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Impact of *Pseudomonas putida* Inoculation on Alleviating Mercury Stress in Turnip Planted on a Saline Soil

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ABSTRACT

Increasing mercury (Hg) accumulation in soil deteriorates cultivated soils, decreases growth and yield of plants, and contaminates the food chain. Therefore, the main objective of this study was to evaluate the effects of three Hg levels (0, 75 and 150 mg. l^{-1}) in a saline soil with and without *Pseudomonas putida* (PTCC 1696) on the growth parameters of the turnip plant. Pots were filled with 3 kg of saline soil (EC: 8.65 $dS.m^{-1}$) and inoculated with *Pseudomonas putida*. Turnip (*Brassica rapa* L.) seeds were sown in all the pots. After 10 days, the soils were treated with either 0, 75 or 150 mg. l^{-1} of Hg until the final Hg concentrations in soil were either 0, 75 or 150 mg/kg. The pots were arranged in a completely randomised design in a greenhouse. After 60 days of planting, soluble sugars, chlorophyll a (Chl_a), chlorophyll b (Chl_b) and catalase enzymes (CAT) in leaf samples were determined while fresh and dry weights of roots and shoots and Hg concentrations in turnip were determined 70 days after sowing. The results showed that inoculated soils produced plants with higher soluble sugars, Chl_a, Chl_b; (are Chla and Chlb the same as Chl_a and Chl_b) fresh and dry weights of roots and shoots were also significantly higher perhaps due to improved nutrients uptake from the stressed soil. At the same time, CAT and total Hg concentrations in the roots and shoots were reduced probably due to efficient nutrient uptake even when Hg was present. The addition of *Pseudomonas putida* to saline soil contaminated with Hg alleviated salinity and Hg toxicity stress of the turnip plants. In conclusion, Hg polluted saline soil inoculated with *Pseudomonas putida* (PTCC 1696) was efficient in increasing the quality and quantity of turnip plants and improving soil health compared to the non-inoculated soil.

Keyword: Mercury, salinity, plant photosynthetic pigments, bacterium inoculation.

INTRODUCTION

Heavy metals, namely mercury (Hg), copper (Cu), chromium (Cr), zinc (Zn), cadmium (Cd), and lead (Pb) are mutagenic, toxic to cells, and induce carcinogenic changes in human beings and other organisms (Naif *et al.* 2019). Mercury (Hg) occurs naturally in the environment in different chemical species with different solubility, reactivity and toxicity, causing differing impacts on ecosystems and human health (UNEP 2002). Hg is a pollutant that has been found to have serious effects on both environmental and human health, mainly in developing countries

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(Nagajyoti *et al.* 2010). Unlike many other pollutants that are simply removed from the environment (Xu *et al*. 2012), Hg metal is considered to be a global pollutant as it accumulates in natural ecosystems and has harmful impacts on humans and wildlife (Driscoll *et al*. 2013; Richardson *et al*. 2013). Driscoll *et al.* (2013) reported that approximately 45% of the atmospheric Hg is deposited on the earth's ecosystems with soils being the greatest reservoir accounting for up to 75% of the Hg stocked in the biosphere (Mason and Sheu 2002). Hg particularly accumulates in the uppermost soil layers due to its high affinity for the thiol groups to soil organic matter (OM) (Skyllberg *et al*. 2006). Therefore, Hg predestination in soils is mainly a function of organic carbon (OC) dynamics (Smith-Downey *et al*. 2010).

Elimination of contamination by heavy metals such as Hg is difficult in a region because the metals cannot be transformed into harmless forms. Many methods have been applied to eliminate heavy metals from the aquatic environment. The common methods include chemical oxidation, chemical precipitation, reduction, filtration, electrochemical treatment, and extraction using solvents (Mortaheb *et al.* 2009). These traditional methods have various drawbacks including the unpredictable removal of heavy metals and the huge amount of sludge generated which is highly toxic.

Heavy metal removal by means of bioremediation is an alternate way, particularly by applying recombinant and naturally available indigenous microorganisms for the effective removal of toxic substances (Mahajan and Kaushal 2018). There is a need for eco-friendly remedial technologies to address global environmental degradation resulting mainly from anthropogenic activities (De-Bashan *et al.* 2012). Bioremediation is environment friendly and is cheaper than chemical methods. Also, the dead biomass of bacteria or living microbes can remove heavy metals through the bioaccumulation and biosorption process (Joutey *et al.* 2015). Biological systems have been adapted for removal of toxic heavy metals from petrochemical wastewaters (Zeroul *et al.* 2001). Bioremoval, a biological system for elimination of metal ions from polluted environments, has the possibility of achieving greater performance with lower cost than treatments that are not biological (Kondoh *et al*. 1998).

