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Effect Of Bacillus Pumilus Strains On Heavy Metal Accumulation In Cabbage Grown On Contaminated Soil

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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.

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 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 Mutan. 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%)

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 10^6cells ml^{-1} was selected for preparing inoculum on NA media (Shahzad *et al.*, 2021).

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FIGURE 3. 1. (A) INDICATES THE BACTERIAL BROTH OF THREE SEQUENCES OBTAINED FROM IBGE. (B) INDICATES THE POURING OF NA MEDIA INTO PLATES IN LAMINAR FLOW HOOD TO AVOID ANY CONTAMINATION. (C) INDICATES THE STREAKING OF BACTERIA ONTO PREPARED NA PLATES FOR BACTERIAL GROWTH.

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

Data regarding growth parameters and heavy metals were recorded, subjected to analysis of variance (ANOVA) and compared with previous work done by other researchers on the effect of Bacillus strains on both mitigating the heavy metals and growth parameters.

DAYS TO GERMINATION OF SEEDS

The data related to days to germination varied between 12 to 27 days. All the Bacillus strains used during this study promoted growth and took lesser days to germinate especially when the chromite percentage was 2.27 and when the inoculated seeds were grown on garden soil (Figure 1). Likewise, it was noted that days to germination was lesser when the inoculated seeds were grown in soil with no heavy metal. Furthermore, when inoculated seeds either with C-2PMW or C-1 SSK-8 and grown in contaminated soil in which chromite percentage was 2.27 it took minimum days i.e. 15.3 days. However, maximum days to seed germination were 27 days recorded for cabbage grown in soil with 7.14% of chromite and these seeds were not inoculated with any bacterial strain.

It was observed that seeds inoculated with Bacillus strains which have plant growth promoting properties exhibited to mitigate the deleterious effect of metal toxicity. On the contrary, when the seeds were inoculated with bacterial strains and grown in contaminated soil (2.27%) resulted in timely germination. It could be concluded that days to germination required by cabbage seeds regardless of *Bacillus pumilus* strain result in decrease in days to germination when the chromite percentage is less.

GERMINATION PERCENTAGE

The germination percentage is significantly different when grown on various chromite mining soil while is non-significant for *Bacillus pumilus* strains. The interaction between mining soil and bacterial strain was found to be significant. The data demonstrated that germination percentage recorded in this study varied from 46.6%- 100%. Also, the data illustrated that treatment in which non-inoculated seed was grown on soil having highest chromite percentage resulted in lowest seed germination percentage (46.6%) however, germination percentage significantly increased by up to 19% when the inoculated seeds were grown on the same soil, when seed was inoculation with sequence C-1 SSK-8 (53.3%), C-2 PMW-8 (55.3%) and sequence C-1 PWK-7 (55.6%) (figure 2). Maximum germination percentage was recorded in seed inoculated with sequence C-1 SSK-8 (100%) followed by seeds inoculated with sequence C-2 PMW-8 (95.3%) and sequence C-1 PWK-7(91.0%) grown on garden soil having no chromite mining soil. A significant increase of about 39.7% inoculated seed grown on garden soil was recorded as compared with control (non-inoculated seeds and garden soil).

Shahzad *et al.*, (2021) reported that when wheat seeds were inoculated with *Bacillus pumilus* were sown in soil with Cd sulphate ($CdSO_4$) it significantly induced the seed germination. However, when the seeds which were non-inoculated and were grown in a soil with concentration of $CdSO_4$ of about 0.75 $mgkg^{-1}$, 39% decline in germination percentage was recorded.

PLANT HEIGHT (CM)

The ANOVA illustrated that plant height is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. However, the interaction between mining soil and bacterial strains was found to be non-significant. The data demonstrated that plant height recorded in this study varied between 11.7- 19.2 cm. It was noted that plant height was less (11.7cm) when the non- inoculated seeds were used however, it was significantly higher in plants grown from inoculated seeds such as 17.8cm in plants inoculated with *Bacillus pumilus* sequence C-2 PMW-8. It was 13.0 cm when the seeds were grown on chromite mining soil with the highest chromite percentage (7.14%) while the plant height (19.2 cm) was comparatively higher when grown on garden soil with no chromite contamination. Two wheat varieties were exposed to Cr stress, and it was reported that wheat plant retain maximum height in control where the plant was not exposed to Cr stress and with increasing Cr concentration considerable decline in plant height was recorded (Habiba *et al.*, 2019).

