

Article

PGPR-Mediated Plant Growth Attributes and Metal Extraction Ability of *Sesbania sesban* L. in Industrially Contaminated Soils

Nida Zainab ¹, Amna ¹, Amir Abdullah Khan ², Muhammad Atif Azeem ¹, Baber Ali ¹, Tongtong Wang ², Fuchen Shi ^{2,*}, Suliman Mohammed Alghanem ³, Muhammad Farooq Hussain Munis ¹, Mohamed Hashem ^{4,5}, Saad Alamri ⁴, Arafat Abdel Hamed Abdel Latef ⁶, Omar M. Ali ⁷, Mona H. Soliman ^{8,9} and Hassan Javed Chaudhary ^{1,*}

- ¹ Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan; nidazainab103@gmail.com (N.Z.); amna_qau@yahoo.com (A.); atifazeem321@gmail.com (M.A.A.); botanist53@gmail.com (B.A.); uafshahbaz@gmail.com (M.F.H.M.)
- ² Department of Plant Biology and Ecology, Nankai University, Tianjin 300071, China; amir_nku@hotmail.com (A.A.K.); 1120180385@mail.nankai.edu.cn (T.W.)
- ³ Biology Department, Faculty of Science, Tabuk University, Tabuk 71491, Saudi Arabia; s-alghanem@ut.edu.sa
- ⁴ Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia; drmhashem69@gmail.com (M.H.); amri555@yahoo.com (S.A.)
- ⁵ Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
- ⁶ Botany and Microbiology Department, Faculty of Science, South Valley University, Qena 83523, Egypt; moawad76@gmail.com
- ⁷ Department of Chemistry, Turabah University College, Turabah Branch, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; om.ali@tu.edu.sa
- ⁸ Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12613, Egypt; monahsh1@gmail.com
- ⁹ Biology Department, Faculty of Science, Taibah University, Al-Sharm, Yanbu El-Bahr, Yanbu 46429, Saudi Arabia
- * Correspondence: fcshi@nankai.edu.cn (F.S.); hassaan@qau.edu.pk (H.J.C.)



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Abstract: The release of harmful wastes via different industrial activities is the main cause of heavy metal toxicity. The present study was conducted to assess the effects of heavy metal stress on the plant growth traits, antioxidant enzyme activities, chlorophyll content and proline content of *Sesbania sesban* with/without the inoculation of heavy-metal-tolerant *Bacillus gibsonii* and *B. xiamenensis*. Both PGP strains showed prominent ACC-deaminase, indole acetic acid, exopolysaccharides production and tolerance at different heavy metal concentrations (50–1000 mg/L). Further, in a pot experiment, *S. sesban* seeds were grown in contaminated and noncontaminated soils. After harvesting, plants were used for the further analysis of growth parameters. The experiment comprised of six different treatments. The effects of heavy metal stress and bacterial inoculation on the plant root length; shoot length; fresh and dry weight; photosynthetic pigments; proline content; antioxidant activity; and absorption of metals were observed at the end of the experiment. The results revealed that industrially contaminated soils distinctly reduced the growth of plants. However, both PGPR strains enhanced the root length up to 105% and 80%. The shoot length was increased by 133% and 75%, and the fresh weight was increased by 121% and 129%. The proline content and antioxidant enzymes posed dual effects on the plants growing in industrially contaminated soil, allowing them to cope with the metal stress, which enhanced the plant growth. The proline content was increased up to 190% and 179% by the inoculation of bacterial strains. Antioxidant enzymes, such as SOD, increased to about 216% and 245%, while POD increased up to 48% and 49%, respectively. The results clearly show that the utilized PGPR strains might be strong candidates to assist *S. sesban* growth under heavy metal stress conditions. We highly suggest these PGPR strains for further implementation in field experiments.

Keywords: phytoremediation; *Sesbania sesban*; heavy metal tolerance; *B. xiamenensis*; *B. gibsonii*; PGPR

1. Introduction

Heavy metal contamination is an environmental problem of great concern that negatively affects human health. Various industrial and anthropogenic activities result in heavy metal soil contamination [1]. Because of the greater content of heavy metals, higher pH level, lower availability of essential nutrients, low organic matter and poor soil structure, metals are difficult to extract from contaminated soils. However, phytoremediation is a useful technique to control these kinds of pollutants [2]. The removal of heavy metals and alleviation of their negative effects using green plants and their associated microbes is called phytoremediation/assisted phytoremediation [3]. Besides the removal of heavy metals, the phytoremediation process can also be used to remediate hydrocarbons, pesticides and polychlorinated biphenyl. Plants usually handle the contaminants via various mechanisms and maintain the fertility of the soil without affecting the topsoil [4]. The word 'phytoremediation' is a combination of two words: phyto (Latin) and medium (Greek), which means to remove evil. In 1983, Chaney proposed the concept of phytoremediation. Phytoremediation is an eco-friendly and low-cost technology used for the remediation of contaminated soils. The main purpose of phytoremediation is to reclaim lands (1) for agricultural purposes, and (2) for the phytoextraction of toxic heavy metals with economic value, i.e., Au, Ni, and Tl [5]. Thus, phytoremediation is the "green clean" of toxic lands [6].

Plants used for the phytoremediation of heavy-metal contamination face extensive stress [7] that reduces the proficiency for phytoremediation [8]. In this context, suitable methods have been used that pose the dual effects of improved phytoextraction and enhanced plant growth under heavy-metal stress. The process of phytoremediation includes various methods, such as phytostabilization, phytoextraction, phyto-volatilization and rhizofiltration. These methods are inexpensive, increase biodiversity, and decrease erosion and energy depletion, which, in turn, reduces the contaminants. Thus, these methods offer an ecofriendly approach [9,10]. In the process of phytoextraction, hyperaccumulating plants are used which tend to tolerate and store larger concentrations of toxic metals. Hyperaccumulators are deep-rooted, fast-growing plants with a comparatively higher biomass [7]. Moreover, extensive root growth is required for the effectiveness of phytoremediation [8]. The efficiency of phytoextraction can be enhanced via association with heavy metal-tolerant plant growth-promoting bacteria. Rhizosphere is an important platform for beneficial bacteria. Rhizospheric bacteria are normally known as plant growth-promoting rhizobacteria (PGPR). This PGPR directly or indirectly affects plants growth and biomass [9]. PGPR results in the production of compounds that increase the biomass of plants and help in the uptake of nutrients from the soil. Bacteria can produce siderophores and phytohormones (IAA); the solubilization of minerals, such as potassium and phosphorus; and a substantial decrease in the excessive ethylene produced during stress conditions [10], while the production of antibiotics and fungicidal compounds assist plants in combatting in host resistance pathogens [11]. PGPR-producing ACC deaminase enzymes are of vital importance because they cleave the precursor of ethylene produced under stress conditions, which directly inhibit plant growth [12]. The PGPR-producing ACC enzyme decreases ethylene production in the plants by splitting 1-aminocyclopropane-1-carboxylate deaminase into α -ketobutyrate and ammonia [13].

PGPR strains endure the harmful effects of toxic heavy metals in plants growing in contaminated soils [14], with a positive influence on plant growth. PGPR strains enhance plant growth by providing a suitable nutrient to their host plants (P, Fe, N and K) through ACC deaminase production and the modification of hormones levels. In polluted soil regimes, PGPR protects their host plants by acting as metal bio-sorption surface or by changing toxic chemical ions into less toxic forms [15]. Moreover, they regulate plant growth under heavy metal stress directly or indirectly by producing chelating agents, an elongated root system, stress-induced resistance against abiotic stress and an increased availability of essential nutrients. In addition, PGPR strains modulate the development of associated microbes in soils via biofilm formation [16,17]. This mechanistic approach makes these stress-tolerant PGPR strains very useful in the reclamation of metal contaminated

soils. The use of such bacteria to remove contaminants from soils is known as bacterial-assisted phytoremediation [18].

Enhanced root density was observed in plants immunized with ACC deaminase-producing rhizobacteria that directly mediate the phytoremediation potential of associated plants [19]. The bioaugmentation of exopolysaccharides (EPS)-producing bacteria [20] in plants under heavy metal stress is also a favorable condition for elevated remediation. In metal-contaminated environments, the production of EPS has been proven to be another defensive approach for the survival of bacteria [21,22]. EPS mainly consists of polypeptides, nucleic acids and polysaccharides secreted by bacteria. In the EPS matrix, carboxyl and hydroxyl groups are negatively charged. This negatively charged bacterial EPS plays a vital role in inhibiting the direct content of toxic metals with cells and chelating metal cations [23,24]. Bacterial secreted EPS assist as the first barrier between the environment and microbial cells. It has been observed that under metal stress, the production of bacterial EPSs prevents metals from direct intercalation with cellular components of bacteria [25]. Metal can be categorized into two main groups from a physiological point of view: (1) nonessential metals to cell life, such as Cd, Pb, Cr, and Sb; and (2) essential metals to cell life, such as Ca, Mg, Fe, Cu and Zn. EPS prevents the entry of metal ions into the cell, and metal homeostasis is also affected by the bacterial secreted EPS [26]. EPS are large particles with mass polymers. These polymers play an important role in the interaction or communication between cells, such as the aggregation of bacterial cells, formation of biofilms around their colonies, organic compound sorption ability and production of inorganic molecules by microbes in response to different environmental conditions in plants [27,28].

Sesbania sesban, is commonly known as *Sesban* L. Merrill, was established as popular leguminous species. *Sesbania sesban* is widely used as fodder and fuel, and the *S. sesban* plant can inhabit saline sites and waterlogged conditions. This plant is found in semi-arid to sub-humid climates with rainfalls between 500–2000 mm per year. *S. sesban* can maintain and restore soil fertility and stop soil erosion. These plants can be effectively used for the reclamation of heavy metal-contaminated soils because of their ability to fix nitrogen via root nodules. The insufficiency of important nutrients and toxicity of heavy metals could be overcome using *S. sesban* plants. The current study focused on the ability of PGPR, i.e., *Bacillus xiamenensis* and *Bacillus gibsonii* to produce ACC-deaminase, EPS- and IAA. These strains play an important role in enhancing plant growth and phytoextraction potential in associated *Sesbania* plants. The present research also focused on the effects of metals on the physiology and growth of the plant. In addition, we assessed *S. sesban* as the best tool for phytoremediation.

