Original Research Article

EFFECT OF *Bacillus pumilus*STRAINS ON HEAVY METAL ACCUMULATION IN LETTUCE GROWN ON CONTAMINATED SOIL

ABSTRACT

Increasing number of heavy metal 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 lettuce grown on contaminated soil” focuses on functional role of Bacillus pumilus strains inoculated with lettuce 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 lettuce seeds 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 effect of chromite mining soil and Bacillus pumilus strains indicated that these both factors have significant influence on growth parameters. For mining soil minimum days to germinate (14 days) was noted in lettuce grown on garden soil inoculated with sequence. Maximum germination percentage noted was (100%) for C-1 SSK-8 grown on garden soil, dry weight of lettuce leaf (5.0 g) for lettuce inoculated with C-1 SSK-8 and C-1 PWK-7 strains, survival percentage (77.9%) for C-1 SSK-8 treated lettuce for sequence C-2 PMW-8. Maximum germination percentage noted was (88%) for garden soil, fresh leaf weight (17.3g), dry leaf weight (6.5g), number of leaf per plant (16), leaf area (49.0 cm2) were noted in garden soil with no chromite mining soil. Results related to heavy metals accumulation showed that minimum chromium was (2.3 mg kg-1) in lettuce and (1.9 mg kg-1) in soil for all three sequences. It can be concluded that chromite mining soil significantly reduced the growth and survival of lettuce, but when lettuce was inoculated with Bacillus.pumilus strains it enhances the growth and survival. Similarly, minimum heavy metal accumulation in plant and soil, regardless of type of *B.pumilus* used, all three sequences has same mitigating effect on heavy metal in both soil and lettuce. All the three Bacillus pumilus strains ensured reduction in heavy metals content (Cr) in lettuce, below the maximum permissible limits of WHO/FAO 2011.

Keywords: Bioremediation, lettuce, heavy metal, permissible limits and Bacillus strains.

1. Introduction

In Heavy metals are group of elements having atomic density of at least 5g cm^{-3}. Heavy metal is ubiquitous on earth crust and concentration of heavy metals along with availability of these elements may vary depending upon its occurrence for instance, availability of heavy metal may differ from less than 1000 parts per million to few parts per billion. Heavy metals include chromium (Cr), Cobalt (Co), Cadmium Cd), Nickel (Ni), Copper (Cu), Manganese (Mn), Lead (Pb), Arsenic (As) and Mercury (Hg) (Torresdey*et al*., 2005).

Some of the major causes of occurrence of heavy metals in our surrounding are increase in urbanization which is causing industrialization and growing population expanding on many folds causing soil modification too. Heavy metals can cause serious threat to earth and creatures living with in. As Pb, Cd, As and Cr are all present in soil they can easily enter plants either through water or nutrient absorption by plant roots .So, these edible plant parts are utilize by human in their daily diet and in this way heavy metals enter human body through ingestion of these fruits and vegetables for instance, human consume Cd almost 70% orally which was initially either part of fruit or vegetable. Toxicity of these metals within human body can cause different symptoms, likewise most prominent common symptom are gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria, ataxia, paralysis, vomiting, convulsion, depression and Pneumonia (Torresday*et al*., 2005). Lettuce is the most utilized leafy vegetable; in addition to this it plays a cardinal role in economy worldwide. Lettuce is a cool season crop which belongs to Cicoreae tribe and its family is Compositae (Pink and Keane, 2012). Lettuce production in Pakistan is merely 391 tons covering area of 367 hectares (MNFSR, 2020). Heavy metal is recorded in higher concentration in lettuce grown in urban areas. Lettuce has capability to absorb heavy metal from soil and it might cause toxicity if consumed by human being because it is considered to be most consumed leafy vegetable (Jean *et al*., 2015).

World health organization (WHO) conducted various researches in order to record quantity of Cd in certain vegetable samples (including lettuce) and got some analytical results which indicated that vegetable sample under observation contained level of Cd above permissible level therefore, it is paramount to introduce a remediation method to mitigate Cd and other heavy metals before it reach a critical noxious level (Achankzai*et al*., 2011).

Several approaches including biological, chemical as well as physical remedies were used previously to alleviate non-biodegradable metals. However, these approaches have certain limitations including high requirement of labor, requirement of vast amount of capita and during these approaches. So there is another promising approach to alleviate heavy metals from environment also, this technique requires less capital, less labor and cause least disruption in micro flora of soil known as phytoremediation (Musa *et al*., 2017). This remediation technique is in situ technology comprises of using endophytic bacteria, rhizospheric bacteria and plants having adaptive mechanisms employed for accumulating high quantity of metals to remove them (Khan *et al*., 1998;Hayes *et al*., 2003).

Phytoremediation is a process which can be used for elimination of organic pollutants and heavy metals as well. Organic pollutants can be removed using phytoremediation so this techniques have huge scope which constitutes of using plants having property to take up metals from topsoil without affecting it and converting these pollutants into products will increase fertility of soil. Thus if using phytoremediation to alleviate pollutant it will undergo through various methods to detoxify pollutants and there is no need to add organic matter to ameliorate fertility because this process improve fertility of soil too (Musa *et al*., 2017).

