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Investigation of PGPR's potential to promote growth in maize (*Zea mays* L.) under chromium and cadmium stress

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Contribution **Zahra, F.** conducted the experiment and data collection, **A. Ahmed** conceptualization and methodology development, **A. Tariq** performed data analysis, **R. Hanif** data analysis, editing, and review.

ABSTRACT

Corn is an important cereal crop after wheat and rice in various regions of the world. It is used by animals as well as human beings. Industrialization, urbanization and other anthropogenic activities have significantly elevated the concentration of heavy metals in the biosphere. Agricultural crops are facing heavy metal contamination. Cadmium (Cd) and chromium (Cr) are particularly concerning heavy metals due to their documented phytotoxicity and zootoxicity even at low levels. In this regard, plant growth promoting rhizobacteria (PGPR) offer a clean and green solution of bioremediation of these heavy metals. During the current study, already isolated chromium and cadmium resistant bacterial strains i.e., *Pseudomonas aeruginosa* (EH2), *Pseudomonas songnensis* (GR3), *Bacillus bingmayongensis* (KH3) and *Bacillus tropicus* (LS5) were selected to observe their beneficial impact on the increase production of corn in chromium and cadmium stress (150 & 250 μg/mL). Minimum inhibitory concentration (MIC) of these bacterial isolates was recorded for chromium and cadmium stress. Plant microbe experiment was conducted and various growth as well as biochemical parameters were measured. Results showed that these PGPR significantly alleviated the toxic effects of chromium and cadmium stress and improved the production. Maximum possible enhancement in growth parameters i.e., root and shoot length, number of leaves and fresh weight was observed up to 47, 41, 70 and 56% in plants treated with *P. songnensis* (GR3), *B. bingmayongensis* (KH3), *P. songnensis* (GR3) and *B. bingmayongensis* (KH3) respectively. Enhancement in total chlorophyll and protein content of treated plants with *P. aeruginosa* (EH2) was noted up to 21 and 13% respectively as compared to control. In chromium and cadmium contaminated soil, these bacterial strains can be employed to ameliorate plant growth and reduce heavy metal phytotoxicity.

Keywords: Bacterial strains, plant growth promoting rhizobacteria, *Zea mays*, bioremediation, heavy metal stress

INTRODUCTION: Corn is third most important cereal crop after wheat and rice in various regions of the world. In Pakistan, it is cultivated in all provinces but mostly produced in Punjab and KPK. It is considered a multipurpose cereal crop and serves as a vital source of fodder, feed, and food. Like all other crops, maize production faces significant challenges from various abiotic factors, particularly heavy metal stress, which can substantially decrease both yield and quality. Heavy metals contamination has become serious eco-toxicological concern worldwide (Rajendran *et al.*, 2022). In the biosphere, concentration of these heavy metals has been increased due to urbanization and industrialization. Soil and aquatic ecosystems are largest reservoir of these heavy metals (Zakaria *et al.*, 2021). Among the heavy metals, chromium and cadmium are of special concern because they are potentially toxic to both animals and plants even at low concentrations. The toxicity in plants caused by chromium (Cr) varies from the inhibition of enzymatic activity to mutagenesis. Leaf chlorosis, stunting, and yield reduction are the major visible symptoms (Ali *et al.*, 2023). High toxicity and great solubility in water makes cadmium a very dangerous pollutant. In addition to inducing lipid peroxidation and chlorophyll breakdown in plants, Cd interacts with the absorption of metal nutrients such as Fe, Zn, Cu, and Mn that results in increased production of reactive oxygen species (ROS) (Khaliq *et al.*, 2019). The corn, an annual flowering plant, is considered a crucial cereal grown as a food and feed crop worldwide. Because of its nutritional content and phytochemical compounds, maize is ranked third among cereals, after rice and wheat, and is used as a staple diet (Rizvi *et al.*, 2022). Like other various cereal crops, corn production is also threatened by heavy metal stresses which reduce its quality and quantity (Zha *et al.*, 2023). Hence to mitigate environmental harm, remediation of heavy metals is essential. For the retrieval and reduction of heavy metals in water and polluted lands, PGPR-assisted bioremediation is an inventive and optimistic technology. Various techniques such as efflux systems, siderophores and chelation, biotransformation, biosorption, bioaccumulation, precipitation, ACC deaminase activity, biodegradation, and biomineralization are used by PGPR to clean up the heavy metal-contamination in the environment (Gupta *et al.*, 2022).

OBJECTIVES: The objective of the current research is to study the potential role of metal resistant PGPR in enhancing corn growth and reducing chromium and cadmium toxicity.

MATERIALS AND METHODS: **The Cr and Cd resistance assay:** Previously isolated bacterial isolates (n=32) were checked for their resistance against chromium (K_2CrO_4) and cadmium stress ($CdCl_2$). Bacterial isolates were grown on L-agar medium supplemented with different concentrations of chromium and cadmium ranging from 100μg/mL to 1000μg/mL to observe their Minimum inhibitory Concentration (MIC) (figure 1).