Developments in biotechnology suggest that bacteria and fungi can remove heavy metals from aquatic solutions by adsorption (Saglam *et al*. 1999). Many species of bacteria are adapted to resist toxic effects of metals, so microbial diversity remains high (Iwasaki *et al.*, 2009; Gough and Stahl 2011; Berg *et al.* 2012; Stanaway *et al*., 2012).

Pseudomonas are ubiquitous soil and water microorganisms dwelling in different environments and have diverse lifestyles. Strains of *Pseudomonas putida* are common inhabitants of soil and are crucial in recycling of organic matter in nature; they possess bioremediation potential as they carry genes to cope with natural and xenobiotic chemicals (Segura *et al*. 2009). Green-Ruiz (2006) observed high levels of passive biosorption of heavy metal ions for nonviable cells of *Pseudomonas putida, Brevibacterium* sp. and *Bacillus* sp. The

microbes synthesise various metabolites to degrade the complex wastes and also develop the ability to survive in the presence of various toxic heavy metals in their environment (Tang *et al*. 2018). The important advantage of this process is high sorption ability, very low operating cost, potent biosorbent revival, and the possibility of metal recovery (Goksungur *et al.* 2005). Therefore, the aims of this research were to study the impact of *Pseudomonas putida* in alleviating Hg and salinity stress in turnip plants planted in Hg contaminated saline soils.

MATERIALS AND METHODS

Soil Sampling and Chemical Analyses

Iran's land area of 65 million hectares covers both arid and semi-arid regions. Of this, about 16 - 23 million hectares of land have saline and sodic/alkaline soils, mostly calcareous in nature. These soils are mostly high in salts, pH, and alkaline carbonates and are low in organic matter (OM) and microorganism population and activity. Hence they do not have the essential nutrient elements needed for agricultural crop production. As most of the heavy metal pollution studies at present are conducted on ordinary soils (without any stresses), we decided to study the harmful effects of Hg levels in a highly stressed saline soil (high salinity, with calcareous nature, low OM, low microbial population and activity, and mostly deficient in plant nutrient elements), which was a novel approach to studying a soil, in this respect.

Soil from a surface layer (0-15 cm depth) was collected from the Chenaran region (mostly having natural different saline soils) in Khrasan Razavi state. In the laboratory, the soil was air-dried in the shade and sieved (≤ 2 mm). The visible plant materials in the sieved soil were then removed. The physico-chemical characteristics of the soil were determined based on international standard methods as listed in Table 1.

Pots containing 3 kg saline soil (EC: 8.65 $dS.m^{-1}$) were inoculated with *Pseudomonas putida*. and turnip (*Brassica rapa* L.) seeds were sown in the pots. After 10 days, the soil was treated with three levels of Hg (0, 75 and 150 mg. l^{-1})

TABLE 1
Chemical properties of soil at the start of experiment

Parameters	Unit	value
Texture	-	clay loam
sand	%	26
silt	%	46
clay	%	28
pH	-	8.96
EC	$dS\ m^{-1}$	8.65
Na^{+}	$meq.l^{-1}$	34.8
Ca^{+2}	$meq.l^{-1}$	29
Mg^{+2}	$meq.l^{-1}$	22
HCO_3^{-2}	$meq.l^{-1}$	2.5
Cl^{-}	$meq.l^{-1}$	37
SO_4^{-2}	$meq.l^{-1}$	45.9

until the final concentrations of Hg were equal to 0, 75 and 150 mg/kg of soil. This experiment was conducted as a completely randomised design (factorial) with three replications (3 Hg levels* 2 bacteria inoculations* 3 replications) under greenhouse conditions.

Agar Preparation for Increasing Microbial Growth and Population

Preparations for nutrient agar to the stock culture in test tubes were similar to the preparation of the growth media. The nutrient agar consisted of 5% of pepton from 3% of meat extract and 12% of agar. The nutrient agar was purchased from Merck Sdn. Bhd. The nutrient for the stock culture was prepared by suspending 20 g in 1 litre of demineralised water and heating in a boiling water bath or in a current of steam and autoclaving about 15 min at 121^{o}C (Azoddein *et al.* 2015). *Pseudomonas putida* (PTCC) 1696) was obtained from Iranian Microbial Research Center in Tehran (due to its highly effective remediation of many heavy metals).