FRESH LEAF WEIGHT (G)

The ANOVA illustrated that fresh leaf weight for cabbage is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. However, the interaction between chromite mining soil and bacterial strains was found to be non-significant. The collected data illustrated that fresh leaf weight (g) recorded for cabbage ranged from 9.3-18.1 g. Maximum fresh leaf weight (17.3 g) recorded was in cabbage grown on garden soil with no chromite mining soil and minimum fresh leaf weight (10.4 g) was recorded in cabbage grown on soil with highest chromite concentration (7.14%). Also, non-inoculated cabbage resulted in lower fresh leaf weight (12.2 g) however; the inoculated cabbage treated with sequence C-1 PWK-7 had highest fresh leaf weight (15 g) which is at per with sequence C-2 PMW-8 (14.9 g) and sequence C-1 SSK-8 (14.7 g). Thus, cabbage if grown on garden soil and treated with *Bacillus pumilus* strains results in maximum fresh leaf weight however, if exposed to chromite mining soil will decline the fresh leaf weight. Shahzad *et al.*, (2021) reported that when wheat seeds grown in garden soil with no heavy metal were inoculated with *Bacillus pumilus* significantly induced plant fresh weight by 34% as compared to the control (non-inoculated seeds and no Cd).

WEIGHT OF DRY LEAF (G)

The ANOVA illustrated that dry leaf weight for cabbage is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. However, the interaction between mining soil and bacterial strains was found to be non-significant. The collected data illustrated that dry leaf weight (g) recorded for cabbage ranged from 2.6-7.1 g. Maximum dry leaf weight (6.5 g) was recorded in cabbage grown on garden soil with no chromite mining soil and minimum fresh leaf weight (2.7 g) was recorded in cabbage grown on soil with highest chromite concentration (7.14%). Additionally, non-inoculated cabbage resulted in lower dry leaf weight (4.0 g) however; the inoculated cabbage treated with sequence C-1 SSK-8 and C-1 PWK-7 had highest dry leaf weight (5.0 g). Hayat *et al.*, (2020) studied the role of *Bacillus pumilus*, plant microbe chelator and ethylene diamine tetraacetic acid (EDTA) in mitigation effect of heavy metal (Cd) in maize. Therefore, maize plants were grown under three soil types such as non-contaminated soil, aged-industrially contaminated soil and Cd spiked soil with 300 mg kg^{-1} Cd. Furthermore, the experiment consists of non-inoculated and untreated control, plant inoculated with *Bacillus pumilus*, plants treated with *Bacillus pumilus*, EDTA treatment and 5 Mm EDTA. Subsequently, the highest dry weight was recorded in plants with both *Bacillus pumilus* and EDTA treatment grown in non-contaminated soil. However, a significant decline in maize dry weight was recorded in plants which were non-inoculated and have no EDTA treatment.

NUMBER OF LEAVES PLANT ^{1}

The ANOVA illustrates that the effect of both mining soil and *Bacillus pumilus* on number of leaves $plant^{-1}$ in cabbage significantly differs from each other. However, the interaction between mining soil and *Bacillus pumilus* strains was found non-significant. Also, the effect of *Bacillus pumilus* strains and chromite soil on mean value and F-value is given in table 1. The collected data illustrated that the number of leaves $plant^{-1}$ recorded for cabbage ranged from5.3-17.3. The highest number of leaves $plant^{-1}$ (16.0) were recorded in soil with highest chromite percentage (7.14%) and lowest number of leaves $plant^{-1}$ were recorded (9.7) in garden soil. On the contrary, lowest number of leaves $plant^{-1}$ recorded were in plants which were non-inoculated; however, cabbage inoculated with C-1 SSK-8 sequence had the highest number of leaves per plant (13.9), which was at par with sequence C-2 PMW-8 (13.7) and sequence C-1 PWK-7 (13.5). Chetan and Ami. 2016 were performed an experiment on leafy vegetables (spinach and amaranthus) and were treated with two dozes (50 and 250 ppm) of $CdCl_2$ and $CuCl_2$ and concluded that both heavy metals when applied in higher quantity have effect on plant physiology.

