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ECOTOXICOLOGICAL EVALUATION OF THE TOLERANCE OF SOME PLANT GROWTH-PROMOTING RHIZOBACTERIA TO ENVIRONMENTAL METAL POLLUTANTS

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Abstract: *The major N_2-fixing systems can play a significant role in improving the fertility and productivity of low-N soils. The plant growth-promoting rhizobacteria (PGPR) have received the most attention and have been examined extensively. Under heavy metal stress, soil microorganisms including PGPR have developed many strategies to evade the toxicity generated by the various heavy metals. In the present study, some environmental factors such as heavy metals (Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}), Al^{3+} were studied at different concentrations (0, 20, 40, 80 and 160 µm) on the ecophysiological properties and their growth using microfermentor technique as well as their tolerances to various hydrogen ion concentration. The results showed that the stress factor suppresses the growth characteristics of the studied PGPR including symbiotic Sinohizobium strains related to R. leguminosarum, R. phaseoli, R. trifolii, and R. loti as well as Sinorhizobium meliloti in comparison with non-symbiotic N_2-fixer Azotobacter and Pseudomonas as PGPR strains which are differentially react with the stress factors at high concentrations. However, the tolerance of several investigated strains, distributed among Sinorhizobium meliloti strains: GH-130, GHR-94, GH-97 and GH-07 are sensitive to the stress of 160 µm for heavy metals effects in comparison with Azotobacter and Pseudomonas strain. None of the strains can tolerate 160 µm of Cd^{2+}, $Pb^{2+}$and Cu^{2+}. The most toxic metal was Cd^{2+}, followed by $Pb^{2+}$and Cu^{2+}. The ecophysiological tolerant Sinorhizobium and Azotobacter as well as Pseudomonas putida strains are suggested to be the ideal solution for the improvement of soil fertility and the rehabilitation of reclaimed soils and are an important direction for future soil protection and quality research. Hence, a better understanding of N_2-fixing bacteria ecophysiological responses to different intrinsic stresses factor is very important to improve crop production by harnessing the growth of the N_2-fixing bacteria process.*

Keywords: *heavy metals, aluminium, pH, relative growth rate, tolerance, N_2-fixing bacteria, Azotobacter, Pseudomonas*

INTRODUCTION

Heavy metals are well known for their toxicity and become significant environmental pollution with a continually rising technology and public outcry to ensure the safest and healthiest environment. Contamination of the environment by heavy metals has resulted in the manifestation of heavy metal tolerant microorganisms in the soil polluted with metals. In addition, such heavy metals, once in the soil, accumulate preferentially in the parts where the plant roots are aggregated and in the forms that are easily available to plants. These heavy metals are then absorbed by the plants thus, ultimately

entering the food chain. Microorganisms use different kinds of mechanisms related to resistance and detoxification of heavy metals [1] thus play prominent part in biogeochemical cycling of harmful heavy metals leading to the remediation of metal contaminated environments [2]. Abo-Amer [3] demonstrated that among *Azotobacter* isolates extracted from the soil contaminated with wastewater, 10 strains exhibited considerable degree of resistance to the heavy metals like Co, Ni, Zn and Cu. The soil microbial community faces extremely high pressure due to adulteration of soil by a range of toxic materials including heavy metals along with other organic contaminants of wastewater, sewage sludge etc. The addition of heavy metals in several forms in the environment results in significant alterations of the microbial diversity and activities, thus directly affecting the soil fertility [4]. Some of the heavy metals are necessary for microbial growth and biochemical reactions in very low concentrations in the cell. However, as the heavy metal concentration increase it becomes largely toxic to microorganisms thereby leading to disturbance in vital ecological processes [5]. Pollution of soil with heavy metals is known to have a negative effect on microbial activities. Metal toxicity is of great environmental concern because of their bioaccumulation and non-biodegradability in nature. Today, heavy metal pollution has become one of the serious issues of concern amongst all environmental crises. Heavy metals and aluminium are one of the major sources of environmental pollutants and exist in soil as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds like oxides, carbonates and hydroxides or they may form a part of silicate materials [6]. Heavy metal arise in soil by repeated applications of sewage sludge, municipal wastes and animal slurries, activity of mining and smelting industries, impurities in fertilizers and decomposition of air pollutants by burning of fossil fuels and various industrial activities [7]. Heavy metals such as Cu, Fe, Mn, Ni and Zn are essential for plant growth and are important constituents of many enzymes, whereas metals such as Al, As, Cd, Cr, Hg, Pb, Sb and Se are nonessential and toxic above certain threshold levels [8]. An increase in metal concentration also influences the soil microbial properties, especially respiration and enzymatic activity that serve as good indicators of metal pollution. Several studies have shown a negative relationship between heavy metal concentration and microbial activities, such as respiration [9], [10], mineralization [11].