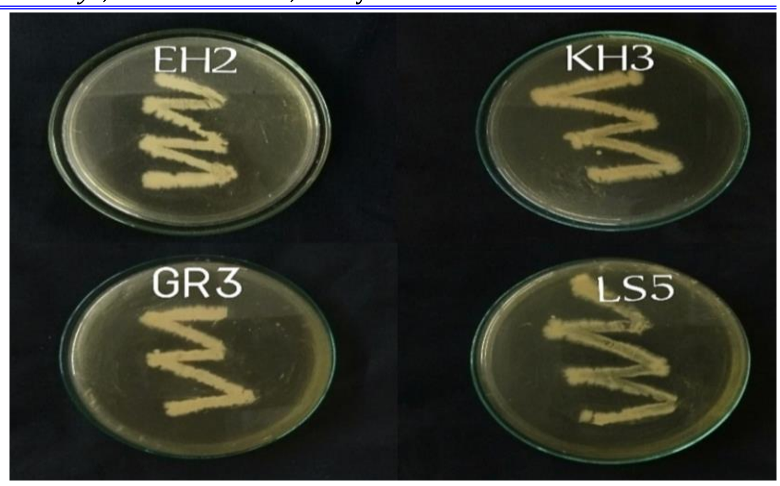

Figure 1: The bacterial strains selected for the current study i.e., *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5).

Plant microbe interaction study: Corn certified seeds (var. DK-6714) were procured from Punjab Seed Corporation, Lahore, were washed with detergent thrice and then surface sterilized using 0.1% $HgCl_2$ solution. Bacterial isolates from 24 h. of incubated cultures were harvested. The cell density was adjusted by taking the O.D at 600nm by using a spectrophotometer. After adjusting the optical density of all cultures to 10^6 to 10^7 CFU/mL, surface sterilized seeds were treated with bacterial cultures for one hour for the pre-germination inoculation treatment of corn seeds. The seeds were treated with autoclaved distilled water for the same period, which served as a control. The growth experiment was conducted in the control laboratory settings with four bacterial strains *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5). After 20 days, plants were harvested and their different growth and biochemical parameters were recorded and compared with control. A modified method described by Wellburn (1994) was employed to estimate the chlorophyll content of treated and non-treated plants. For each treatment, 1g crushed plant material was taken in test tube containing 10 ml of 80% acetone solution and kept in dark. After 24 h. optical density was recorded by using spectrophotometer. A method described by Lowry *et al.* (1951) was used to estimate total soluble protein content of treated and non-treated plants. For each treatment, 1g plant material was taken, crushed and centrifuged at 10,000 rpm for 10 min. 0.4ml of supernatant was collected in test tube and 2ml Folin's mixture was added to it. After this, 0.2ml Folin's Ciocalteus phenol reagent was added and kept at room temperature. Optical density was adjusted at 750nm and protein content was measured by comparing with standard curve.

Statistical analysis: All the results obtained were statistically analysed by applying Duncan's multiple range test and by using Software SPSS v.16.

RESULTS: The Cr and Cd resistance assay: *P. aeruginosa* (EH2) and *P. songnensis* (GR3) showed chromium resistance by exhibiting profound growth up to the stress of 250µg/mL and *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) showed tolerance to chromium up to the stress of 350 µg/mL. *P. aeruginosa* (EH2) and *B. tropicus* (LS5) showed cadmium resistance by exhibiting profound growth up to cadmium stress of 250µg/mL while bacterial isolates *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) showed tolerance to cadmium up to 300 µg/mL. Results indicated that growth and biochemical parameters of corn (*Zea mays* L.) were enhanced due to inoculation of *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) under chromium, cadmium and their combined stress in comparison to control.

The germination percentage of bacterially treated seeds was greater than non-treated seeds. The maximum percentage of germination was expressed by *P. aeruginosa* (EH2) i.e., 80% without chromium stress. Whereas, seeds treated with *Pseudomonas aeruginosa* (EH2), *B. bingmayongensis* (KH3), *P. songnensis* (GR3) and *B. tropicus* (LS5) under chromium stress of 150µg/mL showed 75, 72, 69 and 65% increase in percentage germination respectively, as compared to control. Seeds treated with *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3), *P. songnensis* (GR3) and *B. tropicus* (LS5) under chromium stress of 250µg/mL showed 70, 65, 61 and 55% increase in percentage germination, as compared to control (figure 2). Under 150µg/mL chromium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. tropicus* (LS5) and *B. bingmayongensis* (KH3) treated plants showed increase of 11, 10, 10 and 9% respectively, in shoot length as compared to the control. Under 250µg/mL chromium stress, *B. bingmayongensis* (KH3), *B. tropicus* (LS5), *P. songnensis* (GR3) and *P. aeruginosa* (EH2) treated plants showed increase of 21, 21, 16 and 8% respectively, in shoot length as compared to control (figure 3). Under 150 µg/mL chromium stress, *P. songnensis* (GR3), *B. bingmayongensis* (KH3), *P. aeruginosa* (EH2) and *B. tropicus* (LS5) treated plants showed increase of 15, 14, 14 and 13% respectively, in root length as compared to control. When exposed to the stress of 250µg/mL chromium, *P. songnensis* (GR3), *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. aeruginosa* (EH2) treated plants showed increase of 18, 17, 14 and 12% respectively, in root length as compared to control (figure 3).