SINGLE LEAF AREA (CM^2)

The ANOVA illustrated that single leaf area of cabbage is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. However, the interaction between mining soil and bacterial strains was found to be non-significant. The collected data illustrated that single leaf area (cm^2) recorded for cabbage ranged from 29.6-50.6 cm^2. Maximum single leaf area 49.0 cm^2 was recorded in cabbage grown on garden soil with no chromite mining soil and minimum single leaf area was recorded (37.7cm^2) in cabbage grown on soil with highest chromite concentration (7.14%). Additionally, non-inoculated cabbage resulted in lower leaf area (37.3 cm^2) however; the inoculated cabbage treated with sequence C-1 SSK-8, C-1 PWK-7 and C-2 PMW-8had highest single leaf area (45cm^2). Khan *et al.*, (2016) reported that all the abiotic stresses directly cause the production of reactive oxygen species (ROS) which ultimately cause damage to plant membrane or in severe condition can lead towards plant cell death. To deal with the increasing ROS rice seedling were inoculated with *Bacillus pumilus*, which mitigated the deleterious effects of abiotic stress by increasing the nutrient availability to plant, proper proliferation of root system; increase the water absorption, which increased the leaf surface area.

CHLOROPHYLL CONTENT (SPAD)

The ANOVA illustrated that chlorophyll content for cabbage leaf is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. However, the interaction between mining soil and bacterial strains was found to be non-significant. The collected data related to chlorophyll content is between 18.8- 36.8 SPAD. Here, minimum chlorophyll content (24.9 SPAD) was recorded in non-inoculated cabbage. But when plants were inoculated with sequence C-2 PMW-8 chlorophyll content recorded was 28.5SPAD which is a noticeable increase in chlorophyll content. It was significantly at par with sequence C-1 SSK-8 927.4 SPAD) and sequence C-1 PWK-7 (27.8 SPAD). On the contrary, the highest chlorophyll content (36.2 SPAD) was recorded in cabbage grown in soil with no chromite. However, a rapid decline in chlorophyll content was noted when the plants were grown on soil with 7.14% of chromite mining soil and had the lowest chlorophyll content (19.9 SPAD). Singh *et al.*, (2016) observed that if a plant accumulates heavy metal, then the stress will severely affect the plant on both the molecular and cellular level which will ultimately cause deleterious impact on physiological processes and biochemical processes. As a result, imbalance in biochemical and physiological processes in plants leads towards significant plummets in photosynthetic and pigment synthesis in plants. For instance, tomato seedlings when exposed to heavy metal stress experienced a decline in photosynthetic rate and chlorophyll content. When Brazilian waterweed was grown in soil contaminated with heavy metals it caused significant decline in chlorophyll content and photochemical efficacy of photosystem.

ROOT LENGTH (CM)

Root length (cm) of cabbage was significantly different when treated with mine soil and for *Bacillus pumilus* strain. However, the interaction between mining soil and bacterial strains was found non-significant. The related data of root length illustrated that it ranges between 3.3-8.1cm. Maximum root length (7.6 cm) was obtained in cabbage grown on garden soil with no chromite mining soil, however, minimum root length (3.9 cm) was recorded in cabbage grown on chromite mining soil with highest chromite percentage (7.14%). Similarly, maximum root length (5.6 cm) was obtained in cabbage inoculated with C-2 PMW-8 and C-1 PWK-7. However, minimum root length (4.5 cm) was recorded in non-inoculated cabbage.

Ameliorative character of plant growth promoting bacteria have been used in past in many plant to alleviate the plant inhibitors by synthesizing phytohormones for instance auxin and cytokinin are made available to plant which subsequently surge the plant growth. Moreover, plant growth promoting rhizobacteria improve growth in plants by producing indole acetic acid, which subsequently increase the surface area of root and root length leading to increase of higher nutrient uptake (Abeer *et al.*, 2015)