A total of 120 *Azotobacter* sp. were isolated from soils irrigated with wastewater (contaminated soils) and groundwater (uncontaminated soils). These isolates were screened for resistance to heavy metals. The size of the *Azotobacter* population in heavy metal contaminated soil was lower than that in uncontaminated soil. Of the *Azotobacter* isolates, 64 that were recovered from contaminated soil exhibited high resistance to heavy metals (Hg^{2+}, Cd^{2+}, Cu^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Pb^{2+}) compared to 56 isolates from uncontaminated soil. The majority of *Azotobacter* isolates from contaminated soil showed multiple-resistance to different metal ions. All isolates failed to grow at pH less than 6 [4].

The study thereby highlighted the possible utilization of such bacterial isolates for the bioremediation of metal-contaminated system. Studies by Joshi and Juwarkar [12] revealed that a heavy metal-resistant strain of *Azotobacter* spp. possess a high tendency of binding with Cd and Cr both under in vitro as well as in vivo conditions, and thereby consists of significant control of their uptake by wheat plants raised in heavy metal polluted soils. Resistance to heavy metals in *Azotobcter* spp. is demonstrated to be provided by plasmids [13]. But, in case of *Azotobacter* species particularly, prior to the entry of heavy metal into the cell, they face an encounter with extracellular polymeric substances, which are reported to be produced in large amounts by this bacterium. Extracellular polymeric substances thus clearly play the role of first barrier by chelating the metal ions and restricting their access into the bacterial cells. In addition to its beneficial impact on plant growth promotion, *Azotobacter* is also known to be associated with the suppression of pathogenic diseases of plants. Several examples are present in the literature advocating the importance of disease suppression by different species of *Azotobacter.*

The utilization of PGPR in agriculture is continuously increasing as it offers an effective tool to replace the use of chemical fertilizers, pesticides and other harmful supplements [14], [15], [16]. Growth promoting substances are produced in huge quantities by the action of these rhizosphere microorganisms that directly or indirectly influence the overall morphology and physiology of the crops. Recent advances in the field of sustainable development relies on the use and diversity of PGPR, their colonizing capability and the mechanism of action that may be used to facilitate their application as a dependable element in the management of sustainable agricultural system [15], [16].

MATERIALS AND METHODS

Bacterial strains

- *Sinorhizobium meliloti* strains: GH-130, GHR-94, GH-97 and GH-07
- *Azotobacter* strains: Azot-99 and *Azotobacter* sp
- *Pseudomonas* strain: *Pseudomonas putida*

Aluminium and heavy metal salts:

- Zinc chloride ($ZnCl_2.6\ H_2O$)
- Copper chloride ($CuCl_2$)
- Lead chloride ($PbCl_2$)
- Cadmium chloride ($CdCl_2.2.5H_2O$)
- Aluminium sulphate (Al2(SO4)3

Growth of bacteria in heavy metal treated culture

Using the microfermentor technique, the following investigations were done to determine the relative growth rate (%) of the investigated *Sinorhizobium, Azotobacter* and *Pseudomonas* bacterial strains when the cultural broth media contaminated with heavy metals (Zn, Cu, Pb, Cd) and Al or the pH of the cultural broth media adjusted to be 7; 6.2; 5.2 and 4.5.