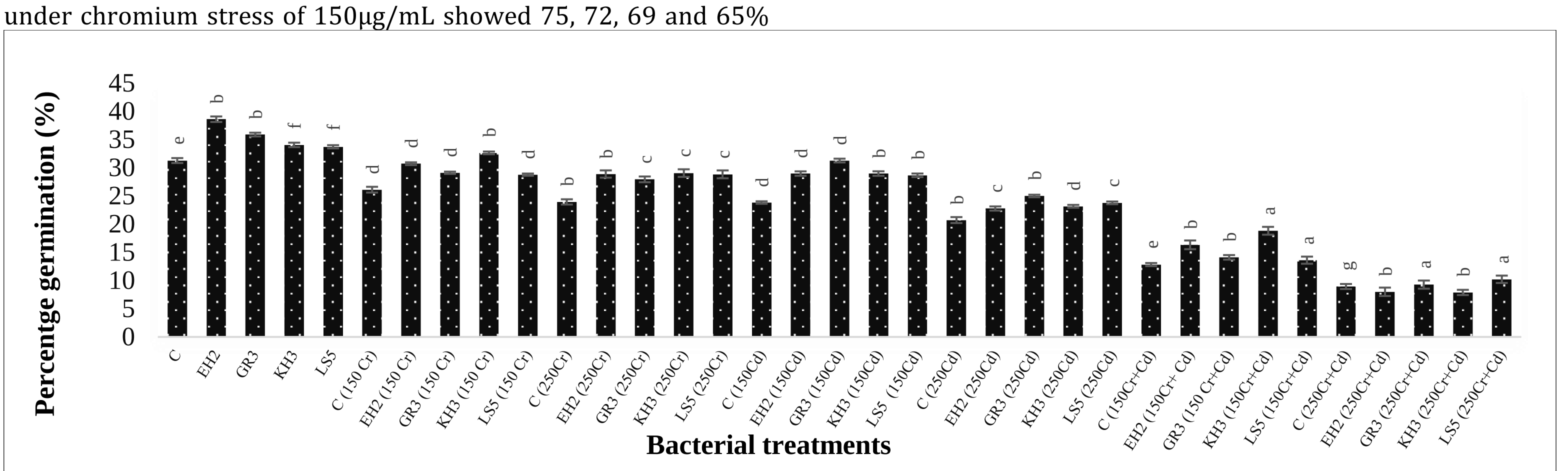

Figure 2: Effects of bacterial treatments on percentage germination of *Zea mays* L. (DK-6714) under chromium and cadmium stress (150 & 250µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5)].