Phytoremediation also involves using free living bacteria which possess certain beneficial traits causing stimulating plant growth and these bacteria colonize plant roots they are known as plant growth promoting bacteria for instance, numerous strains of bacillus, rhizobium erwinia and flavobacterium are all plant growth promoting bacteria. Plant growth promoting bacteria undergo fixation of nitrogen, increasing root surface to absorb more nutrients, solubilization of phosphorus, and synthesis of certain siderophore and production of phytohormone. Plant growth promoting hormones for instance bacillus strains were used in basmati rice varieties as a result increase in yield of rice was noticed because these strains enhanced solubility of phosphorus, potassium and zinc additionally it protect rice from *Pyricularia oryzae* and *Fusarium oryzae* (Masood *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 lettuce growth and survival along with this; current experiment was conducted to study remediation and immobilization of heavy metals using *Bacillus pumilus* strains.

III. MATERIALS AND METHODS

Experimental site

The experiment was conducted at ornamental horticulture nursery and Institute of Biotechnology and Genetic Engineering (IBGE), the University of Agriculture Peshawar. In this research soil samples were collected from chromite mining area situated in Prang Ghar Mohmand Agency, Pakistan. Composite soil was passed through sieve in order to eradicate chunks. It is pre-requisite to determine concentration of heavy metals in each sample thus for this purpose we used spectrophotometer.

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 on the basis of 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).

Pic 1. Showing the preparation of bacterial plates. (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

Lettuce *(Lactuca sativa)* seeds variety Batavia were taken and in order 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

In order to grow lettuce seven seeds were sown in each bag. Lettuce was grown in 48 bags (4.5 kg per bag) containing well-mixed non-contaminated garden soil with contaminated soil having three different concentration of chromite collected from chromite mine except in control.

Initially, we converted 4.5kg into gram to get the percentage of chromite mine soil which was added into each bag varying from 100 gram, 200gram and 300 gram. Weight of garden soil taken for each bag was 4.5 kg which is equal to 4500 grams. Furthermore, first level of chromite mine soil which needed to add into bags was 100 gram. Thus, if 4400 gram soil contains 100 gram of chromite mine soil then 100 percent of 4400 gram soil contains 2.27 % of chromite mine soil in 4.5 kg of soil. Likewise, when the chromite mine soil weight was increased up to 200 gram in soil (4.5 kg) the percentage of chromite mine soil added in bags containing garden soil was 4.56% and for 200 gram of chromite mine soil the percentage was 7.14. Afterward, these pots were placed in wetland research area and cultural practices i.e., irrigation, weeding etc. were performed during the growth of lettuce.

Plantgrowth parameters

After sowing in contaminated mine soils various parameters of all treated bags and control ones were compared. The effect of strains of *Bacillus pumilus* on lettuce was studied by comparing the following parameters:

Days to germination of seeds

One of the parameter recorded for each treatment in each replication was first germination after sowing and their average was calculated. Lettuce seeds were sown on the 7th of December 2022 and data regarding days to germinate was recorded after 3-4 weeks of seed sowing.

Germination percentage

Germination percentage of random three lettuce seeds were recorded from each treatment in each replication after 3-4 weeks of sowing using following formula:

$$\text{Germination percentage} = \frac{\text{Germinated seeds}}{\text{Total No of seeds sown}} \times 100$$

Fresh leaf weight (g)

For fresh leaves weight measurement electric weight balance was used. From each treatment of every replication selection of fresh leaves from per plant (five plants) were done randomly and then average of calculated value was recorded.

Survival percentage

Following formula was used to find the survival percentage of lettuce plants:

$$\text{Survival percentage} = \frac{\text{Number of total plants survived}}{\text{Number of total plants germinated}} \text{x}100$$

Permissible limits of heavy metals (mg kg^{-1}) in soil and plant

The permissible limit of heavy metal (mg kg^{-1}) set by FAO/WHO (WHO, 2011) for both plant and soil. Also, the concentrations exceeding above this limit can cause deleterious effects on both plant and human.

Table 1. Permissible limit of Cd, Cr, Pb and Mn set by FAO/WHO in 2011 for soil and plant.

Permissible limits by FAO/WHO (mg kg^{-1})	Chromium	Cadmium	Lead	Manganese
Maximum permissible limit in soil	8	0.3	13	2000
Maximum permissible limit in plant	2.3	3	0.3	6.61

WHO/FAO 2011

Concentration of heavy metals (mg kg^{-1}) in soil and plants (heavy metal analysis)

Analysis of heavy metals was carried out for both soil and plant by atomic absorption spectrophotometer (AAS) in laboratory of soil science department, University of Agriculture Peshawar with the help of wet digestion methods. For soil heavy metal analysis 15 g of soil from each bag containing chromite mine soil was taken and this soil sample was initially crushed and was passed through a 2 mm sieve to separate the unwanted particles. Eventually, the soil was oven dried at 84.2^{o}C for 10 minutes then it was homogenized by using pestle and mortar. Moreover, it was treated with ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-

