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# Rhizosphere Interactions of *Bacillus pumilus* Regulating Heavy Metal Transport and Detoxification in Cabbage

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## ABSTRACT

Increasing the number of heavy metals on land needs to be addressed through sustainable ways and various species of Bacillus can be used to mitigate heavy metals. The research work entitled "Effect of *Bacillus pumilus* strains on heavy metal accumulation in cabbage grown on contaminated soil" focuses on functional role of *Bacillus pumilus* strains inoculated with cabbage seed in mitigating heavy metal present in chromite mining soil. This experiment was conducted at ornamental horticulture nursery, the University of Agriculture Peshawar. In this experiment, factor A was three *Bacillus pumilus* strains (sequence C-2PMW-8, C-1 SSK-8 and C-1 PWK-7) while soil used for this experiment was collected from Prang Ghar mining site and cabbage were grown in three levels of chromite mining soil (2.27, 4.65 and 7.14 %).The experimental design used during this research was randomized complete block design with two factors and was replicated thrice. The collected data related to the effect of chromite mining soil and *Bacillus pumilus* strains indicated that these both factors have significant influence on growth parameters.

**Keywords:** *Bacillus Pumilus*, Heavy Metal, Cabbage, Phytoremediation

## INTRODUCTION

Cabbage production in Pakistan is merely 391 tons covering area of 367 hectares (MNFSR, 2020). In recent years, for the process of phytoremediation most of the researchers prefer heavy metal-immobilizing bacteria because they are excellent

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heavy metal passivators. Additionally, heavy metal immobilizing bacteria alter the state of existing heavy metal in soil and decrease the absorption of toxic metals as a result it improves the overall growth and quality of crops. About 62 strains of Bacillus indicated strong ability to immobilize Cd and Pb in soil with medium heavy metal pollution. Likewise, total 22 strains of bacteria in which Bacillus was main genus showed ability to immobilize both Cd and Pb in soils which have relatively less heavy metal pollution (Wang *et al*., 2020).

Plant growth promoting bacteria in soil produces plant hormones including auxins, gibberellins and cytokinins. Some of the examples of bacterial species include *Bacillus velezensis*, *B. subtilis*, *B. megaterium* and *B. licheniformis* which produce indole acetic acid. These growth promoting bacteria can sometimes obtain from metal contaminated site for instance one of the bacillus strains producing indole acetic acid Bacillus specie JH2-2 was isolated from heavy metal contaminated site. Saxena *et al*., (2019) reported that Bacillus species JH2-2 can stimulate growth of Indian mustard grown in chromium contaminated soil. *B. pumilus*, *B. licheniformis*, *B. cereus*, *B. macroides*, *B. velezensis* and *B. subtilis* produce gibberellin which is a plant growth hormone. Subsequently, production of gibberellin by some genus of Bacillus strains ultimately causes regulation in both plant growth and flowering in mustard. *Bacillus pumilus* is plant growth promoting bacteria and possesses characteristic stimulating plant growth because it can undergo both direct along with indirect stress tolerance mechanism. *B. pumilus* exhibits plant growth promoting characteristics for instance it has higher capability of Indole acetic production, aminocyclopropane-1-carboxylate deaminase positive activity, phosphate solubilization, and production of ammonia. Additionally, *B. pumilus* exhibits an influential role in stimulating the activity of protease and catalase (Hayat *et al*., 2020). Keeping in mind the heavy metals contamination from mining activities and other sources, it is essential to remediate or immobilize the heavy metals from contaminated soils. Therefore, this study was planned to know the effects of heavy metal contaminated soils on cabbage growth and survival along with this; current experiment was conducted to study remediation and immobilization of heavy metals using *Bacillus pumilus* strains.

## MATERIALS AND METHODS

### Experimental site

The experiment was conducted at ornamental horticulture nursery at Bahauddin Zakariya University Multan. In this research soil samples were collected from chromite mining area situated in Prang Ghar Mohmand Agency, Pakistan.