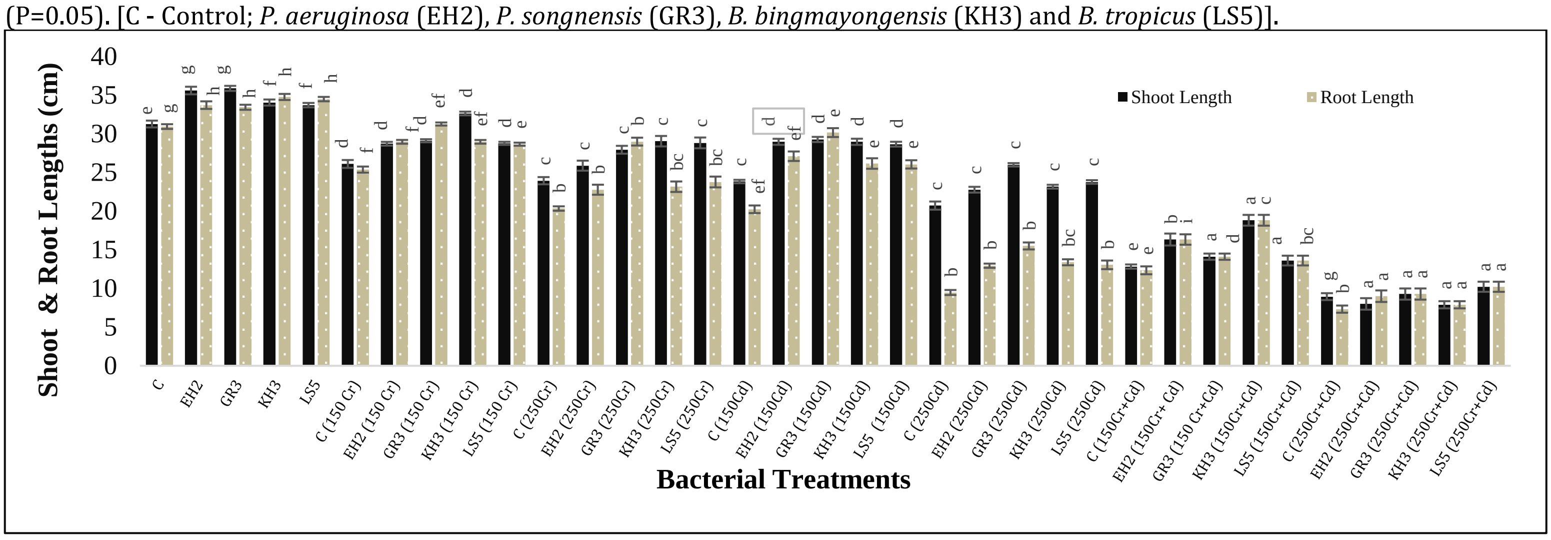

Figure 3: Effects of bacterial treatments on shoot and root lengths of *Zea mays* L. (DK-6714) under chromium and cadmium stress (150 & 250µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5)].

Under 150µg/mL chromium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2) and *B. bingmayongensis* (KH3) treated plants showed increase of 41, 23 and 8% respectively, in the number of leaves as compared to the control. Under 250µg/mL chromium stress, *P. songnensis* (GR3), *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. aeruginosa* (EH2) treated plants showed increase of 49, 23, 23 and 11% respectively, in the number of leaves as compared to control (figure 4). Under 150µg/mL chromium stress, *B. bingmayongensis* (KH3), *P. songnensis* (GR3), *P. aeruginosa* (EH2) and *B. tropicus* (LS5) treated plants showed increase of 38, 13, 11 and 11% in fresh weight respectively, as compared to the 150µg/mL chromium treated plants without bacterial inoculum. Under 250µg/mL chromium stress, *B. bingmayongensis* (KH3), *P. aeruginosa* (EH2), *B. tropicus* (LS5) and *P. songnensis* (GR3) treated plants showed increase of 56, 30, 30 and 25% respectively, in the fresh weight as compared to 250µg/mL chromium treated plants without bacterial inoculum (figure 4).

The maximum percentage of germination was expressed by *B. bingmayongensis* (KH3) i.e., 85% without cadmium stress. Whereas seeds treated with *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3), *P. songnensis* (GR3) and *B. tropicus* (LS5) under cadmium stress of 150µg/mL showed 70, 65, 62 and 58% increase in percentage germination respectively, as compared to control. Seeds treated with *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3), *P. songnensis* (GR3) and *B. tropicus* (LS5) under cadmium stress of 250µg/mL showed 65, 60, 54 and 50% increase in percentage germination respectively, as compared to control (figure 2). Under 150µg/mL cadmium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 23, 21, 21 and 8% in shoot length respectively, as compared to control. Under 250µg/mL cadmium stress, *P. songnensis* (GR3), *B. bingmayongensis* (KH3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 15, 14, 11 and 10% in shoot length, as compared to the control (figure 3).

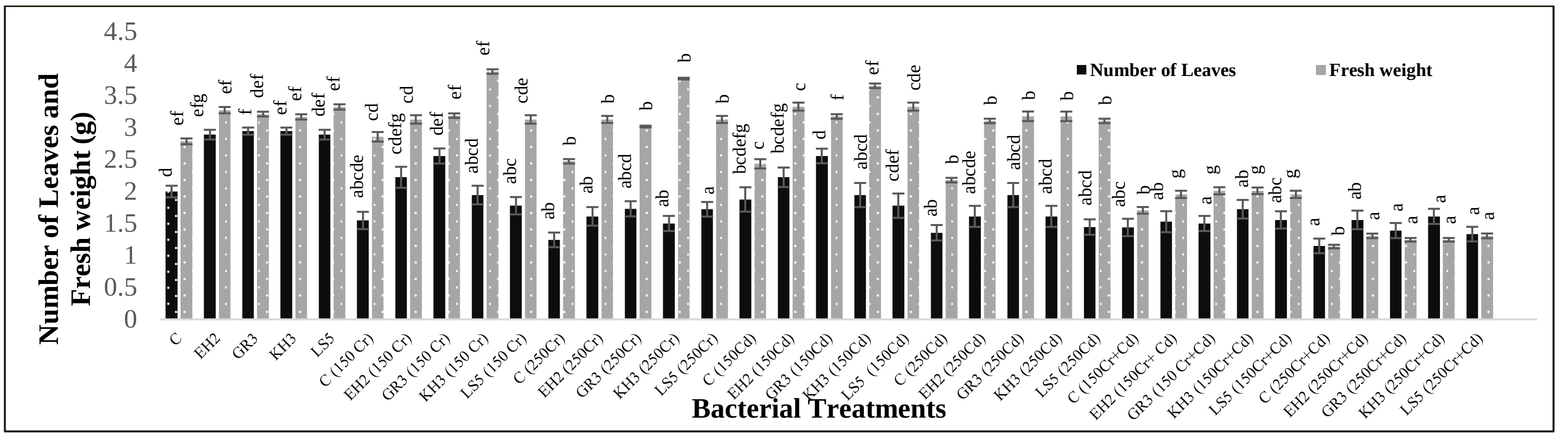

Figure 4: Effects of bacterial treatments on number of leaves and fresh weight of *Zea mays* L. (DK-6714) under chromium and cadmium stress (150 & 250μg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5)].