SURVIVAL PERCENTAGE

The ANOVA illustrated that survival percentage for cabbage is significantly different when grown on chromite mining soil and treated with *Bacillus pumilus* strains. Also, the interaction between mining soil and bacterial strains was found to be significant. The data demonstrated that survival percentage recorded in this study varied from 60.6-91.3 %. Also, the data illustrated that treatment in which non-inoculated cabbage was grown on soil with highest chromite percentage (7.14%) resulted in lowest survival percentage (60.6) however, maximum survival percentage (91.3%) was recorded when inoculated cabbage were grown on garden soil. It is evident through previous research work done on the effect of heavy metal on plant growth that this stress due to heavy metal can impart decline in survival of plant. Thus, to increase survival in such adverse conditions conventional breeding and transgenic technologies are introduced, however, they require more time and labor. Therefore, bioremediation is frequently used to increase survival percentage in plants grown on metalliferous environment. Plant growth regulating bacteria decreases the accumulation of metal either by immobilizing (by binding the heavy metal to outer parts of cell) or detoxifying metals and as a result surge up the survival of plants (Tiwari and Lata., 2018).

CONCENTRATION OF CHROMIUM ($MGKG^{-1}$) IN CABBAGE PLANT

The effect of *Bacillus pumilus* stains and chromite mining soil on concentration of chromium in cabbage plant and soil on both mean value and F-value is given in table 2. The ANOVA represented that mining soil, *Bacillus pumilus* and their interaction were significantly different from each other. The data demonstrated that concentration of chromium (Cr) recorded in this study in cabbage plants varied between 0-6.3 mg kg^{-1}. Minimum Cr concentration recorded was 0 mg kg^{-1} in plants which were grown in soils having no chromite soil and either treated or non-treated with bacterial strains. The data further illustrated that treatment in which non-inoculated seeds were grown on soil having highest chromite percentage (7.14%) resulted in highest Cr concentration, which is around (6.3 mg kg^{-1}) which is above permissible limit of Cr in plants (2.3 mg kg^{-1}) thus if this cabbage plant is consumed may cause lethal and deleterious effects in human. The initial Cr concentration of chromite mining soil used is 20 mg kg^{-1} (table 3.2). Moreover, Cr concentration significantly plummeted when the inoculated seeds were grown in soil with low chromite concentration (2.27%) for instance seed inoculation with sequence C-1 SSK-8 (1.4 mg kg^{-1}), C-2 PMW-8 (1.4 mg kg^{-1}) and sequence C-1 PWK-7 (1.4 mg kg^{-1}) also, it is lower than the permissible Cr concentration in plant prescribed by WHO (table 3.1) and can be consumed by human. Furthermore, Cr concentration was significantly induced when the non-inoculated and inoculated seeds were used in soil with chromite percentage of around 7.14% as compared to the control (figure 4).

It was reported that Cr negatively affects microorganism however, certain Cr toxicity resistant bacteria such as Bacillus sp. can be utilized to plunge its effects. Bacillus and Pseudomonas sp. have the capability to decrease the Cr concentration either by utilizing it or by secreting enzyme which can solubilize this metal (Upadhyay *et al.*, 2017).

CONCENTRATION OF CHROMIUM IN SOIL ($MG\ KG^{-1}$)

The given data related to ANOVA represented that mining soil, *Bacillus pumilus* strains and their interaction are significantly different. The data recorded for concentration of chromium (Cr) in soil indicated that the recorded values were between 0-4.8 mg kg^{-1}. The data depicted that treatment in which non-inoculated seed was grown on soil having highest chromite percentage (7.14%) resulted in highest Cr concentration which is around 4.8 mg kg^{-1}(figure 5).

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On the contrary, minimum Cr concentration (0 mg kg^{-1}) recorded for soil with no chromite treatment and when seeds were either inoculated or not inoculated with bacterial strains followed by the soil with low chromite concentration (2.27%) for instance seed inoculation with sequence C-1 SSK-8 (1.5 mg kg^{-1}), C-2 PMW-8 (1.4 mg kg^{-1}) and sequence C-1 PWK-7 (1.5 mg kg^{-1}) also it is lower than the permissible Cr concentration in soil (8 mg kg^{-1}) prescribed by WHO (table 3.1) .