2. Materials and Methods

2.1. Isolation Source of Bacterial Strains

Two bacterial strains i.e., *B. xiamenensis* and *B. gibsonii*, formerly isolated from rhizosphere of sugarcane, were taken from the Plant-Microbe Interaction Lab, Department of Plant Sciences, Quaid-i-Azam University, Islamabad [29].

2.1.1. Screening of Plant Growth-Promoting Parameters and Biochemical Characterization

Characterization of isolated bacterial strains was performed both morphologically and biochemically by following the standard protocols. Catalase activity was determined by taking a freshly cultured bacterial colony on the slide. The production of catalase enzyme was confirmed with the formation of oxygen bubbles by adding 2–4 drops of hydrogen peroxide on bacterial colony [30]. To determine the solubilization of phosphate, Pikovskaya's medium [31] was used. The bacterial colony was spot-inoculated in the middle of plates containing tricalcium phosphate as an unsolvable source of phosphate. After 7 days of incubation, the development of halo zones around the bacterial colony confirmed P-solubilization activity by bacteria. The ability of the bacterial strains to produce IAA was estimated by the method described by the authors of [32]. The chrome azurol S plate method was used for the siderophores production assay [33]. The zinc solubilization, am-

monia production and cellulose activity abilities of bacteria were determined by methods described by the authors of [34–36]. For ammonia production, bacterial strains were incubated for 24 h at 32 °C and inoculated in peptone broth. Then, 0.5 mL of Nessler's reagent was added to the reaction mixture. Next, the method described by the authors of [37,38] was used to estimate the production of protease, pectinase and amylase production. The ACC deaminase ability of bacterial isolates were determined using the method described by the authors of [39]. Bacterial culture was grown in 5 mL of TSB medium at 28 °C for 24 h in a shaking incubator at 120 rpm. Bacterial cells were collected by centrifugation at 3000 rpm for 5 min, and the pellet was washed twice and resuspended in 0.1 M Tris-HCl (pH 7.5). Then, these cultures were spot-inoculated on Petri plates, with DF media [40] augmented with and without ACC. Plates with ammonium sulfate were used as positive controls. After 3 days, growth on the ACC-augmented plates was compared to the positive and negative controls. The ATCC medium No. 14 was used to determine the EPS production of the bacterial cultures [41]. Streaking of the bacterial culture was done on ATCC medium No. 14, and cultures were incubated for 3 days at 28 °C. After 3 days of incubation, the development of the slimy layer around the bacterial colonies confirmed the production of EPS.

2.1.2. Heavy Metal Tolerance Ability of Strains

To examine the heavy metal tolerance capacity, bacterial isolates were grown on Lauria Bertani (LB) agar plates accompanied with various quantities of heavy metals, such as Copper (Cu), Zinc (Zn), Chromium (Cr) and Nickel (Ni). Quantities ranged from 50 mg/L of heavy metals to 1000 mg/L [42].

2.1.3. Bacterial Growth Curve Analysis and Quantification in Metal Stress

Bacterial cultures were initially grown in a nutrient broth medium at 28 ± 2 °C for 48 h. The respective salts, Cr and Cd, were dissolved in distilled water to prepare various concentrations of heavy metals. A culture of 2 mL growing bacteria ($OD_{600} = 1.0$) was suspended into 100 mL nutrient broth, comprising the respective metals in 250 mL conical flasks incubated for 48 h at 28 °C in a shaking incubator. Bacterial cultures were monitored at an absorbance of 600 nm by a spectrophotometer (752N UV-VIS, Pakistan) to generate the growth curve of both bacterial strains to analyze their heavy metal stress tolerance.

2.1.4. Quantitative Assays of Exopolysaccharide, IAA Acid Production and ACC-Deaminase Activity

Quantification of Exopolysaccharides

To determine the quantitative production of EPS, a 50 mL ATCC No. 14 liquid medium was used for the the growth of strains [43]. Bacterial isolates were incubated at 28 °C for 3 days at 2000 rpm. After 3 days of incubation, cells were harvested at 10,000 rpm for 20 min by centrifugation. Then, two volumes of cold isopropanol were added to the solution and kept overnight at 40 °C. Then, the solution was centrifuged at 10,000 rpm for 20 min. The supernatant was discarded, and the pellet was dried at 1000 °C. Then, the dried pellets were weighed to determine which bacterial strains showed the higher production of exopolysaccharide.

Quantification of IAA

The production of IAA was determined using the method described by the authors of [44]. This method of IAA production was modified by the authors of [45]. In this method, bacterial isolates were grown in 1000 mL L-tryptophan broth, 5 g of NaCl, 10 g of tryptone, 5 g of yeast extract and 1 g of l-tryptophan. The broth was augmented with 50 mg L⁻¹ of heavy metal (either Cu, Pb, As, Ni, Cr, Cd or Mn) stress and without heavy metal stress. The mixture was incubated at 36–38 °C for 72 h. After that, solution was centrifuged for 30 min at 3000 rpm. Then, 2 mL of the supernatant was taken, and two drops of orthophosphoric acid and 4 mL of Salkowski reagent were added to the mixture.

IAA production was confirmed by the production of a pink color. The density of IAA was determined at 530 nm. To estimate the concentration of IAA, a standard curve was prepared in the range of 10–100 µg/mL of IAA.

Quantification of ACC

The determination of ACC deaminase activity was performed by growing bacterial isolates in a 50 mL TSB medium at 28 °C. The late lag phase culture was used to induce the ACC deaminase activity. After that, the mixture was centrifuged. The pellets were washed twice with 0.1 M Tris-HCl (pH 7.5), then dissolved in 2 mL of modified DF (Dworkin and foster salt medium) minimal medium added with either 3 mM final concentration of ACC without stress or supplemented with heavy metal (either Cu, Pb, As, Ni, Cr, Cd or Mn) stress [46] and incubated at 28 °C for 36–72 h. During the breakdown of ACC by ACC deaminase, α -ketobutyrate and ammonia were produced, by which the ACC deaminase activity was determined [47]. After incubation, the mixture was centrifuged for 5 min at 3000 rpm, and the harvested cells were washed two times with 0.1 M Tris-HCl (pH 7.5). The mixture was resuspended in 200 µL of 0.1 M Tris-HCl with (pH 8.5) and 5% toluene. Then, the mixture was vortexed for 30 s. Then, 50 µL of the cell suspension was taken and mixed with 5 µL of 0.3 M ACC in an Eppendorf tube. The tubes were incubated for 30 min at 28 °C. Next, 50 µL of cell suspension without ACC was used as a negative control. The blank included 50 µL of cell suspension mixed with 5 µL of 0.3 M ACC and 50 µL of 0.1 M Tris HCl (pH 8.5).

The samples were mixed with 500 µL of 0.56 N HCl and vortexed at high speed. Then, the cells were harvested by centrifugation at 12,000 rpm for 5 min. Next, 500 µL of the supernatant was taken and transferred to a glass test tube. In total, 0.56 N HCl 400 µL and 150 µL of DNF solution (0.1 g 2,4-dinitrophenylhydrazine in 100 mL of 2 N HCl) were added to the reaction mixture. Before measuring its optic density at 540 nm, 1 mL of 2 N NaOH was added to the samples. The quantification of ACC was conducted by plotting the standard curve of α -ketobutyrate against the different absorbance values of the samples.

2.2. Contaminated Soil

Two soil samples were used in this experiment. The first soil sample was collected from Ittihad Steel Re-Rolling Mills, Islamabad Pakistan, and was characterized as contaminated soil (Zn 9.94 ppm, Mn 8.9 ppm, Cd 5.1 ppm, Cr 26.4 ppm, Cu 13.2 ppm, Ni 18.3 ppm and Pb 42 ppm). A noncontaminated soil sample was obtained from QAU, Islamabad. Soil samples were processed and autoclaved to purify them. Soil analyses, including pH, texture [48] and EC [49] were performed. Soil organic matter was determined using the method described by the authors of [50]. Following this method, 2 g of soil was taken in a 500 mL conical flask. Then, 200 mL of distilled water, 10 mL of 1 N $K_2Cr_2O_7$ and 10 mL of orthophosphoric acid were added to the flask.

After half an hour, 30 drops of Diphenylamine (used as an indicator) were added to the mixture. This solution was titrated against the 0.5 N Mohr's control. The appearance of a green color was the endpoint of the reaction. The metal analysis of soil was conducted following the method described by the authors of [51]. The atomic absorption spectrophotometer was used to analyze the concentrations of heavy metals (Zn, Mn, Cr, Cd, Ni and Pb).

2.3. Greenhouse Experiment

S. sesban seeds were taken from the National Agricultural Research Center (NARC), Islamabad, Pakistan. Seed sterilization was performed by following the protocol described by the authors of [52]. Pots were filled with the respective soils. After the successful germination of seeds, three plants were maintained per pot. Six different treatments were included in three sets of replications, namely C (noncontaminated soil and industrially contaminated soil), T1 (*B. xiamenensis* PM14 in noncontaminated soil and industrially contaminated soil) and T2 (*B. gibsonii* PM11 in noncontaminated soil and industrially contaminated soil).

Then, 20 mL of each bacterial suspension (*B. xiamenesis* and *B. gibsonii*) was mixed in both the noncontaminated and industrial contaminated soils. Next, 20 mL of autoclaved distilled water was added to the control. After 60 days of the experiment, *Sesbania* plants were carefully harvested from the soil. We followed the protocol described below to the determined root length, shoot length, dry weight, fresh weight, chlorophyll content, plant proline content and SOD and POD activity.

2.4. Plant Growth Parameters

S. sesban plants were carefully harvested from the soil at the end of the experiment. To remove the debris from the root surface, plant roots were cleaned with deionized water. Plant shoot length, root length and fresh were observed using a digital balance. All plant parts were dried at 70 °C for the determination of the dry biomass until a constant weight was achieved.