Azotobacter bacterial strains were used to measure their growth under different heavy metal stress. A synthetic medium was used (according to Ueda et al. [17]) which contained 1.0 g K_2HPO_4, 0.2 g $MgSO_4$-$7H_20$, 0.1 g $CaCO_3$, 0.2 g NaC1, 5 mg $Na_2MoO_4.2H_20$, and 10.0 g sucrose per litter of distilled water. In flasks of 150 ml capacity, each, containing 45 ml of the culture broth medium containing different concentration (0, 20, 40, 80, 160 µM) of each tested heavy metal ($ZnCl_2$, $CuCl_2$, $PbCl_2$, $CdCl_2$) and $Al_2(SO_4)_3$). Each flask of the polluted broth medium was inoculated with 5 ml of *Azotobacter* bacterial suspension with the cell density $1X10^6$ CFU/ml. Then the flasks were shaken continuously (150 rotation per min) at 28°C. After 96 hours incubation, aliquots were transferred in a sterile manner to test tubes and the optical density (O.D.) at a wavelength of 650 nm was determined. Experiments were carried out in triplicate.

Sinorhizobium strains were grown and maintained on yeast extract mannitol agar medium (YEMA). *Yeast extract mannitol (YEM) agar and broth for Sinorhizobium (g/L): 0.4 yeast extract, 10 mannitol, 0.1 NaCl, 0. $MgSO_4.7H_2O$, 0.2 K_2HPO_4, 15 agar 1000 ml distilled water, pH 6.8. 3 g $CaCO_3$ is added for buffering. Medium was prepared according to Kleczkowska et al.* [18].

Culture medium used in this investigations were YEMA and in broth (YEMB) without agar. Maintenance of sinorhizobial strains was carried out on YEMA + 3 g/l $CaCO_3$. Incubation temperature was at 28°C. A suspension of $1X10^6$ cell forming unit/ml (CFU/ml) of inoculum (in physiological saline of 0.85% NaCl) of each of *Sinorhizobium* strains was used in the following *in vitro* experimental work and the measurement was completed at the logarithmic stage of reproduction. Similar procedure was carried as in case of *Azotobacter* strains. The incubation time was 48 hours.

For *Pseudomonas* strain, similar technique was carried out as mentioned in case of *Azotobacter* and *Sinorhizobium* strains, but the medium was King's B (*(g/L):* (20 g proteose peptone, 1.5 g $MgSO_4.7H_2O$, 1.5 g KH_2PO_4, 15 g agar, 10 ml glycerol, pH 7.2), broth medium. *Pseudomonas* strain was maintained on King's B agar medium. The incubation time was 48 hours. At the end of each experiment, the relative growth rate (%) was calculated as following: (the inhibition or the stimulation of the growth X 100)/the control.

Growth of bacteria in different pH of culture medium

Similar microfermentor technique was used but the pH of the cultural media was adjusted to be 7 as control, 6.2; 5.2 and 4.5. All cultural media were adjusted with diluted HCl (0.1 N) or diluted NaCl (0.1 N). Relative growth rate (%) was calculated as before.

STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

The experimental work was used completely randomized design with three replications. Measurements presented throughout this study represent average values from triplicate analyses. In Figures, error bars represent the standard deviation of these measurements. The averages and standard deviation calculated and the significant differences were calculated at the 5% of probability level. The results are represented by the values of the mean of three replicates. All data were subjected to one-way analysis of variance. Data were processed by analysis of variance (ANOVA) and Fisher's protected least significant differences (LSD at 5%)

RESULTS AND DISCUSSION

The effects of aluminium and heavy metals on some useful soil microorganisms are shown in the Figures from 1 to 6 as well as useful some statistical analysis (Tables from 1 to 12). The results of microfermenting technique showed that zinc chloride at various concentrations (20, 40, 80, and 160 µM) had influencing effects on the relative growth rates of various plant growth-promoting rhizobacteria as shown in Figure (1). It was found that low concentrations had stimulated effects on some rhizobacteria such as GH-130, GH-97 (*Sinorhizobium*), Azot-99, Azotobacter sp. and *Pseudomonas putida*. While the concentractions 80 and 160 µM reduced the relative growth rate in all strains except *Pseudomonas putida*.