Soil Treatment and Planting

In the greenhouse experiment, turnip seeds (*Brassica rapa* L.) were sown in pots filled with 3 kg of saline soil (diameter 23 cm, height 21.5 cm), and irrigated with tap water. Ten days after germination, the soil in the pots was treated with 200 ml of mercury chloride solutions of Hg treatments (0, 75 and 150 Hg mg. l^{-1}). Simultaneously, the soil in the pots was treated with 50 ml of *Pseudomonas putida* 10 days after planting (plants at three leaf stage). Sixty days after planting, leaf samples were collected and investigated for photosynthetic pigments, soluble sugars, catalase enzymes and chlorophylls *a* and *b*. Plants were harvested 70 days after sowing and fresh and dry weights of roots and shoots and mercury concentration in roots and shoots of turnip were determined. Soil in each pot after plant harvest was collected and determined for total and available mercury concentration.

Measurement of Plant Growth and Physiological Parameters

Sixty days after planting, fresh leaf samples were taken and used for photosynthetic pigments. Leaf chlorophyll a, b was determined (Lichtenthaler 1987). Soluble sugars were determined by the method of Omokolo *et al.* (1996). Catalase enzymes (CAT) were determined by the method of Chance and Maehly (1955). Mercury concentrations in root and shoot of turnip were also determined (Greger *et al.* 2005).

Determination of Mercury in Soil and Plant Materials

Soil samples (0.5g) were treated with 12 ml of Aqua Regia (HNO_3: HCl, 1:3) solution. The mixture was heated at a low temperature initially for 1 h, to which was added 20 ml of 2 % HNO_3. The mixture was then digested at a high temperature for 30 min and subsequently diluted with 25 ml of 2 % HNO_3 and filtered with Whatman No.42 filter paper. To measure Hg, the filtrated solution

was analysed by ICP-OES (SPECTRO ARCOS Model76004SSS Germany) (UNEP/IAEA, UNEP 1985). Dried plant samples (0.5 g) were digested with 5 ml of diacid (HNO_3:$HCIO_4$ 1:3). The mixture was heated at a low temperature for 1 h, and 3 ml of diacid was added. The sample was diluted with 50 ml of 2 % HNO_3 and filtered with Whatman No.42 filter paper. The concentration of Hg was measured by ICP-OES. (SPECTRO ARCOS Model 76004SSS Germany) (Greger *et al.* 2005).

Statistical Analysis

The experiment was performed as a completely randomised design (factorial) with three replications, and included 18 pots for treatment at three levels of Hg (0, 75 and 150 Hg mg. l^{-1}) in which the effect of bacterium inoculation and no inoculation was investigated under greenhouse conditions. Data were subjected to statistical analysis using JMP8 software. The significant differences ($P<0.05$, and $P<0.01$) between treatments and control were statistically evaluated by Student's t-test methods.

RESULTS AND DISCUSSION

Effect of Applied Hg on Turnip Growth under Soil Saline Stress

The effects of single Hg treatment on turnip growth (Table 2), showed that the shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of turnip significantly decreased at Hg levels of 75 and 150 mg. l^{-1} in comparison with the control ($P<0.01$). Earlier studies have indicated that Hg at higher concentrations is highly phytotoxic to cells and induced visible injuries and physiological alterations (Zhou *et al.* 2007). The reduction of plant growth caused by mercury was observed for *Triticum aestivum* and other plant species such as tomato (Cho and Park 2000) and tobacco (Suszcynsky and Shann 1995). Reduced growth was also indicated by a high dry weight to fresh weight ratio in plants grown with 10 and 25 µM Hg. An increased dry weight to fresh weight ratio may be a sign of reduced water uptake, which in turn causes inhibited elongation and enlargement of cells leading to reduced growth (Mukherji and Mukherji 1979).

