACC, which is intermediate in the biosynthetic pathway of ethylene.

3.4. Effect of inoculation with HMT-PGPB on heavy metals uptake by sorghum plants

Data presented in Table 6 clearly indicated that no heavy metals were uptake by either inoculated plants or uninoculated ones cultivated in soil free of heavy metals. Zhuang et al. (2009) clarified these results, who revealed that different cultivars of sorghum have a different ability to accumulate heavy metals from contaminated soils. Moreover, all estimated heavy metals (copper, cadmium, lead, zinc) were generally greater than in shoots that root system. Also, it was clear that roots seem to have a barrier to prevent the transport of cadmium to shoots, but for other metals this barrier is more permeable. A higher metal uptake in roots relative to shoots is reported in grasses, semi resistant, sensitive, and resistant plants including sorghum (Pinto et al., 2010).

Results also showed that Zn^{2+} uptake by uninoculated sorghum was significant higher in soil amended with heavy metals mixture than other treatments. But, Pb^{2+} was uptake at higher amounts by the inoculated plants. This was true in shoot system. Plants can immobilize metals in soil by forming of insoluble compounds as a result of interactions of contaminants with plant exudates in rhizosphere or by absorption on root system (Kidd et al., 2009). Some plant species are also able to accumulate heavy metals in their plant tissues, so the contaminant is removed from site with harvested plant (McGrath and Zhao, 2003).

Also, heavy metals concentration in inoculated plants was less than the uninoculated ones. This may be due to HMT-PGPB cane reduce the absorption of heavy metals by sorghum plants. Madhaiyan et al. (2007) approved these results, who said that when vegetable crops were treated with Cd^{2+} and inoculated with bacterial strains, there was reduction in the accumulations of Cd^{2+} in roots and shoots, with significant increase in the plant growth attributes with bacterial inoculations compared to untreated control. Also, Sinha and Mukherjee (2008) demonstrated that the reduction of Cd^{2+} uptake might be due to bacterial cadmium accumulation or immobilization, leading ultimately to lower availability of Cd^{2+} in soil.

Data also revealed that heavy metals concentrations contents increased in sorghum plant tissue by increasing the concentration of soil heavy metals viz. Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} either individually or in mixture. Otherwise, no increase of any heavy metal uptake after inoculation with HMT-PGPB was observed, but in comparison with the control, the increase was significant. Similar results were obtained by Vadas and Ahner (2009) with *Zea mays* cultivated in contaminated soil

Table 7
Translocation factors (TF) of different heavy metals as affected by inoculation with HMT-PGPB.

Treatments			TF	
			Inoculation with HMT-PGPB	
			Without	With
Control			0.00	0.00
Cu^{2+} (mg/kg)		100	1.27^{p}	2.28^{j}
		200	1.70^{o}	2.11^{k}
		400	1.98^{m}	2.40^{i}
Cd^{2+} (mg/kg)		10	1.81^{n}	2.91^{e}
		20	4.08^{g}	1.82^{n}
		40	2.60^{l}	2.58^{h}
Pb^{2+} (mg/kg)		200	4.40^{e}	2.68^{g}
		400	4.16^{f}	2.76^{f}
		800	3.23^{i}	2.06^{l}
Zn^{2+} (mg/kg)		250	3.97^{h}	5.48^{a}
		500	5.55^{a}	4.49^{b}
		1000	4.47^{d}	3.23^{c}
Mixture (mg/kg)	Cu^{2+}	200	3.06^{j}	1.05^{o}
	Cd^{2+}	20	2.77^{k}	1.94^{m}
	Pb^{2+}	400	5.48^{b}	2.38^{i}
	Zn^{2+}	500	5.17^{c}	3.14^{d}

[a, b, c] Means with different superscript in the same column are significantly different at ($P < 0.05$).

with lead. Also, microbial populations are known to affect trace metal mobility and availability to the plant, through release of chelators, acidification, and redox changes (Abou-Shanab et al., 2003).

3.5. Translocation factors (TF) of different heavy metals as affected by inoculation with HMT-PGPB

Data in Table 7 showed the heavy metals translocations from roots to shoots (TF) of different heavy metals as affected by inoculation with HMT-PGPB. Except control treatment, all TF values were more than 1.0 this means that heavy metals were effectively translocated from the roots to the shoots. As Fayiga and Ma (2006) demonstrated, TF values > 1 indicate that heavy metal is effectively translocated from the roots to the shoots.

In case of cultivation of uninoculated plants in soil individually amended with heavy metals, the highest and the lowest TF values were recorded in soil amended with Zn^{2+} at 500 mg/kg soil and Cu^{2+} at 100 mg/kg soil, respectively. Whereas, in soil amended with heavy metals mixture, Pb^{2+} gave the highest TF followed by Zn^{2+} while Cd^{2+} gave the lowest value. Tang et al. (2009) reported that the TF of *Arabis paniculata* was < 1 in the range of Pb^{2+} concentration between 9 and 296 μmol. Sun et al. (2009) showed that the TF of black nightshade (*Solanum nigrum* L.) was > 1, while Rezvani and Zaefarian (2011) reported that the TF of *Aeluropus littoralis* changed with differing soil Pb^{2+} concentrations.

4. Conclusion

A green-house experiment was designed during summer 2017 to examine the effectiveness of three heavy metal-tolerant plant growth promoting bacterial strains (HMT-PGPB) (*Alcaligenes faecalis* MG257493.1, *Bacillus cereus* MG257494.1 and *Alcaligenes faecalis* MG966440.1 for alleviating the heavy metal's toxic effects on sorghum plant (*Sorghum bicolor,* L.) cultivated in soil amended with four heavy metals, as well as their capability to enhance sorghum plant. Results proved that the application of heavy metals in soil cause significant decrease in all estimated characteristics in plants viz. two plant oxidative enzymes (peroxidase and polyphenol oxidase), photosynthetic pigments, growth characteristics and metals uptake and these activities in were decreased with the increasing of heavy metals concentrations. On the other hand, all parameters were improved in inoculated plants than uninoculated ones. Additionally, except control treatment, all TF values were more than 1.0 this means that the heavy metals were effectively translocated from the roots to the shoots. This because of the beneficial effects of HMT-PGPB for alleviation the toxic effects of heavy metals through production of EPS, biosurfactants, proline, salicylic acid and sidrophores, chelating various metal ions and promoting the growth of sorghum plant (*Sorghum bicolor,* L.).

Author statement

1. **Rasha M. El-Meihy,** this author contributes as Supervision; Formal analysis; Investigation; Roles/Writing – original draft; Writing – review & editing
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5. **Eman A. El-Alkshar,** this author contributes for Data curation; Formal analysis; Investigation; Methodology; Roles/Writing – original draft.

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