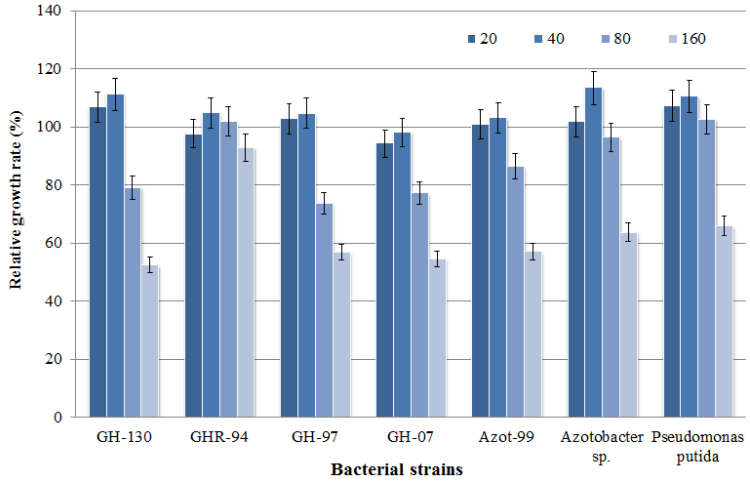


Figure (1): Effect of zinc chloride on the relative growth rates of various plant growths promoting rhizobacteria

Data in Table (1) shows the correlation matrix between the concentrations of zinic chloride in regards to the relative growth rates of the investigated strains. The interaction was significant between the 20 and 40 µM $ZnCl_2$.

Table (1): Correlation matrix between the concentrations of zinc chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.7730*	1		
80	0.1359	0.4464	1	
160	-0.2516	0.0492	0.737	1

Table (2) shows the correlations between the relative growth rates of the investigated strains in regards to the applied of different concentrations on the relative growth rates of used rhizobacteria. High

correlations were found between the strains GH-130 and GH97, GH07 (*Sinorhizobium*), Azot-99, *Azotobacter* sp. and *Pseudomonas putida*. Also, these correlations can be found between GH-97 and GH-07 (*Sinorhizobium*), Azot-99 and *Azotobacter* sp. Similarlly, it was found between GH-07 and Azot-99, *Azotobacter* sp. and *Pseudomonas putida*. Similar correlation was found between Azot-99 and *Azotobacter* sp. as well as *Pseudomonas putida*. All correlations there were ovwer 0.9000.

Table (2): Correlation matrix between the concentrations of zinc chloride and the relative growth rates of various plant growths promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.7097	1					
GH-97	0.9930*	0.6347	1				
GH-07	0.9971*	0.7501	0.9812*	1			
Azot-99	0.9814*	0.7739	0.9537*	0.9925*	1		
Azotobacter sp	0.9480*	0.8858	0.9051*	0.9681*	0.9792*	1	
Pseudomonas putida	0.9209*	0.8378	0.8703	0.9475*	0.9781*	0.9808*	1

Figure (2) shows that copper chloride was more inhibiting the relative growth rates of the tested rhizobacterial strains. The highest concentration of copper chloride had toxic effect on GHR-94 GH-97 GH-07 and Pseudomonas putida than other strains.