2.5. Determination of Photosynthetic Pigments in Plants

The chlorophyll content and carotenoid of the *S. sesban* plant were examined following the method described by the authors of [53]. Following this method, 0.3 g of plant sample was grinded in 5 mL of acetone (80%). The reaction mixture was centrifuged at 3000 rpm for 10 min. Then, chlorophyll-a, chlorophyll-b and carotenoid content were observed at 645 nm, 663 nm and 480 nm wavelengths, respectively. The absorbance was observed using a spectrophotometer (752N UV-VIS, Beijing, China). The following equations were used to determine the chlorophyll and carotenoid content of plants.

$$\text{Chl (a) (mg/g)} = [12.7 (\text{OD}_{663}) - 2.69(\text{OD}_{645})] \times V/1000 \times W$$

$$\text{Chl (b) (mg/g)} = [22.9 (\text{OD}_{645}) - 4.68(\text{OD}_{663})] \times V/1000 \times W$$

$$V = \text{Volume of the extract (mL)}, W = \text{Weight of fresh leaf tissue (g)}$$

$$\text{Carotenoids (mg/g): (OD at 480)} \times 4$$

2.6. SOD and POD Activity of Enzymes

Following the method described by the authors of [53], the SOD and POD activity of the plants were determined. First, 0.1 g of fresh leaves were grinded in 10 mL of 0.1 M phosphate buffer (pH 6.5). Then, the solution was centrifuged for 10 min at 430 nm. The supernatant was taken after centrifugation and 500 µL H₂O₂ solution was added. After that, absorbance was taken at 430 nm for 3 min on the spectrophotometer (752N UV-VIS, Beijing, China). For blank 1%, the H₂O₂ solution was used. At 430 nm, a change in optical density was considered as one unit of POD:

$$\text{POD} = \text{Final reading} - \text{initial reading}$$

Whereas, in SOD, the determination two buffer solutions were prepared. The following buffers were prepared for the SOD analysis: (a) Monosodium Dihydrogen Phosphate was prepared by dissolving 15.6 g of Monosodium dihydrogen phosphate into 600 mL of distilled water, and (b) Disodium Hydrogen Phosphate was prepared in 600 mL of distilled water by dissolving 53.65 g of disodium hydrogen phosphate. The SOD activity of the plant was estimated using the following formula.

$$R4 = R3 - R2$$

$$A = R1 (50/100)$$

$$\text{Final} = R4/A$$

$$R1—\text{OD of Reference}, R2—\text{OD of Blank}, R3—\text{OD of Sample}$$

2.7. Proline Content of *SESBANIA* Plants

The proline content of the leaves was examined using the method described [54]. Following this method, 0.1 g of fresh plants was taken. Then, these leaves were grinded in a 4 mL solution of sulfosalicylic acid. After that, the solution was centrifuged for 5 min at 3000 rpm. Then, 2 mL of supernatant was taken from the reaction mixture in test tubes, and 2 mL of acid ninhydrin solution was added to the test tubes. The test tubes were placed in a water bath for 1 h at 100 °C. After that, the mixture was cooled, and the reaction mixture was extracted by adding 4 mL of toluene. Using toluene as blank, the OD was observed at 520 nm via the spectrophotometer (752N UV-VIS, Beijing, China).

2.8. Metal Analysis of Soil and Plants by Wet Acid Digestion Method

For the metal analysis, 1 g of the sample was taken and mixed with the 12 mL (9 mL HCl + 3 mL HNO₃) of aqua regia. The solution was placed overnight, and the flasks were covered with a watch glass. Afterward, 24-hour-old solution was heated on hot plates. The completion of digestion was confirmed by the detection of brown fumes. Then, the solution was cooled down and the reaction mixture was filtered through No. 42 filter paper. By adding the distilled water, the volume of the reaction mixture was raised to 50 mL. The prepared samples were used to analyze the quantity of heavy metals (Zn, Mn, Cd, Cr, Ni and Pb). Standard SRM 3136-Nickel, SRM 132,239 Manganese, SRM 3128 Pb, SRM 3168 Zinc, SRM 3108 Cadmium, SRM 2701 Chromium were used by the atomic absorption spectrophotometer AAS model spectra AA Varian [51].

2.9. Statistical Analysis

The experiments were designed in replicate (3) and arranged in a completely randomized design (CRD) factorial. Excel sheets were used to build a database for the observed parameters and results. The analysis of variance was performed using Statistics 8.1 to analyze the obtained data [55]. Principal component analysis correlation (PCA) was analyzed using XL-STAT 2010. The LSD value of $p \leq 0.05$ was used to determine the statistical significance among the mean values of treatments.

3. Results

3.1. Plant Growth-Promoting Traits of Bacterial Strains

Both strains were positive for various PGP traits, such as phosphate and zinc solubilization and IAA production, siderophores production, and the ammonia test (Table 1). Moreover, both strains showed the potential to produce EPS, ACC deaminase and extracellular enzymes, such as catalase, protease, amylase, pectinase, chitinase and cellulase. The plant growth-promoting traits of *B. xiamenensis* were also previously reported by [29].

Table 1. Plant growth-promoting traits of *Bacillus* strains.

Strain	Biochemical Analysis													
	Phosphate Solubilization	IAA Production	Siderophore	Exopolysaccharide	Zinc Solubilization	HCN	Ammonia Test	Pectinase	Chitinase	Cellulase	Amylase	ACC Deaminase	Catalase	Protease
<i>B. gibsonii</i>	+	+	+	+	+	–	+	+	+	+	+	+	+	+
<i>B. xiamenensis</i>	+	+	+	+	+	–	+	+	+	+	+	+	+	+

3.1.1. Response of Bacterial Strains to Heavy Metals

Both rhizobacterial strains showed growth in the LB plates amended with various concentrations of heavy metals. Bacterial strain *B. xiamenensis* tolerated Cr, Ni, Cd and Cu up to 1000 mg/L, 150 mg/L, 1000 mg/L and 100 mg/L, respectively. However, bacterial strain *B. gibsonii* tolerated Cr, Ni, Cd and Cu up to 1000 mg/L, 150 mg/L, 700 mg/L and 50 mg/L, respectively (Figures 1 and 2).

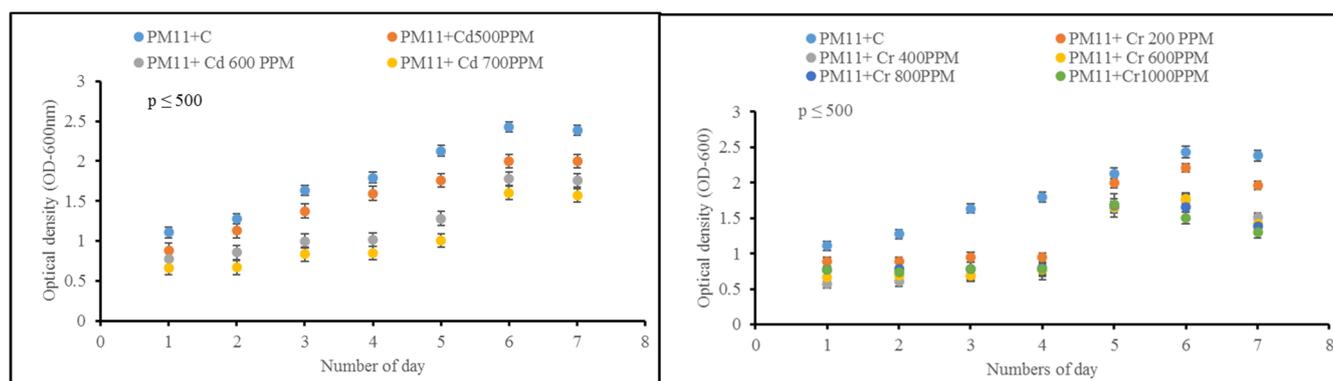


Figure 1. Growth curve of *B. gibsonii* against various concentrations of Cr and Cd.

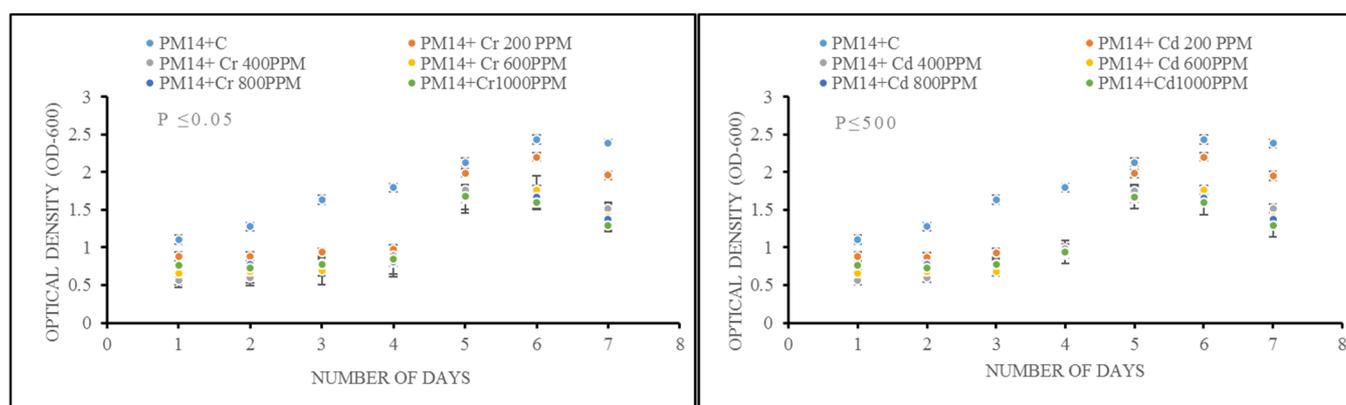


Figure 2. Growth curve of *B. xiamenensis* against various concentrations of Cr and Cd.

3.1.2. Quantitative Assessment of EPS, ACC Deaminase, and IAA Production

To determine the qualitative ability of the isolates to produce the ACC enzyme, both bacterial isolates were grown on agar plates with DF medium as a negative control, ammonium sulfate as a positive control and ACC as a nitrogen source. For both strains, there was variation in the growth patterns on the plates supplemented with ACC. Both bacterial strains produced ACC. Quantitative screening was performed to determine the ability of the bacterial strains to produce a total amount of ACC deaminase. This method was performed in (50 mg L^{-1}) of chromium stress condition and (0 mg L^{-1}) non-stress condition. Between $0.63 \text{ } \mu\text{M/mg protein/h}$ and $0.91 \text{ } \mu\text{M/mg protein/h}$ (Table 2), both bacteria produced ACC. Exopolysaccharides production is an important feature of stress-tolerant bacteria. Bacteria can tolerate stress by forming a biofilm around their colonies. To determine the ability of bacterial isolates to qualitatively produce EPS, PGPR was grown on ATCC No. 14 medium. The results revealed that both bacterial isolates produced EPS. In the quantitative screening, both strains showed a higher production of Exopolysaccharides between 0.5 g/L and 0.6 g/L (Table 2) under stressed conditions. The quantitative screening of IAA was performed in both stress and non-stress conditions. Both strains produced IAA. Statistically, between $93 \text{ } \mu\text{M/mL}$ and $117 \text{ } \mu\text{M/mL}$, both bacterial isolates showed the development of IAA (Table 2).