Under 150μg/mL cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 34, 29, 29 and 28% in root length respectively, as compared to control. Under 250μg/mL cadmium stress, *B. bingmayongensis* (KH3), *B. tropicus* (LS5), *P. aeruginosa* (EH2) and *P. songnensis* (GR3) treated plants showed increase of 41, 37, 36 and 31% respectively, in root length as compared to control (figure 3). Under 150μg/mL cadmium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 70, 48, 29 and 18% in number of leaves respectively, as compared to the control. Under 250μg/mL cadmium stress, *P. songnensis* (GR3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 15, 14 and 7% respectively, in the number of leaves as compared to the control while *B. bingmayongensis* (KH3) showed no significant increase in the number of leaves (figure 4). Under 150μg/mL cadmium stress, *B. bingmayongensis* (KH3), *B. tropicus* (LS5), *P. aeruginosa* (EH2) and *P. songnensis* (GR3) treated plants showed increase of 52, 38, 38 and 32% in fresh weight as compared to the control. Under 250μg/mL cadmium stress, *P. songnensis* (GR3), *B. bingmayongensis* (KH3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 51, 51, 47 and 47% respectively in fresh weight as compared to the control (figure 4).

Under 150μg/mL chromium and cadmium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 40, 21, 15 and 2% in percentage germination, as compared to the control. Under 250 μg/mL chromium and cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3) and *B. tropicus* (LS5) treated plants showed increase of 4, 2 and 1% respectively, in percentage germination as compared to the control while the bacterial isolate *B. bingmayongensis* (KH3) showed no significant increase in percentage germination (figure 2). In the presence of 150μg/mL chromium and cadmium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 41, 27, 15 and 6% respectively, in shoot length as compared to the control. Under 250μg/mL chromium and cadmium stress, *P. songnensis* (GR3) and *P. aeruginosa* (EH2) treated plants showed increase of 4 and 1% respectively, in shoot length as compared to the control, while the bacterial isolates *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) showed no significant increase in shoot length (figure 3). Under the stress of 150μg/mL chromium and cadmium, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 47, 32, 20 and 10% in root length respectively, as compared to the control. Under 250μg/mL chromium and cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. tropicus* (LS5) and *B. bingmayongensis* (KH3) treated plants showed increase of 29, 26, 13 and 7% in root length respectively, as compared to the control (figure 4).

Under 150μg/mL chromium and cadmium stress, *B. bingmayongensis* (KH3), *B. tropicus* (LS5), *P. songnensis* (GR3) and *B. tropicus* (LS5) treated plants showed an increase of 23, 11, 9 and 7% respectively, in the number of leaves as compared to the control. Under the stress of 250μg/mL chromium and cadmium, *B. bingmayongensis* (KH3), *P. aeruginosa* (EH2), *P. songnensis* (GR3) and *B. tropicus* (LS5) treated plants showed an increase of 46, 41, 26 and 21% respectively, in the number of leaves as compared to the control (figure 4). Under 150μg/mL chromium and cadmium stress, *B. bingmayongensis* (KH3), *P. songnensis* (GR3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 18, 18, 15 and 15% in the fresh weight respectively, as compared to control. Under 250μg/mL chromium and cadmium stress, *B. tropicus* (LS5), *P. aeruginosa* (EH2), *P. songnensis* (GR3) and *B. bingmayongensis* (KH3) treated plants showed increase of 18, 18, 14 and 13% in the fresh weight respectively, as compared to control (figure 4).

Under 150μg/mL chromium stress, *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) treated plants showed increase of 9, 7 and 2% respectively, in chlorophyll 'a' content as compared to the control while *P. aeruginosa* (EH2) treated plants showed no significant increase in chlorophyll 'a' content. Under 250μg/mL chromium stress, *B. tropicus* (LS5), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *P. aeruginosa* (EH2) treated plants showed increase of 16, 16, 4 and 3% respectively, in chlorophyll 'a' content as compared to the control (figure 5). Under 150μg/mL chromium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. tropicus* (LS5) and *B. bingmayongensis* (KH3) treated plants showed increase of 20, 14, 13 and 9% respectively, in chlorophyll 'b' content as compared to the control. Under 250μg/mL chromium stress, *B. tropicus* (LS5), *P. songnensis* (GR3), *P. aeruginosa* (EH2) and *B. bingmayongensis* (KH3) treated plants showed increase of 18, 14, 14 and 11% respectively in chlorophyll 'b' content as compared to the control (figure 5).