Effect of Applied P. putida on Turnip Growth under Soil Saline Stress

Single *P. putida* treatment on turnip growth (Table 3) resulted in a significant increase in the shoot and root fresh weights and in the shoot and root dry weights of turnip compared to the control ($P<0.01$). Plant growth-promoting

TABLE 2
Effect of Hg levels on turnip growth

Hg levels (mg .l^{-1})	Shoot fresh weight (g pot^{-1})	Shoot dry weight (g pot^{-1})	Root fresh weight (g pot^{-1})	Root dry weight (g pot^{-1})
0	5.65a	2.26a	18.76a	4.47c
75	3.28b	1.97b	16.65b	3.56b
150	1.58c	1.55c	13.1c	2.96a

Values are the mean of three replicates and different letters within columns indicate significant differences $P<0.01$ by Student's t-test compared to control.

rhizobacteria (PGPR) within genera that are known to stimulate growth of plants are *Azospirillum, Azotobacter, Bacillus*, Enterobacter, and *Pseudomonas*. The latter has been studied in recent years for its beneficial effects when used as organic fertiliser or as an agent for biological control of pathogens (Lugtenberg and Kamilova 2009). Furthermore, the positive response of the plants to the inoculation of the different strains of *P. putida* for both cultivars and in particular Prestige may be due to a hormonal effect of the rhizobacteria, whether produced directly via an auxin and/or gibberellin (Yao *et al*. 2010). *Azotobacter* spp. and *Pseudomonas* spp. are the most important bacteria that increase soil mineral elements with the production of matters increased the dry matter accumulation, number of nodules, seed yield and grain protein by 71%, 86%, 36% and 16%, respectively, compared to noninoculated plants. Nitrogen in roots and shoots increased by 46% and 40%, respectively, at 136 mg Cr/kg that regulate growth and development and yield of plants (Hayat *et al.* 2010). Pseudomonas bacteria are able to produce the hormones, auxin and gibberellic, as well as vitamins. The bacteria lead to increased nutrients uptake resulting in increased plant weight and yield. This increase in plant growth and yield by growth-promoting rhizobacteria is due to its ability to produce siderophore and increase the level of iron in the plant (Bhattacharyya and Jha 2012).

TABLE 3
Effect of *P. putida* levels on turnip growth

P. putida levels	Shoot fresh weight (g pot^{-1})	Shoot dry weight (g pot^{-1})	Root fresh weight (g pot^{-1})	Root dry weight (g pot^{-1})
without *P. putida*	2.83 b	1. 6 b	15.98 b	3.59 b
with *P.putida*	4.17a	2.25a	16.36a	3.74a

Notes: Values are the mean of three replicates and different letters within columns indicate significant differences $P < 0.05$ and $P<0.01$ by Student's t-test when compared to control.

Interaction Effects of Hg and P. putida Treatments on Turnip Growth under Soil Saline Stress

Interaction effects of Hg and *P. putida* treatments on turnip growth (Table 4), showed that the shoot and root fresh weights, shoot and root dry weights of turnip significantly increased at Hg levels of 75 and 150 mg. l^{-1} with *P. putida* in comparison with no *P. putida* (control) ($P<0.01$). Enhanced plant biomass yield in treatments with plant growth-promoting bacteria (PGPB) is supported by previous studies on PGPB inoculation in the presence of organic toxicants and metals (Gurska *et al*. 2009). In the presence of copper, *Pseudomonas* spp. enhanced the growth of canola and common reed (Gurska *et al*. 2009). *Pseudomonas putida* UW4 inoculation improved growth of cucumber (*Cucumis sativus*) (Gamalero *et al*. 2010) and canola under salinity stress (Cheng *et al.* 2012).

TABLE 4
Interaction effect of P. putida levels on turnip growth under Hg levels stress

P. putida levels	Hg levels (mg $.l^{-1}$)	Shoot fresh weight (g pot^{-1})	Shoot dry weight (g pot^{-1})	Root fresh weight (g pot^{-1})	Root dry weight (g pot^{-1})
Without *P. putida*	0	4.4 [b]	1.92 [d]	16.74 [a]	4.12 [b]
	75	2.9[c]	1.8[e]	15.41 [b]	3.74 [c]
	150	1.2[d]	1.76[f]	13.00c	2.91[e]
With *P. putida*	0	6.9[a]	2.6a	18.98 [a]	4.83 [a]
	75	3.66 [bc]	2.14 [b]	16.9 [b]	3.39d
	150	1.37[d]	2.01[c]	13.2[c]	3.01[e]

Notes: Values are the mean of three replicates and different letters within columns indicate significant differences P<0.05 and P<0.01 by Student's t-test compared to control.