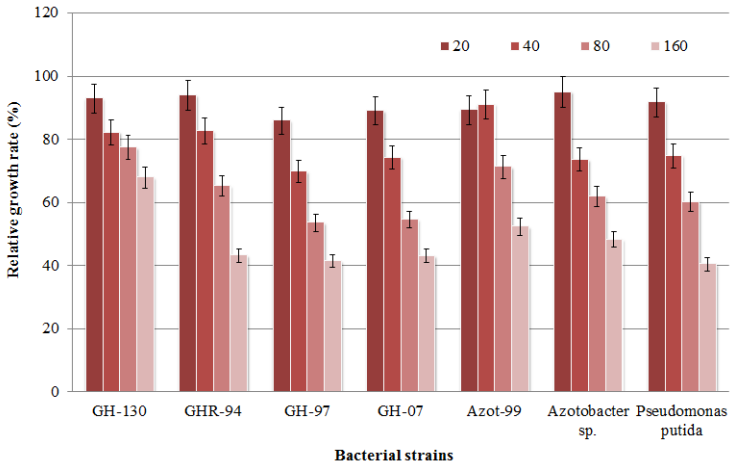


Figure (2): Effect of copper chloride on the relative growth rates of various plant growth-promoting rhizobacteria

Table (3) shows the correlation matrix between the concentrations of copper chloride applied to the broth media and their effects on them. The best correlation was foud between 160 and 80 µM $CuCl_2$.

Table (3): Correlation matrix between the concentrations of copper chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.1990	1		
80	0.4671	0.7917	1	
160	0.2941	0.5012	0.8683*	1

Table (4) illustres the correlation matrix between the plant growth-promoting rhizobacteria in regads to $CuCl_2$ concentrations. The best corrlations were found between GH-130 and other strains except with the strain Azot-99. Similar seults were found between GHR-94, Gh-97 and GH-07 and other

strains. Good correlations were found berween Azot-99 and *Pseudomonas putida* as well as between *Azotobacter* sp. and *Pseudomonas putida*.

Table (4): Correlation matrix between the concentrations of copper chloride and the relative growth rates of various plant growths promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9752*	1					
GH-97	0.9868*	0.9801*	1				
GH-07	0.9766*	0.9802*	0.9983*	1			
Azot-99	0.8892	0.9684*	0.9127*	0.9241*	1		
Azotobacter sp	0.9952*	0.9644*	0.9931*	0.9848*	0.8713	1	
Pseudomonas putida	0.9936*	0.9935*	0.9926*	0.9883*	0.9340*	0.9879*	1

Figure (3) domentstrates that 20 µM of lead chloride can increase the relative growth rates of tested microorganisms except the *Pseudomonas putida* strain. Also, it was found that by increasing the concentration the relative growth rates were decreased.

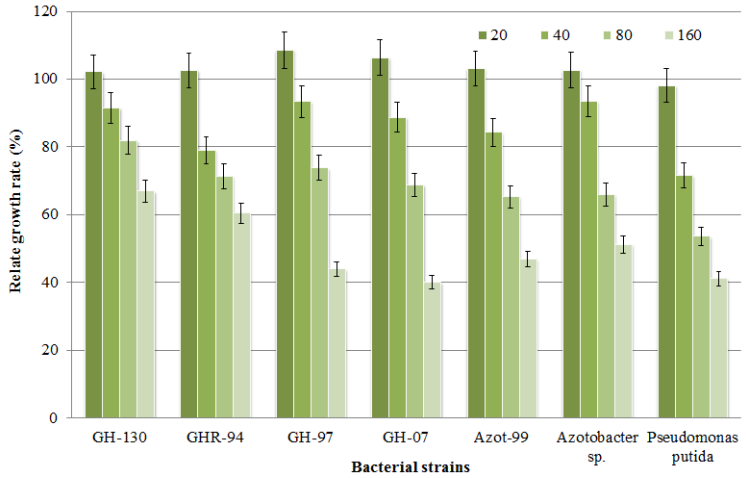


Figure (3): Effect of lead chloride on the relative growth rates of various plant growth-promoting rhizobacteria

Table (5) shows that the correlation between the concentrations of $PbCl_2$ was low. The best correlation was found between 20 and 40 µM.