Table 2. Quantitative assays of ACC-deaminase, Exopolysaccharides and Indole acetic acid under the normal and stress conditions.

Bacterial Isolates	ACC-Deaminase ($\mu\text{M}/\text{mg Protein}/\text{h}$)		EPS (mg/mL)		Indole Acetic Acid ($\mu\text{M}/\text{mL}$)	
	Normal	Under Stress	Normal	Under Stress	Normal	Under Stress
<i>B. xiamenensis</i>	0.493 ^c \pm 0.07	1.829 ^a \pm 0.89	0.5933 ^b \pm 0.02	2.953 ^a \pm 0.04	107 ^a \pm 1.632	91 ^c \pm 0.816
<i>B. gibsonii</i>	0.489 ^c \pm 0.081	1.701 ^b \pm 0.008	0.58 ^b \pm 0.024	2.967 ^a \pm 0.04	96 ^b \pm 1.632	79 ^d \pm 1.632

Zainab et al. (2020). Each value is the mean of replicates ($n = 3$); the different letters with mean values in each column indicate significant differences, detected by LSD test ($p < 0.05$).

3.2. Physiochemical Properties of Soil

The industrial soil was sandy loam, while the other soil sample was loamy. The industrially contaminated soil was marginally acidic with a pH of 7.6, electric conductivity of 0.005 (dsm^{-1}) and organic matter of 0.92. The pH and EC values for the noncontaminated soil were 6.35 and 0.015 (dsm^{-1}), respectively. In total, 0.74 ppm of organic matter was present in the normal soil. In the normal soil, there was a higher amount of potassium (109 ppm), phosphorous (7 ppm) and nitrate (7.24 ppm), whereas in the industrial soil, there were fewer organic nutrients. Potassium (7 ppm) and nitrate (5.32 ppm) were observed in the industrial soil, while the amount of phosphorus was slightly high (12 ppm). Through the atomic absorption spectrometry, the heavy metal analysis showed that there was high concentration of metals in the industrial soil compared to the noncontaminated soil.

3.3. Effects of Bacterial Isolates on Plant Growth and Biomass Production

The *S. sesban* plant was used to estimate plant growth and biomass after 45 days of the experiment. The results showed that in stress and non-stress conditions, *S. sesban* plants inoculated with PGPR showed a one-fold-higher development of the plant compared to the un-inoculated controls. PGPR inoculation increased the growth of the *S. sesban* plant to a certain extent. In non-stressed plants, shoot growth and root growth were enhanced by 53% (Figure 3A) and 75% due to the inoculation of *B. xiamenensis*, respectively. In addition, shoot length and root length were enhanced by 44% and 75% (Figure 3B) due to the inoculation of *B. gibsonii* in comparison with non-bacterial inoculated non-stressed *S. sesban* plants. However, in metal stress conditions, the shoot and root length of the *S. sesban* plant were reduced compared to the plant inoculated with PGPR strains. *B. xiamenensis* increased the shoot length and root length by up to 133% and 105% respectively. *B. gibsonii* enhanced to the shoot length and root length by up to 75% and 80% compared to the non-inoculated stressed *S. sesban* plant. Under non-stress conditions, the fresh weight of *S. sesbania* increased in PGPR-inoculated plant as compared to the control. The fresh weight of the *S. sesban* plant increased by up to 121% with the inoculation of bacterial isolate *B. xiamenensis*. Moreover, the fresh weight increased by up to 129% with the inoculation of bacterial isolate *B. gibsonii* (Figure 3C). Similarly, the fresh weight of *S. sesban* increased in heavy metal stress conditions (Figure 3C) when the plant was treated with heavy metal-tolerant PGPR strains. The fresh weight of *S. sesban* was increased by up to 156% and 165% with the inoculation *B. xiamenensis* and *B. gibsonii*, respectively, as related to the non-PGPR-inoculated stressed plant. Moreover, the dry weight of *S. sesban* was also affected by the heavy metal stress conditions. The inoculation of heavy metal-tolerant PGPR strains increased the dry weight of *S. sesban* compared to the non-inoculated stressed plant (Figure 3D).

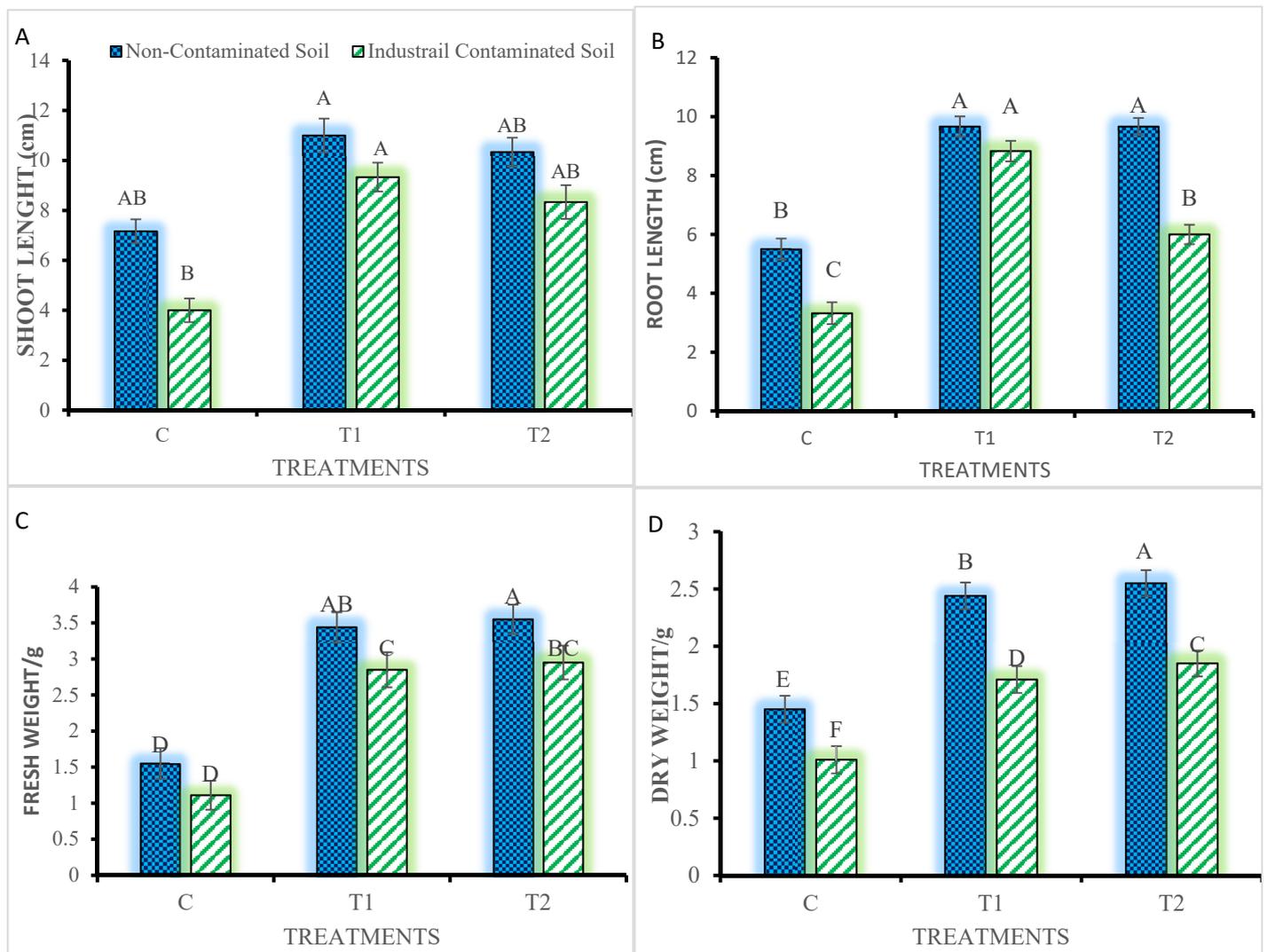


Figure 3. Effects of *B. xiamenensis* and *B. gibsonii* on the shoot length (A) and root length (B), and fresh weight (C) and dry weight (D) of *S. sesban* in noncontaminated and contaminated soil. Control, T1 = *B. xiamenensis* and T2 = *B. gibsonii*. Each value is the mean of replicates ($n = 3$); the different letters with mean values indicate significant differences, detected by LSD test ($p < 0.05$).

3.4. Effects of Different Treatments on Photosynthetic Pigments and Proline Contents

In current research, we observed that in non-stressed PGPR-inoculated plants, the chlorophyll and carotenoid content of *S. sesban* plant were enhanced compared to the non-PGPR non-stressed conditions. Augmentation with *B. xiamenensis* increased chlorophyll-a, chlorophyll-b and carotenoid content by up to 168% (Figure 4A), 144% (Figure 4B) and 101% (Figure 4C), respectively. *B. gibsonii* inoculation increased the chlorophyll-a, chlorophyll-b and carotenoid content by up to 162%, 138% and 126%, respectively. It was observed that the photosynthetic pigments of the *S. sesban* plant were decreased under heavy metal stress conditions. However, inoculation of the plant with heavy metal-tolerant PGPR strains increased the photosynthetic pigments of the plants as compared to the control group.