In general, mercury phytotoxicity exerted some effects on the biomass of the plant, while inhibiting plant growth and showing long-terms impacts on the fertility of the soil (Sahi *et al.* 2006). This study noted that *P. putida* had a positive impact on turnip growth parameters. In general, the analysis of variance (Table 8) with respect to the shoot and root fresh and dry weights results obtained under the *P. putida* effect showed that both the shoot and root fresh and dry weights were significant (P<0.01) respectively. In this experiment, with p. putida inoculation, there was a significant increase in the shoot (47.35%) and root fresh (2.37%) and shoot (40.62%) and root (4.17%) dry weights. In general, inoculation of bacteria under Hg stress promoted plant growth and biomass. The increased biomass can be attributed to absorption of nutrients such as nitrogen and phosphorus due to the increase in root development (Goenadi *et al*. 2000). As indicated by Azoddein *et al*. (2015), the mercury was removed and the percentage of reduction was about 89% for two days. This result indicates that mercury Hg^{2+} is volatile to Hg^0. The final value of mercury concentration was about 0.001 mg l^{-1}. The safety limit in wastewater is about 0.005 mg l^{-1}. So, the results of our study proved that mercury can be removed with high efficiency using *P. putida*, thus improving the growth of the plant in a saline soil.

Effect of Applied Hg levels on Mercury Concentration of Turnip under Soil Saline Stress

Hg treatment (Figures 1 and 2) showed that mercury concentration in shoot and root significantly increased at Hg levels of 75 and 150 mg. l^{-1} respectively in comparison with the control (P<0.01). Hg accumulation, both in leaves and roots of the treated turnip plants increased with increased concentration of $HgCl_2$. There was a comparatively higher amount of Hg in roots than in leaves. The level of Hg found in 7-day old leaves with 2.5, 5, 10 and 25 µ M $HgCl_2$ treated plants were 2.32, 5.39, 14.72 and 41.32 mg Kg^{-1} DW respectively. In roots of these treated plants, the concentrations of Hg were 11.26, 47.25, 187.18 and 721.54 mg Kg^{-1} DW respectively (Gopal *et al*. 2012).

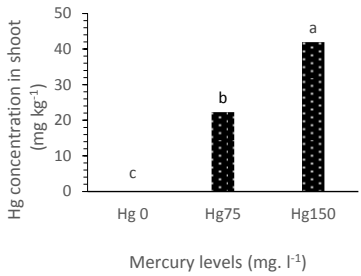

Figure 1. Effect of Hg levels in shoot Hg concentration

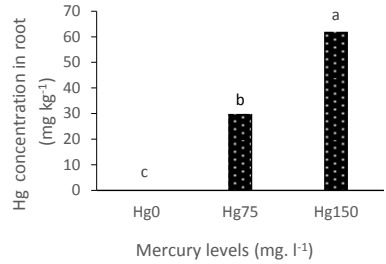

Figure 2.Effect of Hg levels in root Hg concentration

Effect of P. putida Inoculation on Mercury Concentration of Turnip under Soil Saline Stress

The effects of *P. putida* treatment on mercury concentration in turnip (Figures 3 and 4), showed that mercury levels in both shoot and root decreased significantly with *P. putida* inoculation in comparison with no inoculation (control) ($P < 0.01$). Azoddein *et al*. (2015) conducted a study to remove mercury using *P. putida* pure culture ATTC 49128 at optimum growth parameters such as techniques of culture, acclimatisation time and speed of incubator shaker. The removal of two different mercury concentrations, 1 and 4 mg. l^{-1} showed that the overall levels of mercury removal in this study were between 80 and 89.

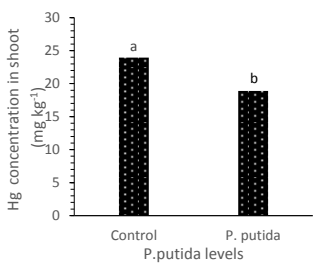

Figure 3.Effect of P. putida levels in shoot Hg concentration

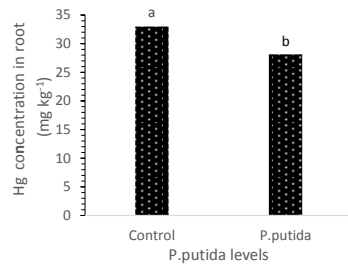

Figure. 4. Effect of P. putida levels in root Hg concentration

Interaction Effects of Hg and P. putida Treatments on Mercury Concentration of Turnip Plant under Soil Saline Stress

Interaction effects of Hg levels and *P. putida* treatments on mercury concentration of root and shoot turnip plant (Figures 5 and 6) showed that mercury concentration in shoot and root were significantly decreased at Hg levels of 75 and 150 mg. l^{-1} respectively compared to *P. putida* treatment with no inoculation (control) ($P<0.01$). Efficient removal of mercury using *P. putida* was successfully achieved by mercury-resistant bacteria, *P. putida*, in the laboratory study. The overall levels of mercury removal in this study were between 80% and 89% indicating that