Under 150μg/mL chromium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. tropicus* (LS5) and *B. bingmayongensis* (KH3) treated plants showed an increase of 21, 15, 12 and 10% respectively, in total chlorophyll content as compared to the control. Under 250μg/mL chromium stress, *P. songnensis* (GR3), *B. bingmayongensis* (KH3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed an increase of 12, 10, 8 and 7% respectively, in total chlorophyll content as compared to the control (figure 5). Under 150μg/mL chromium stress, *P. songnensis* (GR3), *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 4, 3, 2 and 1% respectively in total soluble protein content as compared to control. Under 250μg/mL chromium stress, *P. aeruginosa* (EH2), *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) treated plants showed increase of 4, 3, 2 and 1% respectively in total soluble protein content as compared to the control (figure 6).

Under 150μg/mL cadmium stress, *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 2 and 2.3% in chlorophyll 'a' content as compared to the control while *P. songnensis* (GR3) and *B. bingmayongensis* (KH3) showed no significant increase in chlorophyll 'a' content. Under 250μg/mL cadmium stress, *B. tropicus* (LS5), *B. bingmayongensis* (KH3), *P. aeruginosa* (EH2) and *P. songnensis* (GR3) treated plants showed increase of 8, 5, 5 and 3 % in chlorophyll 'a' content respectively, as compared to the control (figure 5).

Under 150μg/mL cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. tropicus* (LS5) and *B. bingmayongensis* (KH3) treated plants showed increase of 11, 10, 3 and 2% in chlorophyll 'b' content as compared to the control. Under 250μg/mL cadmium stress, plants inoculated with *P. aeruginosa* (EH2), *B. tropicus* (LS5), *P. songnensis* (GR3) and *B. bingmayongensis* (KH3) treated plants showed increase of 33, 24, 14 and 13% respectively, in chlorophyll 'b' content as compared to the control (figure 5).

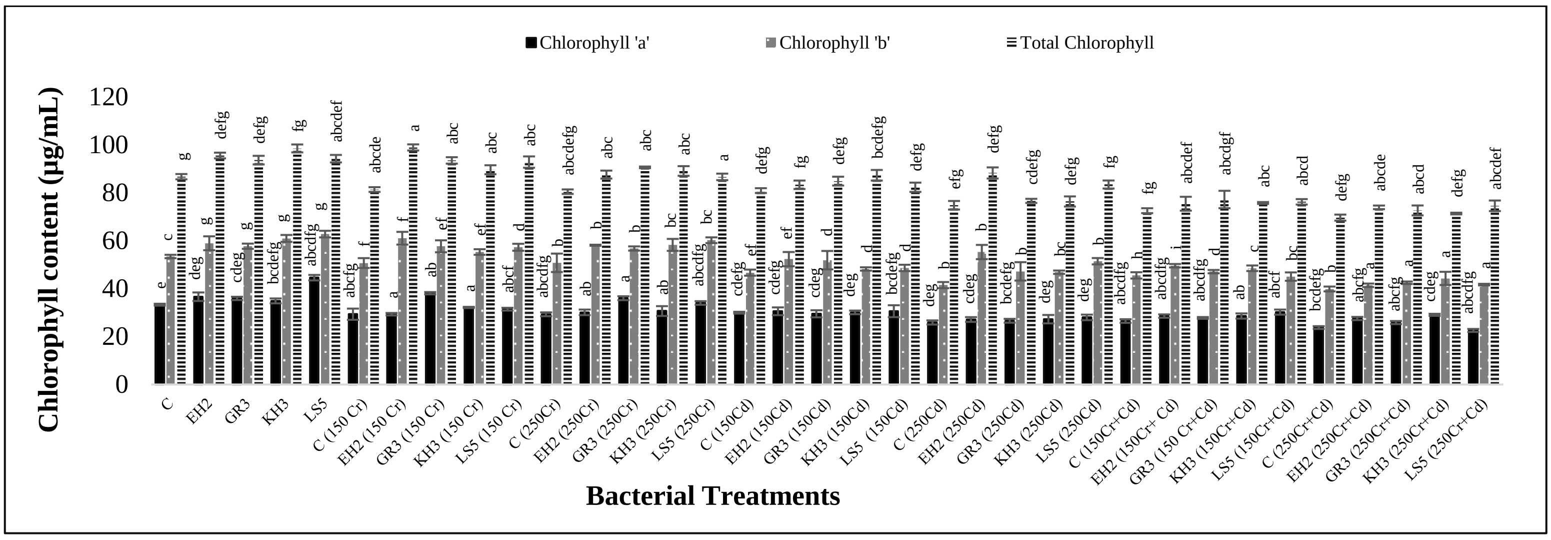

Figure 5: Effects of bacterial treatments on chlorophyll content (a, b and total) of *Zea mays* L. (DK-6714) under chromium and cadmium stress (150 & 250µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5)].