CONCENTRATION OF CADMIUM (MG KG^{-1}) IN CABBAGE PLANT

The ANOVA of given data depicts that mining soil, *Bacillus pumilus* strains and their interaction are significantly different. The recorded data for concentration of cadmium (Cd) accumulated in cabbage plant lies in between 0-3.2 mg kg^{-1}. Highest Cd concentration (3.2 mg kg^{-1}) accumulation were in plants which were non-inoculated and grown on soil with highest chromite percentage (7.14%) and thus the Cd concentration in these cabbage plants was above the permissible Cd concentration limit (3 mg kg^{-1}) set by World Health Organization (WHO). However, minimum Cd concentration (0 mg kg^{-1}) accumulated was in cabbage grown on garden soil when seeds were either inoculated or non-inoculated with bacterial strains. Initial concentration of Cd in chromite mining soil before inoculating it with bacterial strain was 2.3 mg kg^{-1}(table 3.2). They were followed by 1.1 mg kg^{-1} which is same for all cabbage grown on soil with 2.27% chromite mining soil regardless of *Bacillus pumilus* sequence used (figure 6). For instance, Cd accumulation was 1.1 mg kg^{-1} for cabbage plants inoculated with C-2 PMW-8, C-1 SSK-8 and C-1 PWK-7. However, when the chromite percentage was increased the Cd accumulation also surge up and it was seen that Cd concentration for cabbage grown on 7.14% chromite soil was 2.6 mg kg^{-1} for cabbage inoculated with C-2 PMW-8, 2.8 mg kg^{-1} for sequence C-1 SSK-8 and C-1 SSK-8, respectively which is approximately close to permissible Cd concentration set by WHO (3.0 mg kg^{-1}). Hayat *et al.*, (2020) showed that inoculating *Bacillus pumilus* and Ethylenediamine tetraacetic acid in corn not only have ameliorative effect on growth parameters but it also alleviates the phytotoxic effect of Cd.

CADMIUM CONCENTRATION IN SOIL (MG KG^{-1})

The data recorded for concentration of cadmium (Cd) stored in soil indicated that the recorded values ranged in between 0-0.9 mg kg^{-1}. The data depicted that treatment in which non-inoculated seed was grown on soil having highest chromite percentage (7.14%) resulted in highest Cd concentration which is around 0.9 mg kg^{-1} which is above the permissible Cd concentration in soil (0.3 mg kg^{-1}) as set by WHO/ FAO 2011 (table 3.1). On the contrary, minimum Cd concentration (0 mg kg^{-1}) was recorded in soil with no chromite treatment and when seeds were either inoculated or not inoculated with bacterial strains (table 7). They are followed by the soil with low chromite concentration (2.27%) for instance seed inoculation with sequence C-1 SSK-8 (0.1 mg kg^{-1}), C-2 PMW-8 (0.1 mg kg^{-1}) and sequence C-1 PWK-7 (0.1 mg kg^{-1}) also it is lower than the permissible Cd concentration in soil (0.3mg kg^{-1}) prescribed by FAO also mentioned in table 3.1. Ali *et al.*, (2021) delineate that Bacillus sp. are being used to reduce Cd because it has capability to mitigate the Cd content in soil by increasing heavy metal solubility either by organic acid production or by producing hormones which stimulate the growth.

MANGANESE CONCENTRATION (MG KG^{-1}) IN CABBAGE PLANT

The concentration of manganese (Mn) accumulated by cabbage ranged from 0-10.4 mg kg^{-1}. Moreover, the table indicates that the maximum recorded data (10.4 mg kg^{-1}) was in treatment where cabbage used was non-inoculated and was grown in soil with highest chromite concentration (7.14%) which is above the permissible limit of Mn concentration (6.61 mg kg^{-1}) in plants given by WHO. Consequently, consuming cabbage from these treatments could have fatal effects on humans. However, minimum data recorded (0 mg kg^{-1}) was for all treatments where cabbage was grown in garden soil and whether the seeds were either inoculated or not inoculated with bacterial strains (figure 8). They are followed by 4.6 mg kg^{-1} which is same for all cabbage grown on soil with soil having 2.27% chromite soil regardless of *Bacillus pumilus* strain used. Furthermore, Mn accumulation was 5.7 mg kg^{-1} for all cabbage plants inoculated with C-2 PMW-8, C-1 SSK-8 and C-1 PWK-7 when grown on soil with 4.65% chromite soil concentration. However, when the chromite percentage was increased the Mn accumulation also increased, Mn concentration for cabbage grown on 7.14% chromite soil was 7.5 mg kg^{-1} for cabbage inoculated with sequence C-2 PMW-8, 7.5 mg kg^{-1} for sequence C-1 SSK-8 inoculated seeds and C-1 PWK-7 respectively which is higher as compare to permissible Mn concentration set by WHO/ FAO 2011 (table 3.1).