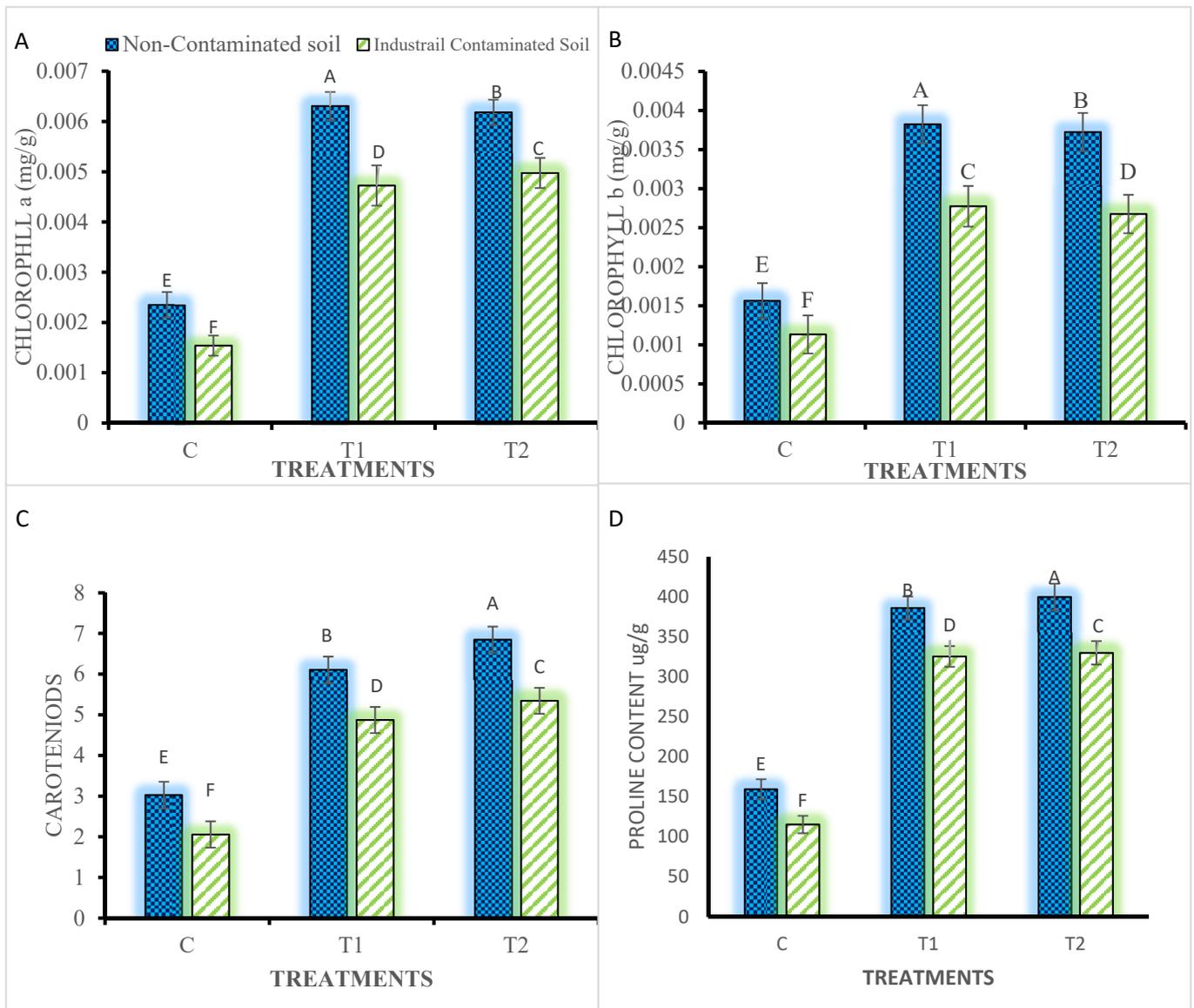


Figure 4. Effects of *B. xiamenensis* and *B. gibsonii* on the chlorophyll-a (A), chlorophyll-b (B), carotenoids content (C) and proline content (D) of *S. sesban* in noncontaminated and contaminated soil. C = Control, T1 = *B. xiamenensis* and T2 = *B. gibsonii*. Each value is the mean of replicates ($n = 3$); the different letters with mean values indicate significant differences, detected by LSD test ($p < 0.05$).

S. sesban plants inoculated with *B. xiamenensis* showed an enhanced chlorophyll-a content (206%), chlorophyll-b content (144%) and carotenoid content (136%) in correspondence to their non-PGPR-inoculated stressed counterparts. The augmentation of *B. gibsonii* enhanced the chlorophyll-a, chlorophyll-b and carotenoid content up to 223%, 136% and 159% respectively. Un-inoculated non-stressed plants showed a lower production of proline compared to the PGPR-inoculated plants. The inoculation *B. xiamenensis* and *B. gibsonii* increased the proline content up to 190% and 179%, respectively (Figure 4D). In heavy metal stress conditions, the *S. sesban* plant showed a lower production of proline, but an increased content of proline was observed (Figure 4D) when *S. sesban* plants were treated with heavy metal-tolerant PGPR strains, which indicates a protective behavior. *B. xiamenensis* enhanced the proline content up to 117%, and *B. gibsonii* increased the proline content up to 112%.

3.5. Response of Plants for SOD and POD Activity

Antioxidant activity (SOD, POD) was estimated under various heavy metal stress treatments. In the non-PGPR-inoculated non-stress *S. sesban* plant, a lower accumulation of antioxidant enzymes was observed as compared to the PGPR-immunized non-stressed plant. PGPR enhanced enzyme production in the bioaugmented plants. SOD and POD activity were increased by *B. xiamenensis* up to 216% and 48%, respectively. Similarly, *B. gibsonii* increased SOD activity up to 245% and POD activity up to 49%. Under heavy metal stress conditions, the plants' capacity to produce antioxidant enzymes was reduced. However, the inoculation of plants with heavy metal-tolerant PGPR strains increased the plants' ability to produce antioxidant enzymes such as SOD and POD. SOD activity was enhanced up to 117% (Figure 5A), whereas POD (Figure 5B) activity was increased up to 80% by the individual inoculation of the bacterial strain *B. xiamenensis*. With the inoculation of *B. gibsonii*, the SOD activity was increased up to 206%, and POD activity was increased up to 96%. Hence, the results revealed that under heavy metal stress conditions, the inoculation of PGPR increased the production of enzymes.

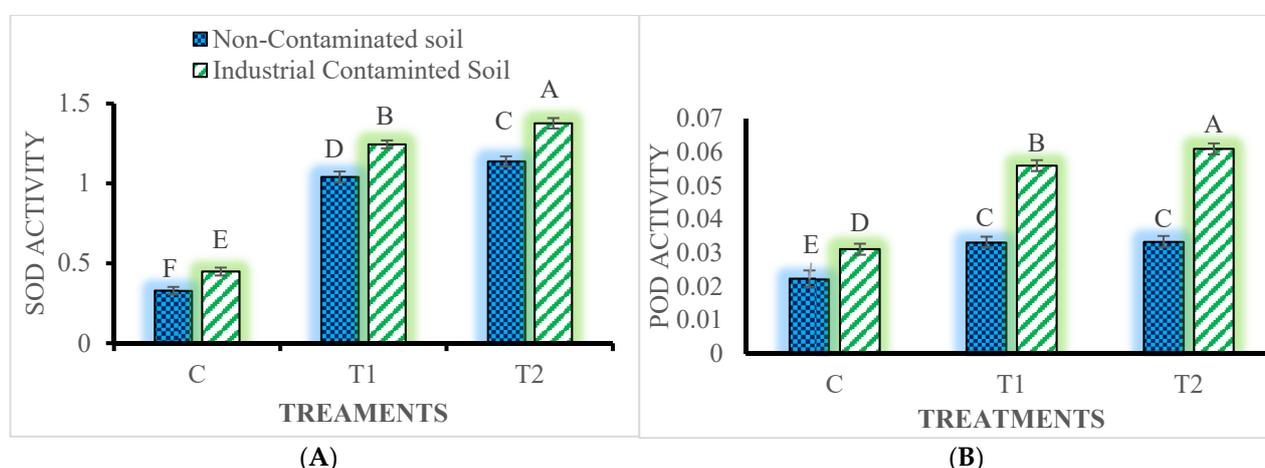


Figure 5. Effects of bacterial strains *B. xiamenensis* and *B. gibsonii* on the SOD (A) and POD activity (B) of *S. sesban* in noncontaminated and contaminated soil. C = Control, T1 = *B. xiamenensis* and T2 = *B. gibsonii*. Each value is the mean of replicates ($n = 3$); the different letters with mean values indicate significant differences, detected by LSD test ($p < 0.05$).

3.6. Analysis of Plant for Uptake of Heavy Metals

Differential effects on growth and metal uptake of *S. sesban* plants were observed for the contaminated industrial soil and bacterial inoculation (Table 3). In the current research, it was also found that the phytoextraction ability of *S. sesban* plants was increased by the inoculation of both PGPR strains. Heavy metal-tolerant bacterial isolates, such as *B. xiamenensis* and *B. gibsonii*, increased the growth of the *S. sesban* plant and the phytoextraction of heavy metals from the soil. A higher absorption of heavy metal Zn (15.74 ppm), Mn (16.4 ppm), Cd (20.5 ppm), Ni (36 ppm) and Cu (25 ppm) was observed by the immunization of *B. gibsonii* compared to *B. xiamenensis*. The immunization of *B. xiamenensis* increased the uptake of Pb (64 ppm) and Cr accumulation in *S. sesban* plants.

Table 3. Role of the PGPR strains in the differential uptake of toxic heavy metals from industrially contaminated soil.

Treatments	Zn (ppm)	Mn (ppm)	Cd (ppm)	Cr (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)
<i>Sesbania sesban</i>							
C	9.94 ^c ± 0.02	8.9 ^c ± 0.32	5.1 ^c ± 0.32	26.4 ^c ± 0.24	13.2 ^c ± 0.32	18.3 ^b ± 0.2	42 ^c ± 0.49
T1	10.03 ^b ± 0.01	13.3 ^b ± 0.40	14.1 ^b ± 0.32	35.3 ^b ± 0.33	17.4 ^b ± 0.4	13.9 ^c ± 0.24	64 ^a ± 0.30
T2	15.74 ^a ± 0.02	16.4 ^a ± 0.16	20.5 ^a ± 0.16	38.5 ^a ± 0.24	25.2 ^a ± 0.32	36 ^a ± 0.02	52 ^b ± 0.19

Each value is the mean of replicates ($n = 3$); the different letters with mean values in columns indicate significant differences, detected by LSD test ($p < 0.05$).