Table (5): Correlation matrix between the concentrations of lead chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.7045*	1		
80	0.5216	0.6864	1	
160	-0.2141	0.1832	0.6779	1

Table (6) shows the correlation matrix between the plant growth-promoting rhizobacteria in regads to $PbCl_2$ concentrations. The best corrlations were found between all tested plant growth promoting rhizobacteria GH-130, GHR-94, GH-97, Azot-99, *Azotobacter* sp. and *Pseudomonas putida* under the application of lead chloride concentrations.

Table (6): Correlation matrix between the concentrations of lead chloride and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9545*	1					
GH-97	0.9973*	0.9303*	1				
GH-07	0.9993*	0.9436*	0.9993*	1			
Azot-99	0.9945*	0.9697*	0.9874*	0.9926*	1		
Azotobacter sp	0.9749*	0.9165*	0.9773*	0.9790*	0.9836*	1	
Pseudomonas putida	0.9687*	0.9917*	0.9505*	0.9617*	0.9871*	0.9566*	1

Figure (4) shows the effect of cadmium chloride on the investigated rhizobacterial strains under microfermenting technique. Regarding to the relative growth rates of tested bacteria, it was found the effect of cadmium chloride more toxic than copper chlorids.

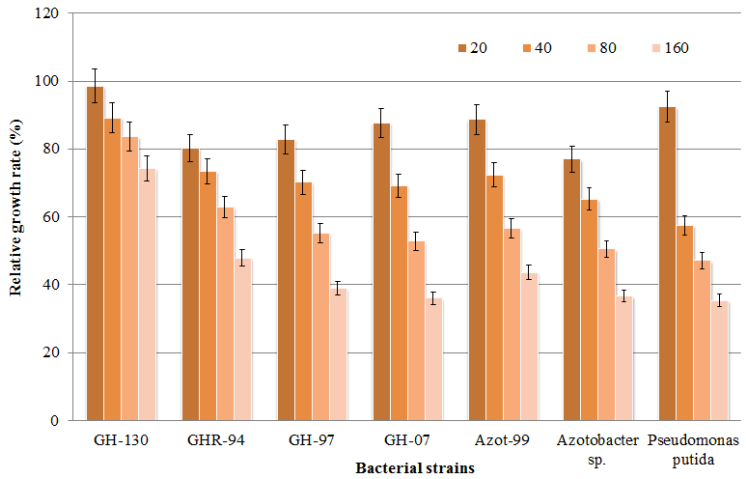


Figure (4): Effect of cadmium chloride on the relative growth rates of various plant growths promoting rhizobacteria

Table (7) shows the correlation matrix between the applied concentrations of cadmium chloride and their effects on the growth of the tested rhizobacteria. The best correlation was found between 160 and 40 and 80 µM.

Table (7): Correlation matrix between the concentrations of cadmium chloride on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.4274	1		
80	0.5260	0.9616*	1	
160	0.5968	0.9215*	0.9874*	1

Table (8) shows the correlation matrix between the plant growth-promoting rhizobacteria in regads to $CdCl_2$ concentrations. The best corrlations were found between all tested plant growth-promoting rhizobacteria GH-130, GHR-94 (except with *Pseudomonas putida*), GH-97, Azot-99, *Azotobacter* sp. and *Pseudomonas putida* under the application of lead chloride concentrations. Most of correlation matrix was laid over 0.9900.