### Experimental design and factors

The experiment was conducted using randomized complete block design with two factors having 16 treatments combination and it was replicated thrice.

| **Factor A=*Bacillus pumilus* strains** | **Factor B=Mining soil** |
|---|---|
| $B_0$=No bacterial strain | $MS_0$=Garden soil |
| $B_1$=Sequence C-2PMW-8 | $MS_1$=Chromite soil (2.27%) |
| $B_2$=Sequence C-1 SSK-8 | $MS_2$=Chromite soil (4.65%) |
| $B_3$=Sequence C-1 PWK-7 | $MS_3$=Chromite soil (7.14%) |

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**Isolation of *Bacillus pumilus* strains**

The bacteria used in this study were from genus bacillus and have antagonistic properties towards heavy metals. The *Bacillus pumilus* strains having sequence isolate of C-2PMW-8, C-1 SSK-8 and C-1PWK-7 used in this study were taken from IBGE bioinformatics laboratory based on its accession number. For the preparation of inoculum, the nutrient agar (NA) media was prepared.

**Inoculation of strain in culture media**

The isolated strains were inoculated in NA media and incubated in shaker incubator at 150 rpm for 48–72 h. After that, the culture was centrifuged for 10 min at 3000 rpm. Afterwards, nine plates of pure culture were taken to determine the optical density (OD) of each strain. For this purpose, Eppendorf tube was filled with distil water (1.8ml). Then, earbuds were used to streak the bacterial growth from plates and these buds were dipped in distil water afterwards, further transfer this into cuvette. This cuvette was then placed in spectrometer and OD calculated varied between 0.15-0.8 and bacterial strain having OD of 0.100 at 660nm and bacterial density of $10^6$cells $ml^{-1}$ was selected for preparing inoculum on NA media (Shahzad *et al*., 2021).

**Seed inoculation**

Cabbage seeds were taken and to achieve surface sterilization seeds were washed with ethanol (95% constituted of 90ml ethanol and 5ml distil water), followed by soaking in 10% Chlorox for 2–3 minutes and subsequently the seeds were washed successively 2–3 times with autoclaved distilled water. After surface sterilization seeds were dipped in *B. pumilus* inoculum for 2-3 hours.

**Soil bag preparation**

To grow cabbage seven seeds were sown in each bag. Cabbage was grown in 48 bags (4.5 kg per bag) containing well-mixed non-contaminated garden soil with contaminated soil having three different concentrations of chromite collected from chromite mine except in control.

## STATISTICAL ANALYSIS

To verify the significant difference in various parameters data were analyzed and their analysis of variance was computed through statistical program statistix-8.1. Least significant difference test was carried out when needed and reported values were considered significantly different at $P \leq 0.01$ and 0.05(Jan *et al*., 2009).

## RESULTS AND DISCUSSION

**Days to Seed Germination**

The number of days required for cabbage seed germination varied between 12 and 27 days depending on chromite concentration and bacterial inoculation. In general, seeds inoculated with *Bacillus pumilus* strains germinated earlier compared with non-inoculated seeds. The shortest germination time (15.3 days) was recorded when seeds inoculated with C-2 PMW-8 or C-1 SSK-8 were grown in soil containing 2.27% chromite. In contrast, the longest germination period (27 days) occurred in non-inoculated seeds grown in soil containing 7.14% chromite, indicating the inhibitory

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effect of heavy metal stress on seed germination. The results demonstrate that *Bacillus pumilus* strains can mitigate the toxic effects of chromite and promote earlier germination. Plant growth promoting rhizobacteria (PGPR) enhance germination by producing phytohormones and improving nutrient availability. Similar findings were reported by Shahzad et al. (2021), who observed improved germination of wheat seeds inoculated with *Bacillus pumilus* under cadmium stress.