3.7. PCA Analysis

The Principal Component Analysis clustered the treatments and variables into various groups based on differences in their correlation. In the current study, PCA analysis clustered the response variables of *Sesbania* plants under heavy metal stress and bacterial strain inoculation. Almost all the growth traits of *Sesbania* plants were grouped, showing their similar trend of increasing responsibility for bacterial strain inoculation under heavy metal stress. The PCA analysis was also performed to compare the different treatments of the pot experiment to evaluate their accumulative effects on plant response (Figures 6A,B and 7A,B). The analysis separated the control treatment from the other treatments, implying that these treatments were different than control treatments.

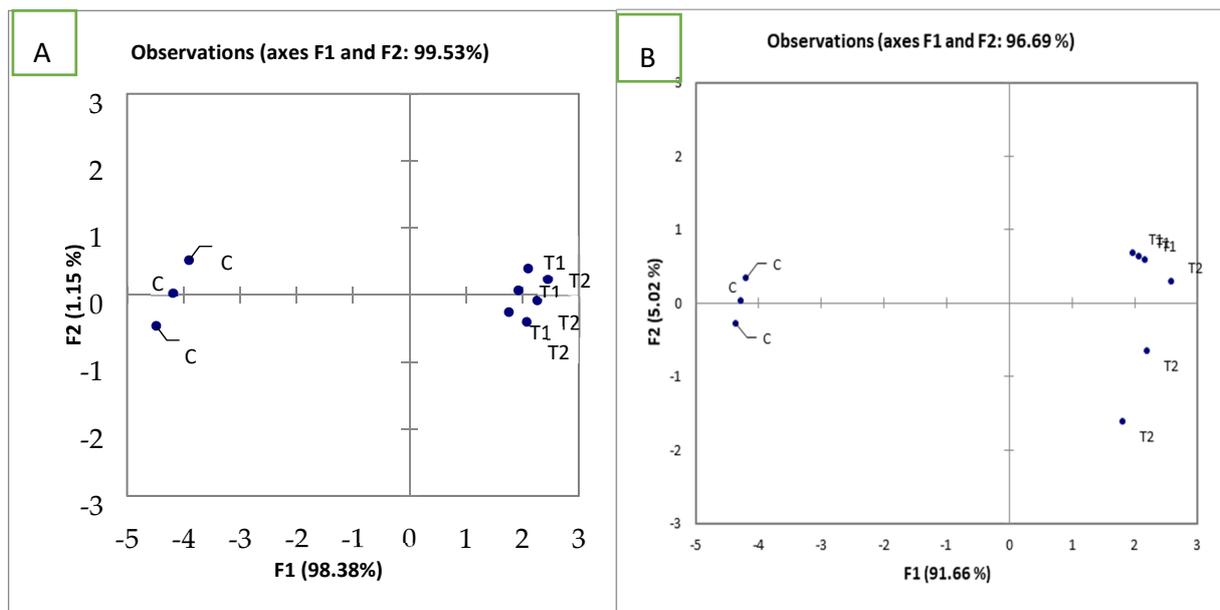


Figure 6. (A,B) PCA analysis among the various treatments in plants growing in noncontaminated soil and industrially contaminated soils.

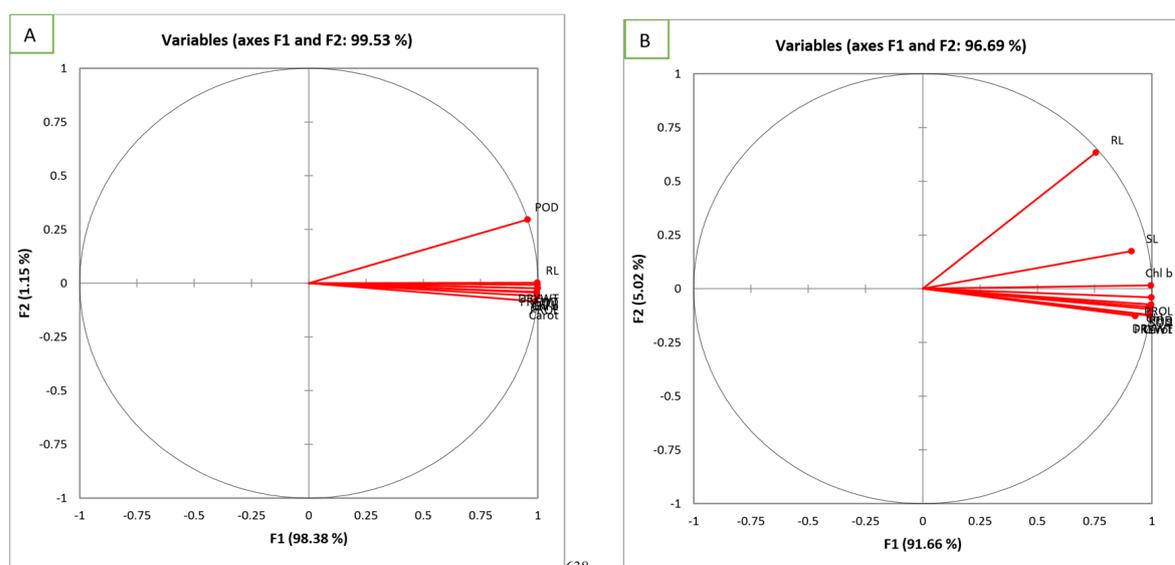


Figure 7. PCA analysis among the various analyzed parameters in plants growing in noncontaminated soil (A) and industrially contaminated soils (B).

4. Discussion

In the current research, the phytoextraction ability of *S. sesban* was assessed under industrially contaminated soil. In addition, we determined the effect of heavy metal-tolerant PGPR strains, i.e., *B. gibsonii* and *B. xiamenensis*, on the root shoot length, fresh and dry biomass, proline content and chlorophyll content, as well as the antioxidant activity of plants under both normal and industrially contaminated soils. Under the stressed condition, increased plant growth and physio-biochemical activity revealed the different stimulating mechanisms that bacteria can elicit. By accelerating the process of phytoremediation through increased plant growth and biomass production, both PGPR strains can be used in industrially contaminated soils for the proficient remediation of harmful substances.

Decreased plant growth is the first indicator of prevailed stress in contaminated soils compared to plants grown in normal soils [29]. There are higher levels of harmful metals in industrially contaminated soils that are accumulated by roots via adsorption in the plants. Then, these harmful metals are translocated to different parts of plants, reducing the metabolism, plant growth and uptake of nutrients from the soil. Plant growth and nutrient metabolism are enhanced using PGPR strains. Through various mechanisms, plant growth-promoting bacteria can promote EPS formation, ACC deaminase activity, the production of IAA, the release of ROS scavengers and siderophores, nutrient absorption and the production of organic substances for the chelation of phosphorus (P), iron (F) and potassium (K). Phytoremediation plays an important role in the remediation of the environment from contaminants, but metal toxicity makes this tactic less effective. Under these conditions, PGPR strains play an important role, as they the process of bioremediation, acculamate or transform contaminants in plants, and increase the growth of plants by producing increased growth hormones, EPS, and ACC production.

PGPR strains regulate the levels of hormones that promote the appearance of different stress-related proteins. These hormones are essential for the protection of plants under biotic and abiotic stress conditions. One of these hormones, ethylene, plays a prominent role in the normal growth and development of plants. However, under stress conditions, higher levels of ethylene are produced, which has a negative impact on plant growth. The use of ACC-deaminase can overcome the negative impact of excessive ethylene. Thus, plants can survive under different environmental stress conditions. These ACC-deaminase producing microorganisms cleave ACC, the immediate precursor of ethylene. In the current study, higher levels of ethylene production were reduced under heavy metal toxicity. Therefore, the growth and development of the plant were observed to be increased as compared to the non-inoculated plants.

Exopolysaccharide production is another strategy used by microbes to fight against heavy metals. It is thought that heavy metal stress stimulates bacteria to increase EPS production, which shields cellular components from the ill effects of heavy metals. EPS is a cell-associated microbial polymer, containing macromolecules such as proteins and phosphates. EPS acts as a biofilm former that can be used for the remediation of polluted soils. By biofilm formation, EPS increases the water attention of microbes, controls the dispersal of organic carbon sources, forms soil aggregates and enables the uptake of water and nutrients across the root [56].

EPS production is an important tool for the removal of heavy metals and can provide an economic and eco-friendly system. In metal-contaminated soil, EPS production is a protective strategy for promoting the survival and growth of biofilm-forming bacteria. The physiochemical properties and growth of plants can be improved using EPS-producing PGPR strains [57]. Under stress and non-stress conditions, IAA is a multifunctional hormone. IAA increases the growth and development of the plants. In addition, the production of IAA promotes root elongation by heavy metal-tolerant PGPR strains and increases nutrient and water uptake, which directly enhances production and growth of associated plants. IAA can also be used to reduce the toxic effects of heavy metals by increasing the phytoextraction of Pb, Zn and Cd by enhanced shoot and root growth [58]. Application of IAA enhanced the growth of *Brassica juncea* and *Oryza sativa* in Arsenic (As)-contaminated

soils. In soil contaminated with lead (Pb), it was also observed that the root biomass of *Helianthus annulus* increased using IAA. In swamp and non-swamp macrophytes, IAA enhances the process of phytoremediation. Moreover, the formation of IAA reduces hydrogen peroxide absorption, controls the activity of defense enzymes activity and promotes the carboxylation of proteins [59]. In industrially contaminated areas, the reduced growth of plants is common due to higher concentrations of heavy metals whose toxic effects lower the production of the necessary enzymes and inhibit the basic physiological processes required for the plant growth. Under stress conditions, a large amount of ROS is produced, which reduces cells' metabolic activities and directly intercalates with the cellular membranes and genetic makeup, inhibiting the growth and biomass of plants [60]. However, PGPR strains eliminate the toxic effects of metals and enhance nutrients absorption from metal-contaminated soil [49,61].