Table (8): Correlation matrix between the concentrations of cadmium chloride and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9819*	1					
GH-97	0.9933*	0.9933*	1				
GH-07	0.9965*	0.9817*	0.9968*	1			
Azot-99	0.9928*	0.9758*	0.9945*	0.9992*	1		
Azotobacter sp	0.9917*	0.9902*	0.9996*	0.9975*	0.9964*	1	
Pseudomonas putida	0.9613*	0.8941	0.9352*	0.9602*	0.9629*	0.9383*	1

Figure (5) demonstrates the effect of aluminium sulphate on the tested plant growth-promoting rhizobacterial strains under microfermenting technique. Regarding to the relative growth rates of tested rhizobacteria, it was found the effect of aluminium sulphate less toxic than cadmium and copper chlorids. Lower concentration (20 µM) can be act as lettel stimulationg doses to increase the relative growth rates of the investigated strain except the strain of *Pseudomonas putida*, while 40 µM increases the relative growth rates of GH-130 and *Azotobacter* sp. The highest concentration (160 µM) acts as inhibitor for all strains except GH-130.

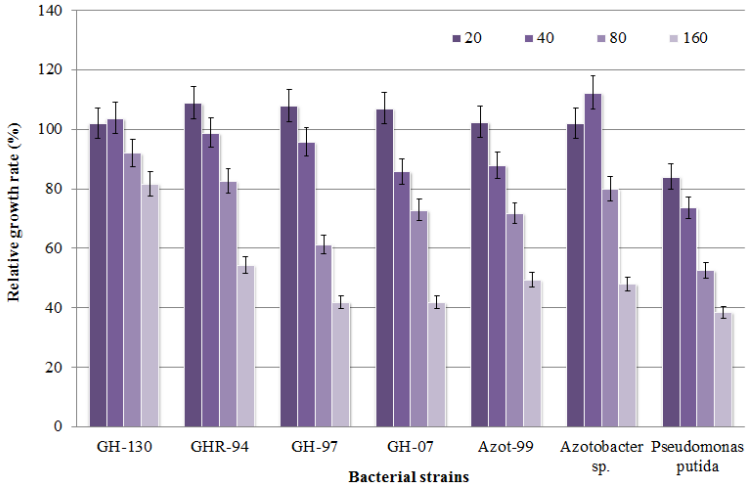


Figure (5): Effect of aluminium sulphate on the relative growth rates of various plant growth-promoting rhizobacteria

Table (9) illustrates the correlation matrix between the applied concentrations of aluminium sulphate and their effects on the growth of the investigated soil hizobacteria. The best correlation was found between 40 and 80 µM. This correlation was not completely significant.

Table (9): Correlation matrix between the concentrations of aluminium sulphate on the relative growth rates of various plant growth-promoting rhizobacteria

	20	40	80	160
20	1			
40	0.5651	1		
80	0.5359	0.7520	1	
160	0.1980	0.5213	0.8232*	1

Table (10) demonstrates the correlation matrix between the soil useful microbiomes as plant growths promoting rhizobacteria in regads to aluminium sulphate concentrations. The best corrlations were

found between all tested plant growth-promoting rhizobacteria GH-130, GHR-94, GH-97, Azot-99, *Azotobacter* sp (with an exception with GH-07), and *Pseudomonas putida* under the application of lead chloride concentrations. Most of correlation matrix was laid between 0.9200 and 0.9900.

Table (10): Correlation matrix between the concentrations of aluminium sulphate and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9677*	1					
GH-97	0.9610*	0.9704*	1				
GH-07	0.9218*	0.9888*	0.9597*	1			
Azot-99	0.9463*	0.9926*	0.9826*	0.9952*	1		
Azotobacter sp	0.9951*	0.9486*	0.9289*	0.8911	0.9156*	1	
Pseudomonas putida	0.9537*	0.9754*	0.9988*	0.9709*	0.9894*	0.9196*	1

Figure (6) shows the relative growth rates of the investigated soil microbiomes as plant growth promoting rhizobacter at different pH values. It was foud that all the investigated soil microbiomes grow well at pH 7, while at pH 6.2 reduced the relative growth rates of GH-94, GH-07 Azot-99 and *Pseudomonas putida*. At pH 5.2 the relative growth rates of the rhizobacteria *Azotobacter* sp. was stimulated. *Pseudomonas putida* strain at pH 4.5 was reduced significantly in comparison with other strains.