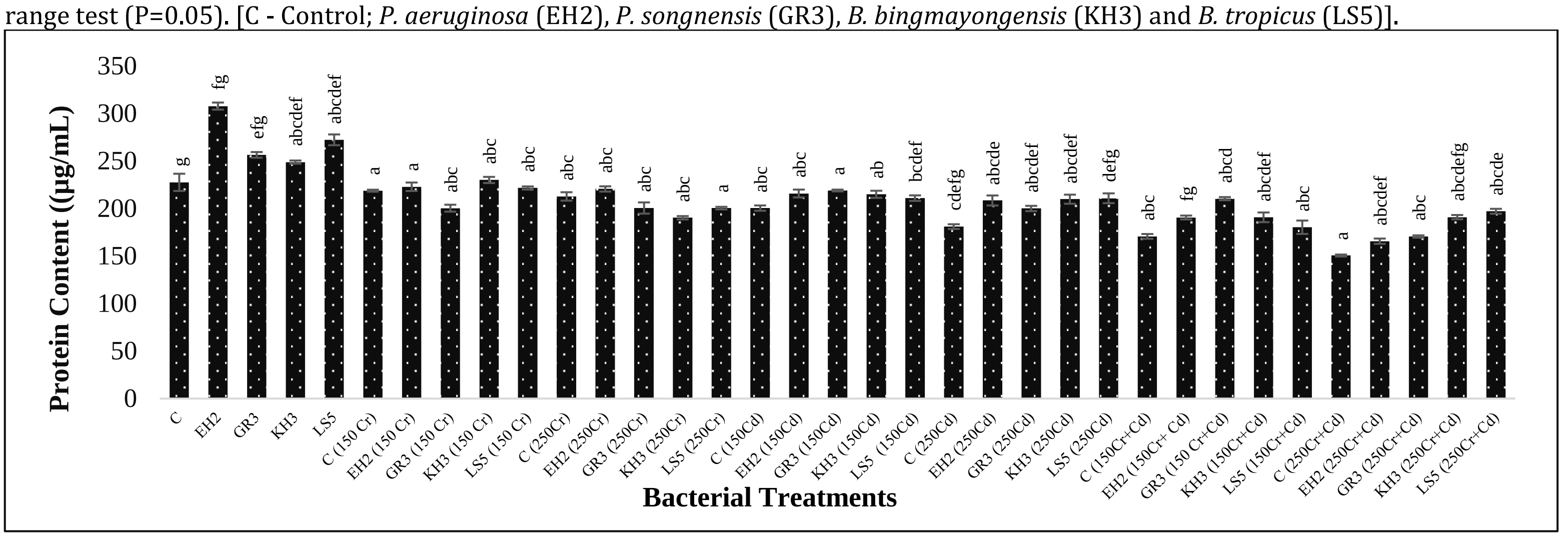

Figure 6: Effects of bacterial treatments on protein content of *Zea mays* L. (DK-6714) under chromium and cadmium stress (150 & 250µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5)].

Under 150µg/mL cadmium stress, *B. bingmayongensis* (KH3), *P. songnensis* (GR3), *P. aeruginosa* (EH2) and *B. tropicus* (LS5) treated plants showed an increase of 7, 4, 3 and 1% in total chlorophyll content respectively, as compared to control. Under 250µg/mL cadmium stress, *P. aeruginosa* (EH2), *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) treated plants showed an increase of 18, 11, 3 and 2% respectively, in total chlorophyll content as compared to the control (figure 5). Under 150µg/mL cadmium stress, *P. aeruginosa* (EH2), *B. tropicus* (LS5), *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) treated plants showed increase of 3, 2, 1 and 1% in total soluble protein content as compared to control. Under 250µg/mL cadmium stress, bacterial isolates *B. tropicus* (LS5), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *P. aeruginosa* (EH2) treated plants showed increase of 5, 4, 3 and 2% respectively in total soluble protein content as compared to control (figure 6).

Under 150µg/mL chromium and cadmium stress, *B. bingmayongensis* (KH3), *P. aeruginosa* (EH2), *B. tropicus* (LS5) and *P. songnensis* (GR3) treated plants showed increase of 8, 8, 6 and 5% respectively, in chlorophyll 'a' content as compared to the control. Under 250µg/mL chromium and cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 16, 9, 4 and 1 % respectively, in chlorophyll 'a' content as compared to the control (figure 5). Under 150µg/mL chromium and cadmium stress, *P. aeruginosa* (EH2), *B. bingmayongensis* (KH3) and *P. songnensis* (GR3) treated plants showed increase of 9, 6, and 3% respectively in chlorophyll 'b' content as compared to the control. *B. tropicus* (LS5) showed no significant increase in chlorophyll 'b 'content. Under 250µg/mL chromium and cadmium stress, *B. bingmayongensis* (KH3), *P. songnensis* (GR3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed increase of 12, 6, 5 and 4 % respectively in chlorophyll 'b' content as compared to control (figure 5).