**Table 1. Effect of Bacillus strains and chromite concentration on days to germination**

| Treatment | Chromite (%) | Bacillus strain | Days to germination |
|---|---|---|---|
| **Garden soil** | 0 | Non-inoculated | 16.8 |
| **Garden soil** | 0 | C-1 SSK-8 | 12.0 |
| **Garden soil** | 0 | C-2 PMW-8 | 13.4 |
| **Chromite soil** | 2.27 | Non-inoculated | 18.7 |
| **Chromite soil** | 2.27 | C-1 SSK-8 | 15.3 |
| **Chromite soil** | 2.27 | C-2 PMW-8 | 15.3 |
| **Chromite soil** | 7.14 | Non-inoculated | 27.0 |

**Germination Percentage**

Germination percentage ranged between 46.6% and 100%. The lowest germination (46.6%) occurred in non-inoculated seeds grown in soil containing 7.14% chromite, whereas the highest germination (100%) was observed in seeds inoculated with strain C-1 SSK-8 grown in uncontaminated garden soil. Bacterial inoculation significantly improved germination even under contaminated conditions. When seeds were inoculated with C-1 SSK-8, C-2 PMW-8, and C-1 PWK-7, germination increased to 53.3%, 55.3%, and 55.6%, respectively, under high chromite stress. This represents approximately 19% improvement compared with the non-inoculated control. These findings agree with previous studies reporting that *Bacillus pumilus* enhances seed germination under heavy metal stress by producing plant growth regulators and improving nutrient uptake.

**Table 2. Germination percentage under different treatments**

| Treatment | Germination (%) |
|---|---|
| **Garden soil (control)** | 72.3 |
| **Garden soil + C-1 SSK-8** | 100 |
| **Garden soil + C-2 PMW-8** | 95.3 |
| **Garden soil + C-1 PWK-7** | 91.0 |
| **7.14% chromite (control)** | 46.6 |
| **7.14% chromite + C-1 SSK-8** | 53.3 |
| **7.14% chromite + C-2 PMW-8** | 55.3 |
| **7.14% chromite + C-1 PWK-7** | 55.6 |

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## Growth Parameters of Cabbage

### Plant Height

Plant height ranged between 11.7 cm and 19.2 cm. The maximum plant height (19.2 cm) was recorded in plants grown in uncontaminated garden soil, whereas the minimum (11.7 cm) occurred in non-inoculated plants grown under chromite stress. Inoculation with C-2 PMW-8 significantly increased plant height (17.8 cm) compared with the control. Heavy metal stress reduced plant growth, which is consistent with the findings of Habiba et al. (2019), who reported a decrease in wheat plant height under chromium stress.

**Table 3. Effect of treatments on plant growth parameters**

| Treatment | Plant height (cm) | Fresh leaf weight (g) | Dry leaf weight (g) |
|---|---|---|---|
| **Control (non-inoculated)** | 11.7 | 12.2 | 4.0 |
| **C-1 PWK-7** | 16.9 | 15.0 | 5.0 |
| **C-2 PMW-8** | 17.8 | 14.9 | 4.9 |
| **C-1 SSK-8** | 17.2 | 14.7 | 5.0 |
| **Garden soil** | 19.2 | 17.3 | 6.5 |
| **7.14% chromite soil** | 13.0 | 10.4 | 2.7 |

### Leaf Growth Characteristics

Leaf development was significantly affected by both chromite contamination and bacterial inoculation. The number of leaves ranged between 5.3 and 17.3 leaves per plant, whereas the single leaf area ranged from 29.6 to 50.6 $cm^2$. Inoculation with C-1 SSK-8 produced the highest number of leaves (13.9 leaves $plant^{-1}$) and larger leaf area (45 $cm^2$). Conversely, non-inoculated plants exhibited reduced leaf number and leaf size.

**Table 4. Leaf growth characteristics of cabbage**

| Treatment | Leaves $plant^{-1}$ | Leaf area ($cm^2$) | Chlorophyll (SPAD) |
|---|---|---|---|
| **Control** | 10.2 | 37.3 | 24.9 |
| **C-1 SSK-8** | 13.9 | 45.0 | 27.4 |
| **C-2 PMW-8** | 13.7 | 45.0 | 28.5 |
| **C-1 PWK-7** | 13.5 | 45.0 | 27.8 |
| **Garden soil** | 9.7 | 49.0 | 36.2 |
| **7.14% chromite soil** | 16.0 | 37.7 | 19.9 |

Heavy metal stress significantly reduced chlorophyll content and photosynthetic activity. Singh et al. (2016) reported that heavy metals disrupt physiological and biochemical processes, leading to decreased chlorophyll synthesis.