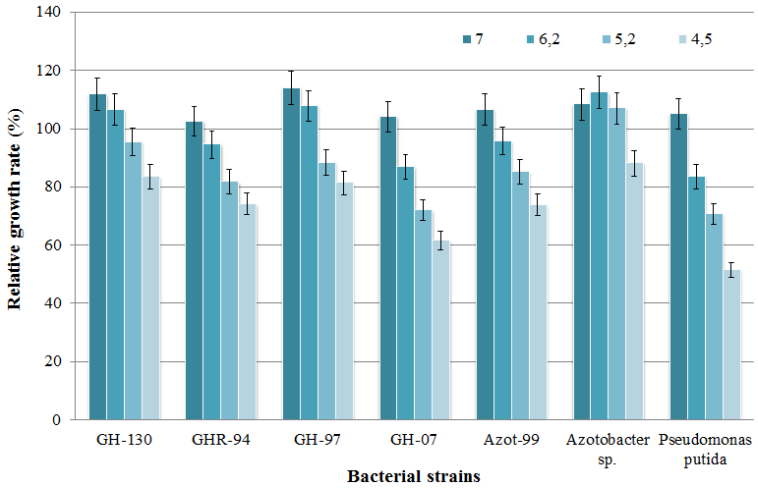


Figure (6): Effect of hydrogen ion concentrations on the relative growth rates of various plant growth-promoting rhizobacteria

Table (11) shows the correlation matrix between the pH values and their effects on the growth of the investigated soil hizobacteria. The best correlation was found between 5.2, 4.5 and 6.2. These correlations at these pH values were completely significant.

Table (11): Correlation matrix between hydrogen ion concentrations on the relative growth rates of various plant growth-promoting rhizobacteria

	7	6.2	5.2	4.5
70	1			
6.2	0.7454	1		
5.2	0.5652	0.9466*	1	
4.5	0.6124	0.9636*	0.9281*	1

Table (12) demonstrates the correlation matrix between the soil useful microbiomes as plant growths promoting rhizobacteria in regads to their relative growth rates at different pH values. The best corrlations were found between all tested plant growth promoting rhizobacteria with GH-130 (with the exception with *Azotobacter* sp.), GHR-94 (except with the strain *Azotobacter* sp.), GH-97 (except with the strain *Azotobacter* sp.), GH-07 (except with the strain *Azotobacter* sp.), and Azot-99 (with the exception with *Azotobacter* sp).

Table (12): Correlation matrix between hydrogen ion concentrations and the relative growth rates of various plant growth-promoting rhizobacteria

	GH-130	GHR-94	GH-97	GH-07	Azot-99	Azotobacter sp	Pseudomonas putida
GH-130	1						
GHR-94	0.9859*	1					
GH-97	0.9767*	0.9930*	1				
GH-07	0.9651*	0.9913*	0.9724*	1			
Azot-99	0.9879*	0.9937*	0.9742*	0.9925*	1		
Azotobacter sp	0.8720	0.7784	0.7706	0.7199	0.7987	1	
Pseudomonas putida	0.9734*	0.9826*	0.9536*	0.9921*	0.9963*	0.7725	1

CONCLUSIONS AND RECOMMENDATIONS

Consequently, the strains selected in the present study can be used as a biomonitor of these heavy metal polluted soils. Moreover, it could be used as a bioremediator, taken into consideration their use in all soil as biofertilizer purposes. None of the strains can tolerate 160 µm of Cd^{2+}, $Pb^{2+}$and Cu^{2+}. The most toxic metal was Cd^{2+}, followed by $Pb^{2+}$and Cu^{2+}. The ecophysiological tolerant *Sinorhizobium* and *Azotobacter* as well as *Pseudomonas putida* strains are suggested to be the ideal solution for the improvement of soil fertility and the rehabilitation of reclaimed soils and are an important direction for future soil protection and quality research.

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