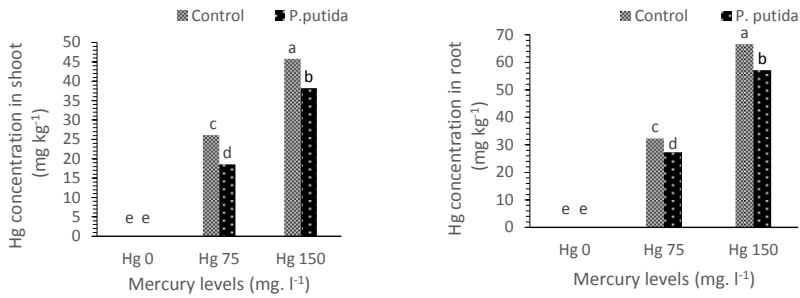

Figure 5. Interaction effect of Hg and P. putida treatments in shoot Hg concentration.
Figure. 6. Interaction effect of Hg and P. putida treatments in root Hg concentration

the microbial detoxification system for mercury was highly effective under these conditions (Azoddein *et al*. 2015).

In general, our study results agreed with many previous studies (Ren *et al.* 2014; Cheng *et al.* 2012), which found that Hg was mainly accumulated in roots. The high Hg concentration in turnip roots is attributed the direct exposure of roots to Hg; moreover, a large amount of Hg was absorbed by roots along with other essential micronutrients (Greger *et al.* 2005; Chen and Yang 2012), and thus immobilised in root cells. However, a significant inhibition of Hg uptake by turnip was observed when plants were exposed to Hg and application of *P. putida* (P<0.01) (Figures 5 and 6). This finding could be explained by the transport of Hg^{+2} to Hg^{0}, bacteria that possess the *mer* operon and are able to enzymatically reduce Hg^{+2} to the volatile and less toxic form of mercury Hg^{0} (Barkay and Wagner-Dobbler 2005). The gene *mer* A is part of an operon which comprises regulatory genes encoding transport proteins (Narita *et al.* 2003). In general, many mercury resistant isolates possess the *mer* R, *mer* P and *mer* genes encoding proteins for regulatory function, transport and extracellular binding, and mercuric (II) reductase, respectively (Silver and Hobman 2007). Ghosh *et al*. (1996) found the ability of microorganisms to remove mercury from the culture medium to be affected by high concentrations of $HgCl_2$, suggesting that it may be due to sequestering of intracellular Hg by cell components that bind to the metal.

Effect of Applied Hg Levels on Photosynthetic Pigments and Some Enzymatic Antioxidant Activity

The effects of Hg levels on chlorophylls a, b, soluble sugars and catalase enzymes (CAT) (Table 5), showed that the chlorophylls a, b and soluble sugars of turnip significantly decreased at Hg levels of 75 and 150 mg. l^{-1} respectively compared to the control (P<0.01). Gopal *et al*. (2012) reported that for roots and leaves of wheat plants with increased concentration of Hg up to 10 µM, low activities of these enzymes were observed at 25 µM Hg. In contrast, results of this research showed that with increasing levels of Hg treatments, there was a significant increase in CAT content compared to the control (P<0.01). Skrebsky *et al.* (2008)

reported an increase in both root and shoot biomass of *Pfaffia glomerata* plantlets at low Cd levels. On the other hand, the reduction in root growth, chlorophyll content and total soluble protein was caused by the increased concentration of Hg to 10 and 25 µM in their study. Many organic compounds including chlorophyll have been used as biomarkers for the early detection of metal toxicity in plants (Prasad 2003). The biosynthesis of chlorophyll is known to be inhibited by Hg that interacts with δ- aminolevulenic acid dehydratase (Prasad and Prasad 1987). CAT activity increased in roots of wheat plants grown with 2.5 and 5 µM $HgCl_2$. Also, the enhancement of enzyme activity in leaves of plants grown with 2.5, 5 and 10 µM $HgCl_2$ was recorded compared to control plants. In the leaves of wheat plants treated with 2.5, 5 and 10 µM $HgCl_2$, CAT activity increased by 18, 27 and 36 % respectively. However, CAT activity decreased by 23 % in the leaves of plants grown with 25 µM $HgCl_2$. In roots, the activity was elevated by 36% and 74 % with 2.5 and 5 µM $HgCl_2$, while it decreased by 24% and 36 % respectively with 10 and 25 µM $HgCl_2$ (Gopal *et al*. 2012). Furthermore, sugar depletion normally occurs during ontogeny of plants. For instance, variations in environmental factors such as light, water or temperature and attacks by pathogens or herbivores may lead to a significant decrease in the efficiency of photosynthesis in source tissues and thus reduce the supply of soluble sugars to sink tissues. Under conditions of sugar deprivation, substantial physiological and biochemical changes occur to sustain respiration and other metabolic processes (Mariana *et al.* 2009). Drought, salinity, low temperature and flooding, in general, increase soluble sugar concentrations, whereas high light irradiance (PAR, UVBR), heavy metals, nutrient shortage and ozone decrease sugar concentrations (Gill *et al*. 2001).