### Root Length and Survival Percentage

Root length varied between 3.3 cm and 8.1 cm, while plant survival ranged from 60.6% to 91.3%. The highest survival (91.3%) was observed in inoculated plants

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grown in garden soil, whereas the lowest survival (60.6%) occurred in non-inoculated plants grown in highly contaminated soil.

**Table 5. Root length and survival percentage**

| Treatment | Root length (cm) | Survival (%) |
|---|---|---|
| **Control** | 4.5 | 68.4 |
| **C-1 PWK-7** | 5.6 | 82.3 |
| **C-2 PMW-8** | 5.6 | 84.1 |
| **C-1 SSK-8** | 5.4 | 83.7 |
| **Garden soil** | 7.6 | 91.3 |
| **7.14% chromite soil** | 3.9 | 60.6 |

The increased survival rate in inoculated plants indicates that *Bacillus pumilus* reduces heavy metal toxicity through immobilization, detoxification, or metal sequestration mechanisms (Tiwari and Lata, 2018).

**Heavy Metal Concentration in Plants**

Chromium, cadmium, manganese, and lead concentrations were significantly influenced by chromite contamination and bacterial inoculation.

**Table 6. Heavy metal concentration in cabbage plants (mg kg$^{-1}$)**

| Treatment | Cr | Cd | Mn | Pb |
|---|---|---|---|---|
| **Garden soil** | 0 | 0 | 0 | 0 |
| **2.27% chromite + Bacillus** | 1.4 | 1.1 | 4.6 | 0.4 |
| **4.65% chromite + Bacillus** | 3.8 | 2.0 | 5.7 | 0.9 |
| **7.14% chromite + Bacillus** | 5.2 | 2.6 | 7.5 | 1.3 |
| **7.14% chromite control** | 6.3 | 3.2 | 10.4 | 1.7 |

The highest chromium (6.3 mg kg$^{-1}$), cadmium (3.2 mg kg$^{-1}$), manganese (10.4 mg kg$^{-1}$), and lead (1.7 mg kg$^{-1}$) concentrations were observed in non-inoculated plants grown in highly contaminated soil. These values exceed the permissible limits recommended by WHO, indicating potential health risks. Inoculation with *Bacillus pumilus* significantly reduced heavy metal accumulation in plant tissues. This reduction may be attributed to bacterial biosorption, enzymatic transformation, and immobilization mechanisms.

**Heavy Metal Concentration in Soil**

**Table 7. Heavy metal concentration in soil (mg kg$^{-1}$)**

| Treatment | Cr | Cd | Mn | Pb |
|---|---|---|---|---|
| **Garden soil** | 0 | 0 | 0 | 0 |
| **2.27% chromite + Bacillus** | 1.5 | 0.1 | 8.9 | 4.3 |
| **4.65% chromite + Bacillus** | 3.2 | 0.4 | 13.9 | 9.8 |
| **7.14% chromite + Bacillus** | 4.5 | 0.6 | 18.5 | 13.2 |
| **7.14% chromite control** | 4.8 | 0.9 | 20.3 | 16.1 |

Bacterial inoculation significantly reduced heavy metal concentration in soil compared with non-inoculated treatments. Previous studies have reported that Bacillus species possess metal-binding proteins and organic acid production

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capabilities, which facilitate metal immobilization and detoxification (Ali et al., 2021).

## CONCLUSIONS

Chromite mine soil contains cadmium (2.3mgkg$^{-1}$), chromium (14mgkg$^{-1}$), lead (7.2mgkg$^{-1}$) and manganese (40mgkg$^{-1}$). Chromite mining soil when used for growing cabbage significantly inhibited the growth and survival of cabbage. Cabbage when inoculated with *Bacillus pumilus* strains including C-2PMW-8, C-1 SSK-8 and C-1PWK-7 sequence enhances the growth and survival of cabbage. Cabbage when inoculated with *Bacillus pumilus* strains including C-2PMW-8, C-1 SSK-8 and C-1PWK-7 sequence has higher potential towards heavy metal alleviation.

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