Phytoremediation is one of the most effective processes to clean nature from harmful effects of metals using likable plants. Oxidative stress is caused by the excessive accumulation of metals in plant tissues. In this way, the affected plant processes, such as alterations in plants' cellular homeostasis, result in harmful effects on the proper growth and development of plants. Synchronizing the proper functioning of antioxidant enzymes that alleviate cellular damage by restricting ROS is the only way to combat stress conditions. ROS are inevitable byproducts of various abiotic stresses that are directly associated with cellular metabolism. There are two phases of ROS: redox biology and oxidative stress. In redox biology, small levels of ROS act as signaling molecules for the preservation of physiological processes, whereas in oxidative stress, elevated level of ROS are produced in bursts when triggered by some external stress that results in DNA, protein and lipid damage. However, the negative effects of oxidative stress can be overcome using antioxidant enzymes produced by the inoculation of heavy metal-tolerant PGPR strains.

Under heavy metal stress conditions, previous researchers have observed a decreased concentration of plant chlorophyll contents, photosynthetic pigments, fluctuations in structural compounds and inhibition of gas exchange factors. This decrease in plant chlorophyll content might be related to the chlorophyll activation, changes in key thylakoid membrane components, their manifestation mechanism and changes in the ultrastructure of proteins and DNA owing to ROS interference. [62]. In the current study, it was found that combining *S. sesban* plants with PGPR strains such as *B. xiamenensis* and *B. gibsonii* increased the plants' chlorophyll content. The increase of the plants' chlorophyll content was confirmed by the organic minerals observed to be assembled and dissolved by the PGPR strains [63], with nitrogen being the most essential ingredient of chlorophyll [64]. As a result, the chlorophyll content of the studied plants increased [65]. Higher levels of chlorophyll facilitated plant growth and development, as evidenced by the fact that the dry weight of PGPR-inoculated *S. sesban* plants increased with PGPR strains such as *B. xiamenensis* and *B. gibsonii* [29,66]. Moreover, enhanced chlorophyll contents were examined in the PGPR-inoculated plants [57]. Carotenoids may act as a photo-protective pigment. In heavy metal stress condition, ROS inhibit carotenogenesis to produce carotenoids. A decrease in carotenoids content has been previously specified in different plants under different treatments [58,59]. Under stressed and non-stressed conditions, the carotenoid content of *S. sesban* treated with PGPR strains was observed to be enhanced as compared to non-inoculated stressed plants.

As a result of abiotic stress, ROS is produced. Under stress conditions, a balance must be maintained between the formation and retrieval of ROS for the proper production of plants. SOD, POD, catalase and proline play important roles in decreasing ROS accumulation, preserving cell membrane integrity, removing malondialdehyde (MDA), preventing lipids peroxidation and protecting the plant from the oxidation of cellular substances by scavenging the ROS. In industrially contaminated soil, the inoculation of heavy metal-tolerant PGPR in *S. sesban* such as *B. xiamenensis* and *B. gibsonii* increased the antioxidant activity, including the SOD, POD and proline content, compared to nonimmunized plants. In the current study, we observed that the inoculation of PGPR in stressed plants enhanced

the antioxidant system in a very useful way, allowing the plant to overcome the destructive effects of heavy metals. Nowadays, the application of multivariate investigation has gained significant importance in tracing possible trends and relationships among datasets, as simple statistical analysis may not be enough to show significant differences among different treatments. However, multivariate analysis separates these treatments based on their overall effects on the different responses of variables [67].

Interaction between plants and microbes in heavy metal contaminated soil increases the process of phytoremediation [68]. In heavy metal-contaminated soils, plants could be protected from the injurious effects of metals by inoculation with PGPR strains that help to clean the contaminated sites. Thus, in industrially contaminated soils, the toxicity of metals could be overcome by the application of PGPR. The present work revealed that the inoculation with heavy metal-tolerant PGPR strains, such as *B. xiamenensis* and *B. gibsonii*, enhanced the process of phytoremediation in *S. sesban* plants to remediate the industrially contaminated soil. This process of phytoremediation is a sustainable approach [44]. The principal component analysis showed that all metrics showed a substantial association ($\alpha = 0.05$) in a biplot between chlorophyll contents and plant growth parameters. It was observed that plant growth parameters, such as the chlorophyll content, fresh weight, shoot length and root length, had a significant correlation. In addition, the dry weight, carotenoids, chlorophyll and chlorophyll-a, all showed a linear relationship. Antioxidant enzymes, such as SOD, POD and catalase, were considerably correlated with the soil toxicity. All parameters presented in the same quadrat and showed a correlation with each other.

5. Conclusions

The results showed that *S. sesban*'s growth, biomass, and photosynthetic pigments were decreased upon its introduction into industrially contaminated soil. However, with the inoculation of heavy metal-tolerant ACC-, IAA- and EPS-producing PGPR strains (*B. xiamenensis*, *B. gibsonii*) alleviated the adverse effects of metals and enhanced the plant growth and biomass. The increase in plant biomass and growth was associated with decreased osmotic stress and increased antioxidant activities during PGPR inoculation. Furthermore, heavy metal accumulation was higher in the bacterial inoculated plants as compared to their uninoculated controls. The present research suggests that PGPR strains can alter the toxicity of metals on *S. sesban*, which makes these strains excellent candidates for the enhanced restoration of contaminated soils through bacterial-assisted phytoremediation. The process of phytoremediation is eco-friendly and could be used to reclaim the industrial soil from contaminants.

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References

1. Chibuikwe, G.U.; Obiora, S.C. Heavy metal polluted soils: Effect on plants and bioremediation methods. *Appl. Environ. Soil Sci.* **2014**, *2014*, 752708. [[CrossRef](#)]
2. Ma, Y.; Rajkumar, M.; Luo, Y.; Freitas, H. Phytoextraction of heavy metal polluted soils using *Sedum plumbizincicola* inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. *Chemosphere* **2013**, *93*, 1386–1392. [[CrossRef](#)]
3. Ullah, A.; Heng, S.; Munis, M.F.H.; Fahad, S.; Yang, X. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. *Environ. Exp. Bot.* **2015**, *117*, 28–40. [[CrossRef](#)]
4. Mench, M.; Schwitzguébel, J.-P.; Schroeder, P.; Bert, V.; Gawronski, S.; Gupta, S. Assessment of successful experiments and limitations of phytotechnologies: Contaminant uptake, detoxification and sequestration, and consequences for food safety. *Environ. Sci. Pollut. Res.* **2009**, *16*, 876–900. [[CrossRef](#)]
5. Vangronsveld, J.; Herzog, R.; Weyens, N.; Boulet, J.; Adriaensen, K.; Ruttens, A.; Thewys, T.; Vassilev, A.; Meers, E.; Nehnevajova, E. Phytoremediation of contaminated soils and groundwater: Lessons from the field. *Environ. Sci. Pollut. Res.* **2009**, *16*, 765–794. [[CrossRef](#)] [[PubMed](#)]
6. Pilon-Smits, E. Phytoremediation. *Annu. Rev. Plant Biol.* **2005**, *56*, 15–39. [[CrossRef](#)]
7. LeDuc, D.L.; Terry, N. Phytoremediation of toxic trace elements in soil and water. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 514–520. [[CrossRef](#)]
8. Saleem, M.; Arshad, M.; Hussain, S.; Bhatti, A.S. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 635–648. [[CrossRef](#)]
9. Vessey, J.K. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* **2003**, *255*, 571–586. [[CrossRef](#)]
10. Jha, B.; Gontia, I.; Hartmann, A. The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. *Plant Soil* **2012**, *356*, 265–277. [[CrossRef](#)]
11. Van Loon, L. Plant responses to plant growth-promoting rhizobacteria. In *New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 243–254.
12. Gontia-Mishra, I.; Sasidharan, S.; Tiwari, S. Recent developments in use of 1-aminocyclopropane-1-carboxylate (ACC) deaminase for conferring tolerance to biotic and abiotic stress. *Biotechnol. Lett.* **2014**, *36*, 889–898. [[CrossRef](#)]
13. Hontzeas, N.; Zoidakis, J.; Glick, B.R.; Abu-Omar, M.M. Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* UW4: A key enzyme in bacterial plant growth promotion. *Biochim. Biophys. Acta (BBA) Proteins Proteom.* **2004**, *1703*, 11–19. [[CrossRef](#)] [[PubMed](#)]
14. Long, X.-X.; Chen, X.-M.; Wong, J.W.-C.; Wei, Z.-B.; Wu, Q.-T. Feasibility of enhanced phytoextraction of Zn contaminated soil with Zn mobilizing and plant growth promoting endophytic bacteria. *Trans. Nonferrous Met. Soc. China* **2013**, *23*, 2389–2396. [[CrossRef](#)]
15. Glick, B.R. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* **2012**, *2012*, 963401. [[CrossRef](#)]
16. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [[CrossRef](#)] [[PubMed](#)]
17. Ma, Y.; Rajkumar, M.; Zhang, C.; Freitas, H. Beneficial role of bacterial endophytes in heavy metal phytoremediation. *J. Environ. Manag.* **2016**, *174*, 14–25. [[CrossRef](#)] [[PubMed](#)]
18. Glick, B.R. Using soil bacteria to facilitate phytoremediation. *Biotechnol. Adv.* **2010**, *28*, 367–374. [[CrossRef](#)]
19. Huang, X.-D.; El-Alawi, Y.; Penrose, D.M.; Glick, B.R.; Greenberg, B.M. Responses of three grass species to creosote during phytoremediation. *Environ. Pollut.* **2004**, *130*, 453–463. [[CrossRef](#)]
20. Safronova, V.I.; Stepanok, V.V.; Engqvist, G.L.; Alekseyev, Y.V.; Belimov, A.A. Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fertil. Soils* **2006**, *42*, 267–272. [[CrossRef](#)]
21. Wei, X.; Fang, L.; Cai, P.; Huang, Q.; Chen, H.; Liang, W.; Rong, X. Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria. *Environ. Pollut.* **2011**, *159*, 1369–1374. [[CrossRef](#)]
22. Li, W.-W.; Yu, H.-Q. Insight into the roles of microbial extracellular polymer substances in metal biosorption. *Bioresour. Technol.* **2014**, *160*, 15–23. [[CrossRef](#)] [[PubMed](#)]
23. Sheng, G.-P.; Yu, H.-Q.; Li, X.-Y. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnol. Adv.* **2010**, *28*, 882–894. [[CrossRef](#)]
24. Webb, H.E.; Brichta-Harhay, D.M.; Brashears, M.M.; Nightingale, K.K.; Arthur, T.M.; Bosilevac, J.M.; Kalchayanand, N.; Schmidt, J.W.; Wang, R.; Granier, S.A. Salmonella in peripheral lymph nodes of healthy cattle at slaughter. *Front. Microbiol.* **2017**, *8*, 2214. [[CrossRef](#)]
25. Sheng, G.-P.; Yu, H.-Q.; Yue, Z.-B. Production of extracellular polymeric substances from *Rhodospseudomonas acidophila* in the presence of toxic substances. *Appl. Microbiol. Biotechnol.* **2005**, *69*, 216–222. [[CrossRef](#)]
26. Baptista, M.S.; Vasconcelos, M.T. Cyanobacteria metal interactions: Requirements, toxicity, and ecological implications. *Crit. Rev. Microbiol.* **2006**, *32*, 127–137. [[CrossRef](#)] [[PubMed](#)]
27. Ali, N.; Masood, S.; Mukhtar, T.; Kamran, M.A.; Rafique, M.; Munis, M.F.H.; Chaudhary, H.J. Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of *Linum usitatissimum* in association with *Glomus intraradices*. *Environ. Monit. Assess.* **2015**, *187*, 311.