Under 150µg/mL chromium and cadmium stress, *B. bingmayongensis* (KH3), *P. songnensis* (GR3), *B. tropicus* (LS5) and *P. aeruginosa* (EH2) treated plants showed an increase of 7, 6, 5 and 4% respectively, in total chlorophyll content as compared to control. Under 250µg/mL chromium and cadmium stress, *B. tropicus* (LS5), *P. aeruginosa* (EH2), *Pseudomonas songnensis* (GR3) and *B. bingmayongensis* (KH3) treated plants showed an increase of 7, 6, 4 and 2% respectively, in total chlorophyll content as compared to the control (figure 5). Under 150µg/mL chromium and cadmium stress, *B. tropicus* (LS5), *P. songnensis* (GR3), *P. aeruginosa* (EH2) and *B. bingmayongensis* (KH3) treated plants showed an increase of 7, 5, 4 and 3% respectively in total soluble protein content as compared to control. Under 250µg/mL chromium and cadmium stress, *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) treated plants showed increase of 5, 4, 1 and 1% respectively in total soluble protein content as compared to control (figure 6).

DISCUSSION: Heavy metal stress has gained much attention due to increased environmental pollution. The highest concentrations of heavy metals are found in soil due to which agricultural crops are badly affected (Sperdouli, 2022). The current purpose of the study is to propose a strategy for the bioremediation of chromium and cadmium-contaminated soil with the help of PGPR. *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) were selected PGPR. These bacterial isolates showed their maximum growth at 37°C after 24 hours of incubation in the presence of K_2CrO_4 and $CdCl_2$. It was observed that when seeds were grown in Cd and Cr contaminated soil, the growth attributes of corn were decreased. But the application of PGPR promoted plant growth by showing significant effect on growth and biochemical parameters. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in germination percentage of the inoculated plant was 80, 75 and 72% respectively, as shown by *P. aeruginosa* (EH2). Under 0, 150 and 250µg/mL cadmium stress, the maximum increment in germination percentage of inoculated plants was 70, 66 and 59% respectively, as shown by *P. songnensis* (GR3). It may be due to fact that PGPR produce gibberellins hormone which is involved in breaking seed dormancy. Abdel Latef *et al.* (2020) also noted the increment in germination percentage when seeds were inoculated with *Azobacter*. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in shoot length of inoculated plants was up to 70, 54 and 41% respectively, as shown by *P. songnensis* (GR3). In the presence and absence of cadmium stress, the shoot length of treated seeds was greater than untreated seeds. Under 0, 150 and 250µg/mL cadmium stress, the maximum increment in shoot length of inoculated plants was up to 70, 56 and 40% respectively, as shown by *B. bingmayongensis* (KH3). It might be due to the reason that PGPR solubilize nutrients such as phosphates and make them more available to plants. (Jamil *et al.*, 2022) also observed the same results. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in root length of inoculated plants was up to 70, 61 and 56% respectively, shown by *P. aeruginosa* (EH2). Under 0, 150 and 250µg/mL cadmium stress, the maximum increment in root length of inoculated plants was up to 101, 80 and 41% respectively, shown by *P. songnensis* (GR3). It might be due to the reason PGPR produce phytohormones such as auxins (e.g., indole-3-acetic acid, IAA), which directly stimulate root elongation and overall root development. Abd El-Mageed *et al.* (2022) also observed the increment in root length and number of leaves due to bacterial inoculation. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in fresh weight of inoculated plants was up to 196, 141 and 73% respectively, as shown by bacterial isolate *P. aeruginosa* (EH2). Under 0, 150 and 250µg/mL cadmium stress, the maximum increment in fresh weight of inoculated plants was up to 196, 120 and 79% respectively, as shown by bacterial isolate *P. songnensis* (GR3). It might be due to the combined effects of better nutrient acquisition, enhanced hormonal balance and improved root development (Habib *et al*., 2021). Jahangir *et al.* (2020) observed increment in fresh weight due to PGPR inoculation. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in chlorophyll content of inoculated plants was up to 56, 42 and 18% respectively, as shown by *P. songnensis* (GR3). In the presence of 150 µg/mL chromium and cadmium, *B. tropicus* (LS5) treated seeds showed maximum increase of 34% in chlorophyll content as compared to the control. It is due to fact that PGPR enhance the availability of essential nutrients like nitrogen, iron, and magnesium, which are crucial for chlorophyll production. Naqqash *et al.* (2022) reported increment in chlorophyll content when plants were treated with PGPR under chromium stress. Under 0, 150 and 250µg/mL chromium stress, the maximum increment in protein content of inoculated plants was up to 120, 82 and 60% respectively, as shown by *B. tropicus* (LS5). Under 0, 150 and 250µg/mL cadmium stress, the maximum increment in protein content of inoculated plants was up to 100, 75 and 60% respectively, as shown by *P. aeruginosa* (EH2). This increment may be due to phytohormones production. Abd El-Mageed *et al.* (2022) also observed the increment in biochemical parameters due to bacterial inoculation.

CONCLUSION: This study suggests the use of above-mentioned chromium and cadmium resistant bacterial strains i.e., *P. aeruginosa* (EH2), *P. songnensis* (GR3), *B. bingmayongensis* (KH3) and *B. tropicus* (LS5) as biofertilizer for increasing growth of maize (*Zea mays* L.) and reducing chromium and cadmium toxicity. It will help to reduce the usage of chemical fertilizers.

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