TABLE 5
Effect of Hg levels on chlorophylls, soluble sugars and CAT of turnip

Hg levels ($mg.l^{-1}$)	Chl_a (µ g/g FW)	Chl_b (µ g/g FW)	Soluble sugars (mg/g FW)	CAT (mg/g FW)
0	1.55^a	0.3^a	0.29^a	0.52c
75	1.38^b	0.16^b	0.24^b	0.73^b
150	1.22^c	0.11^c	0.10^c	1.29a

Notes: Values are the mean of three replicates and different letters within columns indicate significant differences P<0.05 and P<0.01 by Student's t-test compared to control.; Chl_a : Chlorophyll a; Chl_b: chlorophyll b; CAT: catalase enzymes

Effect of P. putida Inoculation on Photosynthetic Pigments and Some Enzymatic Antioxidants Activity under Soil Salinity Stress

The effects of *P. putida* treatment on chlorophylls a, b, soluble sugars and CAT (Table 6), showed that the chlorophylls a, and b of turnip significantly increased with *P. putida* inoculation compared to no *P. putida* (control) (P<0.01). However, soluble sugars of turnip significantly increased with *P. putida* compared to the control (P<0.05). In contrast, our results showed a significant reduction in CAT content compared to no *P. putida* (control) (P<0.01). Delshad *et al*. (2017), who worked on a comparison of different treatments of bio-fertilisers, reported that

the highest increases in chlorophyll a, chlorophyll b and total chlorophyll were related to treatment of *P. putida* compared to the control treatment (without *P. putida*).

TABLE 6
Effect of P. putida levels on chlorophylls, soluble sugars and CAT of turnip

Hg levels (mg .l^{-1})	Chl_a (μ g/g FW)	Chl_b (μ g/g FW)	Soluble sugars (mg/g FW)	CAT (mg/g FW)
0	1.55^{a}	0.3^{a}	0.29^{a}	0.52c
75	1.38^{b}	0.16^{b}	0.24^{b}	0.73^{b}
150	1.22^{c}	0.11^{c}	0.10^{c}	1.29a

Notes: Values are the mean of three replicates and different letters within columns indicate significant differences P<0.05 and P<0.01 by Student's t-test when compared to control. Note: Chla : Chlorophyll a; Chlb: chlorophyll b; CAT: catalase enzymes

Interaction Effects of Hg and P. putida Treatments on Photosynthetic Pigments and some Enzymatic Antioxidants Activity under Soil Salinity Stress

Interaction effects of Hg and *P. putida* treatments on chlorophylls *a, b*, soluble sugars and CAT (Table 7) showed that chlorophylls *a, b* and soluble sugars of turnip significantly increased at Hg levels of both 75 and 150 mg. l^{-1} with *P. putida* inoculation compared to no *P. putida* (P<0.01). With respect to CAT content, results showed that with increasing levels of Hg with and without P. putida inoculation, a significant (P<0.01) increase in CAT content was noted with *P. putida* inoculation compared to control (without *P. putida* inoculation) which showed a lesser increasing trend (Table 7). The induction of enzymatic antioxidants catalase (CAT), ascorbate peroxidase(APX), peroxidase(POX) and superoxide dismutase (SOD) was found in the roots and leaves of wheat plants with increasing concentrations of Hg up to 10 μM but low activities of these enzymes were observed at 25 μM Hg (Gopal *et al*. 2012).