28. Gupta, P.; Diwan, B. Bacterial exopolysaccharide mediated heavy metal removal: A review on biosynthesis, mechanism and remediation strategies. *Biotechnol. Rep.* **2017**, *13*, 58–71. [[CrossRef](#)] [[PubMed](#)]
29. Amna; Xia, Y.; Farooq, M.A.; Javed, M.T.; Kamran, M.A.; Mukhtar, T.; Ali, J.; Tabassum, T.; ur Rehman, S.; Munis, M.F.H.; et al. Multi-stress tolerant PGPR *Bacillus xiamenensis* PM14 activating sugarcane (*Saccharum officinarum* L.) red rot disease resistance. *Plant Physiol. Biochem.* **2020**, *151*, 640–649. [[CrossRef](#)]
30. Schaad, N.W.; Jones, J.B.; Chun, W. *Laboratory Guide for the Identification of Plant Pathogenic Bacteria*; American Phytopathological Society (APS Press): St. Paul, MN, USA, 2001.
31. Pikovskaya, R. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya* **1948**, *17*, 362–370.
32. Gordon, S.; Paleg, L. Observations on the quantitative determination of indoleacetic acid. *Physiol. Plant.* **1957**, *10*, 39–47. [[CrossRef](#)]
33. Schwyn, B.; Neilands, J. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **1987**, *160*, 47–56. [[CrossRef](#)]
34. Di Simine, C.; Sayer, J.; Gadd, G. Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. *Biol. Fertil. Soils* **1998**, *28*, 87–94. [[CrossRef](#)]
35. Gajbhiye, A.; Rai, A.R.; Meshram, S.U.; Dongre, A. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. *World J. Microbiol. Biotechnol.* **2010**, *26*, 1187–1194. [[CrossRef](#)]
36. Naseem, H.; Bano, A. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. *J. Plant Interact.* **2014**, *9*, 689–701. [[CrossRef](#)]
37. Babu, N.J.; Nangia, A. Solubility advantage of amorphous drugs and pharmaceutical cocrystals. *Cryst. Growth Des.* **2011**, *11*, 2662–2679. [[CrossRef](#)]
38. Melkamu, T.; Diriba, M.; Gezahegn, B.; Girma, A. Antagonistic effects of rhizobacteria against Coffee Wilt Disease caused by *Gibberella xylarioides*. *Asian J. Plant Pathol.* **2013**, *7*, 109–122.
39. Glick, B.R. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **1995**, *41*, 109–117. [[CrossRef](#)]
40. Dworkin, M.; Foster, J. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.* **1958**, *75*, 592–603. [[CrossRef](#)]
41. Subair, H. Isolation and Screening Bacterial Exopolysaccharide (EPS) from potato rhizosphere in highland and the potential as a producer Indole Acetic Acid (IAA). *Procedia Food Sci.* **2015**, *3*, 74–81.
42. Din, B.U.; Rafique, M.; Javed, M.T.; Kamran, M.A.; Mehmood, S.; Khan, M.; Sultan, T.; Munis, M.F.H.; Chaudhary, H.J. Assisted phytoremediation of chromium spiked soils by *Sesbania Sesban* in association with *Bacillus xiamenensis* PM14: A biochemical analysis. *Plant Physiol. Biochem.* **2020**, *146*, 249–258. [[CrossRef](#)]
43. Ehsan, S.; Ali, S.; Noureen, S.; Mahmood, K.; Farid, M.; Ishaque, W.; Shakoob, M.B.; Rizwan, M. Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Ecotoxicol. Environ. Saf.* **2014**, *106*, 164–172. [[CrossRef](#)]
44. Weyens, N.; van der Lelie, D.; Taghavi, S.; Vangronsveld, J. Phytoremediation: Plant–endophyte partnerships take the challenge. *Curr. Opin. Biotechnol.* **2009**, *20*, 248–254. [[CrossRef](#)] [[PubMed](#)]
45. Goswami, D.; Vaghela, H.; Parmar, S.; Dhandhukia, P.; Thakker, J.N. Plant growth promoting potentials of *Pseudomonas* spp. strain OG isolated from marine water. *J. Plant Interact.* **2013**, *8*, 281–290. [[CrossRef](#)]
46. Belimov, A.; Hontzeas, N.; Safronova, V.; Demchinskaya, S.; Piluzza, G.; Bullitta, S.; Glick, B. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.* **2005**, *37*, 241–250. [[CrossRef](#)]
47. Penrose, D.M.; Glick, B.R. Methods for isolating and characterizing ACC deaminase—Containing plant growth—Promoting rhizobacteria. *Physiol. Plant* **2003**, *118*, 10–15. [[CrossRef](#)] [[PubMed](#)]
48. McLean, E.O. Soil pH and lime requirement. *Methods Soil Anal. Part 2 Chem. Microbiol. Prop.* **1983**, *9*, 199–224.
49. Xin, G.; Zhang, G.; Kang, J.W.; Staley, J.T.; Doty, S.L. A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biol. Fertil. Soils* **2009**, *45*, 669–674. [[CrossRef](#)]
50. Nelson, D.W.; Sommers, L. Total carbon, organic carbon, and organic matter. *Methods Soil Anal. Part 2 Chem. Microbiol. Prop.* **1983**, *9*, 539–579.
51. Achakzai, K.; Khalid, S.; Bibi, A. Determination of heavy metals in agricultural soil adjacent to functional brick kilns: A case study of Rawalpindi. *Sci. Technol. Dev.* **2017**, *34*, 122–129. [[CrossRef](#)]
52. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1. [[CrossRef](#)]
53. Hayat, K.; Ali, S.; Ullah, S.; Fu, Y.; Hussain, M. Green synthesized silver and copper nanoparticles induced changes in biomass parameters, secondary metabolites production, and antioxidant activity in callus cultures of *Artemisia absinthium* L. *Green Process. Synth.* **2021**, *10*, 61–72. [[CrossRef](#)]
54. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [[CrossRef](#)]
55. Duncan, O.D.; Duncan, B. A methodological analysis of segregation indexes. *Am. Sociol. Rev.* **1955**, *20*, 210–217. [[CrossRef](#)]
56. Maxton, A.; Singh, P.; Masih, S.A. ACC deaminase-producing bacteria mediated drought and salt tolerance in *Capsicum annum*. *J. Plant Nutr.* **2018**, *41*, 574–583. [[CrossRef](#)]
57. Gontia-Mishra, I.; Sapre, S.; Kachare, S.; Tiwari, S. Molecular diversity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing PGPR from wheat (*Triticum aestivum* L.) rhizosphere. *Plant Soil* **2017**, *414*, 213–227. [[CrossRef](#)]

58. Sytar, O.; Kumari, P.; Yadav, S.; Brestic, M.; Rastogi, A. Phytohormone priming: Regulator for heavy metal stress in plants. *J. Plant Growth Regul.* **2019**, *38*, 739–752. [[CrossRef](#)]
59. Bücken-Neto, L.; Paiva, A.L.S.; Machado, R.D.; Arenhart, R.A.; Margis-Pinheiro, M. Interactions between plant hormones and heavy metals responses. *Genet. Mol. Biol.* **2017**, *40*, 373–386. [[CrossRef](#)]
60. Faisal, M.; Hasnain, S. Bacterial Cr (VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. *Biotechnol. Lett.* **2005**, *27*, 943–947. [[CrossRef](#)] [[PubMed](#)]
61. Paungfoo-Lonhienne, C.; Lonhienne, T.G.; Yeoh, Y.K.; Webb, R.I.; Lakshmanan, P.; Chan, C.X.; Lim, P.E.; Ragan, M.A.; Schmidt, S.; Hugenholtz, P. A new species of Burkholderia isolated from sugarcane roots promotes plant growth. *Microb. Biotechnol.* **2014**, *7*, 142–154. [[CrossRef](#)] [[PubMed](#)]
62. Zayed, A.M.; Terry, N. Chromium in the environment: Factors affecting biological remediation. *Plant Soil* **2003**, *249*, 139–156. [[CrossRef](#)]
63. Swan, H. *Relationships between Nutrient Supply, Growth and Nutrient Concentrations in the Foliage of White and Red Spruce*; Pulp and Paper Research Institute of Canada: Vancouver, BC, Canada, 1971.
64. Bojović, B.; Marković, A. Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujev. J. Sci.* **2009**, *31*, 69–74.
65. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals—Concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [[CrossRef](#)] [[PubMed](#)]
66. Kim, K.-C.; Lai, Z.; Fan, B.; Chen, Z. Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell* **2008**, *20*, 2357–2371. [[CrossRef](#)]
67. Middleton, E.M.; Teramura, A.H. The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. *Plant Physiol.* **1993**, *103*, 741–752. [[CrossRef](#)]
68. Shahid, M.; Nadeem, M.; Bakhat, H.F. Environmental toxicology and associated human health risks. *Environ. Sci. Pollut. Res.* **2020**, *27*, 39671–39675. [[CrossRef](#)]