Thus, to alleviate the Mn level China commence using chemical method but they are not environmentally friendly however, another environmentally friendly method where Mn oxidizing bacteria for instance majority of Bacillus sp is used to alleviate the rising level of Mn (Ran *et al.*, 2021).

CONCENTRATION OF MANGANESE (MG KG^{-1}) IN SOIL

Data related to accumulation of manganese (Mn) in soil stipulated that the data ranged from 0-

20.3mgkg^{-1}. Furthermore, maximum Mn concentration recorded was in soil (20.3 mg kg^{-1}) with highest chromite percentage (7.14%) and cabbage grown was non-inoculated. Conversely, minimum concentration noted was 0 mg kg^{-1} for all the soil having chromite mining soil regardless of non-inoculated or inoculated seeds. They were followed by 8.9 mg kg^{-1} Mn concentration which is same for all cabbage grown on soil with 2.27% chromite mining soil regardless of *Bacillus pumilus* strains used (figure 9). Furthermore, Mn accumulation was 13.9 mg kg^{-1} for all cabbage plants inoculated with C-2 PMW-8, C-1 SSK-8 and C-1 PWK-7 grown in chromite mining soil (4.65%). However, when the chromite percentage was increased the Mn concentration increased too in cabbage grown on 7.14% chromite soil it was 18.5 mg kg^{-1} for cabbage inoculated with any Bacillus sequences however, it was 20.3 mg kg^{-1} when non-inoculated cabbage was grown on this same soil and this value is lower as compare to permissible Mn concentration (2000 mg kg^{-1}) set by WHO/FAO 2011(table 3.1).

Thus, an environmentally friendly method which requires bacteria to ameliorate soil fertility and reduce heavy metal in soil called phytoremediation is being introduced, likewise, by combining microorganism and applying it on Mn polluted soil can mitigate the Mn content making soil less detrimental to plants. Bacillus sp. for instance *Bacillus cereus* HM5 and *Bacillus thuringiensis* HM7 can be used to increase the efficacy of Mn remediation (Huang *et al.*, 2020).

CONCENTRATION OF LEAD (MG KG^{-1}) IN CABBAGE PLANT

Data related to lead (Pb) concentration in cabbage plants deduced that it ranges from 0-1.7 mg kg^{-1}. Data in table 17 illustrates that maximum Pb content (1.7 mg kg^{-1}) was recorded when cabbage was grown soil with highest chromite concentration (7.14%) and seeds were non-inoculated with bacterial strains which was comparatively higher than the permissible limit of Pb in plant (table 1). Thus, if this cabbage is consumed by human or animal might cause health issues. On the contrary, minimum Pb concentration (0 mg kg^{-1}) was recorded for cabbage grown on garden soil and when seeds were either inoculated or non-inoculated with bacterial strains (figure 10).

Murthy *et al.*, (2019) reported that *Bacillus cereus* have the highest capability to decrease Pb concentration and it was inferred from data that *Bacillus cereus* can be used as bio-agent to mitigate the Pb accumulation from industrial effluents. Moreover, it was concluded that with the increasing percentage of Pb in soil sample the efficacy of Bacillus sp.to remove Pb plummeted. Also, it was inferred that the bio-adsorption of Pb depends on soil pH and when Bacillus sp. was exposed to lower pH such as 5 Pb bio-adsorption was maximum.

CONCENTRATION OF LEAD (MG KG^{-1}) IN SOIL

Data related to lead (Pb) content in soil deduced that it ranges from 0-16 mg kg^{-1}. Data in table 18 illustrates that maximum Pb concentration was (16.1 mg kg^{-1}) in soil with highest chromite concentration (7.14%) and seeds were non-inoculated with bacterial strains. Maximum Pb concentration recorded was comparatively higher than the permissible limit of Pb in soil (13 mg kg^{-1}). On the contrary, minimum Pb concentration (0 mg kg^{-1}) was recorded in soil with no chromite mining soil and either was inoculated or non-inoculated with bacterial strains (figure 11).

Bacillus subtilis has the highest bio-sorption efficiency and has highest binding capacity towards Pb (Gupta *et al.*, 2020).

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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