TABLE 7
Effect of P. putida levels on chlorophylls, soluble sugars and CAT of turnip under Hg stress

P. putida levels	Hg levels (mg .l^{-1})	Chl_a (μ g/g FW)	Chl_b (μ g/g FW)	Soluble sugars (mg/g FW)	CAT (mg/g FW)
without *P.putida*	0	1.19^{c}	0.21 ^{c}	0.29 ^{b}	0.12 ^{e}
	75	0.9^{e}	0.16^{d}	0.24 ^{d}	0.29 ^{c}
	150	0.71^{f}	0.09^{f}	0.101^{e}	0.66^{a}
with *P. putida*	0	1.92^{a}	0.4a	0.3 ^{a}	0.10 ^{f}
	75	1.41 ^{b}	0.23 ^{b}	0.25 ^{c}	0.21d
	150	1.02^{d}	0.11^{e}	0.109^{f}	0.42^{b}

Notes: Values are the mean of three replicates and different letters within columns indicate significant differences P<0.05 and P<0.01 by Student's t-test compared to control; *Note:* Chl_a : Chlorophyll $_a$; Chl_b: chlorophyll *b*; CAT: catalase enzymes

In this research, we noticed a reduction in the photosynthetic pigments under Hg stress. In general, the high concentrations of this heavy metal are extremely phytotoxic to the plant cells and could cause perceptible damage, as well as physiological disorders (Zhou *et al*. 2007). In plants, the metal ions in the photosynthetic pigments may be replaced by mercury ions, thereby reducing photosynthesis rates (Kupper *et al*. 1998). Our findings showed that high concentrations of mercury ion had significant phytotoxic effects on the plant cells, and also decreased chlorophyll a and b levels especially in the presence of soil salinity. Furthermore, the results demonstrated that the mercury ion could easily accumulate in higher plants (Israr *et al.* 2006). In addition, bacterial inoculation also improved plant physiological parameters such as chlorophyll a, soluble sugar, malondialdehyde, and proline content. A salt-tolerant strain *K. oxytoca* Rs-5 was also able to produce Indole-3-acetic acid (IAA) (Yue *et al*. 2007). The efficiency of plant enhanced phytoremediation (PEP) is dependent on different factors such as PGPB inoculum biomass, plant species, plant-microbe specificity and type of contaminants (Seniyat and Lesley 2019).

TABLE 8

Analysis of variance of Hg and P. putida levels on photosynthetic pigments, some antioxidants enzymatic activity, Hg concentrations in shoot and root of turnip plant

S. V.	Shoot fresh weight	Shoot dry weight	Root fresh weight	Shoot dry weight	Hg concentration in shoot	Hg concentration in root	*Chla*	Chl_b	Soluble sugars	CAT
Mercury (Hg)	24.99**	0.76**	49.1**	3.48**	2645.6**	5749.12**	0.729**	0.090**	0.06**	0.945**
P. putida	8.133**	1.88**	0.63**	0.10**	114.45**	105.27**	3.541**	0.092**	0.0003*	8.40**
Hg × *P. putida*	1.5*	0.125**	0.035**	0.42**	28.61**	33.67**	0.025**	0.002**	0.0000005^{ns}	0.705**
Error	0.25	0.001	0.09	0.004	0.41	1.38	0.0004	0.00003	0.000045	0.0045

Notes: ns non -significant; * and ** Significant at 5% and 1% probability levels respectively; Chl_a : Chlorophyll a; Chl_b: chlorophyll b; CAT: catalase enzymes

CONCLUSION

According to our results, exposure to mercury levels of 75, and 150 mg l^{-1} significantly increased the mercury uptake by turnip (P<0.01), while mercury levels of 0, 75, and 150 mg. l^{-1} significantly reduced growth of turnip through diminishing the chlorophylls and soluble sugars (P<0.01), but in contrast, increasing the catalase enzyme. Also, results showed that inoculated plants with *P. putida* had a better visual appearance, minimising Hg toxicity by increasing photosynthetic pigments, soluble sugars, chlorophylls *a* and *b*. However, with respect to CAT content, with increasing levels of Hg with and without P. putida inoculation, a significant (P<0.01) increase in CAT content was noted; *P. putida* inoculation, compared to control (without *P. putida* inoculation) showed a lesser increasing trend. This bacterium inoculation led to a significant decrease in Hg concentration in the root and aerial parts of the turnip plant (P<0.01). Inoculation with selected soil microbial complex improves plant physiological behaviour, which is important for plant establishment and soil protection. Also, the efficiency of PEP is dependent on different factors such as PGPB inoculum biomass, plant species, plant-microbe specificity and type of contaminants.

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