DTPA) solution, for preparing 1 liter of AB-DTPA we used ammonia hydrogen bicarbonate (79.06 g), DPTA (1.97 g) and 2m L of ammonia solution. Afterwards, pH adjustment of the solution was done to 7.6 and then to make 1000m L of solution we added distil water. Around 30m L of AB-DTPA solution was extracted from 1 liter of whole solution and 15 g of soil was mixed in this solution. In order to make a homogenous mixture of the added soil sample with AB-DTPA it was placed on orbital shaker for 30 minute at 100 rpm (revolution per minute). Subsequently, after getting a homogenized solution we passed the solution through 1500 mm qualitative Whatman filter paper and the filtrate was collected into conical flaks. After getting filtrate we diluted the soil samples with distil water to make the solution up to 50 m L for AAS analysis (Dasgupta *et al*., 2021). Analysis of following heavy metals was done;

- Chromium (Cr)

Table 2. Properties of chromite soil used for the experiment

pH	**Cd (mg kg^{-1})**	**Cr (mg kg^{-1})**	**Pb (mg kg^{-1})**	**Mn (mg kg^{-1})**
7.7	2.3	20	14	40

Statistical analysis

All the recorded data in this research work are actually the means of the three replicates. In order 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 when needed and reported values were considered significantly different at P≤0.01 and 0.05(Jan *et al*., 2009).

IV. 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. Here, minimum days recorded to germinate were lettuce seeds grown in control soil and when seeds were inoculated with C-1 SSK-8 (12 days). 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 lettuce grown in soil with 7.14% of chromite and these seeds were not inoculated with any bacterial strain.

In this study, the lettuce grown on chromite soil was affected by heavy metal toxicity however; 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, plants that were not inoculated grown in soil that was amended with different concentrations of heavy metals were stipulated to have lower growth parameters. When the seeds were inoculated with bacterial strains and grown in contaminated soil (2.27%) resulted in timely germination however; increase in contaminated soil concentrations resulted in increased in days to germination in lettuce. It could be concluded that days to germination required by lettuce seeds regardless of *B.pumilus* strain will result in decrease in days to germination when the chromite percentage is less.

Increasing the concentration of heavy metal imposes stress on seeds which can affect seed germination either by causing membrane alteration, decreases in the level of soluble proteins and is manifested by low germination leading to low yield in both agronomic and horticultural crops. Likewise, exposure of seeds to heavy metals or stress can lead towards altering normal germination in seeds and metal toxicity also leads to abnormal seed physiology. Furthermore, soil having a high level of chromium is reported to cause a delay in germination and also cause mineral leakage in seeds leading to loss of accumulated nutrients in seed (Sethy and Ghosh., 2014).

Germination percentage

Figure 2 indicates 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 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 upto 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%). Furthermore, seed germination significantly induced when the inoculated seeds were used in soil with chromite percentage of around 2.27% and about 24% increase was recorded when compared with the control (figure 2). Likewise, germination was significantly induced when the chromite percentage was increased for instance; germination percentage for seeds inoculated with three sequences resulted in germination around 70% or above when chromite percentage was low however it decline ranging between 50-60%. 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. Significant increase of about 39.7% inoculated seed grown on garden soil was recorded as compare with control (non-inoculated seeds and garden soil).

Minaxi *et al*., (2013) reported that Bacillus spp.RM2 when inoculated with cowpea plant significantly increased the seed germination when compared with the control. Majority of Bacillus species can produce phytohormones for instance auxin, cytokinin and gibberellin due to which they act as growth promotors. Likewise, *Bacillus pumilus* have potential to produce auxin and gibberellin. Thus, it has potential to produce indole acetic acid (IAA) which facilitates the seed germination. It elucidates that strains of *Bacillus pumilus* can be utilize in seeds with less germination (Miljakovic*et al*., 2020).

Fresh leaf weight (g)

The ANOVA illustrated that fresh leaf weight for lettuce 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 non-significant.

The collected data illustrated that fresh leaf weight (g) recorded for lettuce ranged from 9.3-18.1 g. Maximum fresh leaf weight (17.3 g) recorded was in lettuce grown on garden soil with no chromite mining soil and minimum fresh leaf weight (10.4 g) was recorded in lettuce grown on soil with highest chromite concentration (7.14%). Also, non-inoculated lettuce resulted in lower fresh leaf weight (12.2 g) however; the inoculated lettuce 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, lettuce 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.

Heavy metals have deleterious effect on plants for instance Cu once accumulated in plants in excess amount can lead towards imbalance in normal metabolic pathways which lead towards lower growth, inhibit biomass and low fresh weight. The toxic effects of heavy metal stress on plant and how it declines the plant yield and quality of plant biomass production. Although heavy metals including Cd, Cr and Mn are non-essential for plant growth they are still absorbed by plants and are accumulated by plant shoot this leads towards hindrance in normal transport of crucial nutrients. Thus, accumulation of heavy metals and abnormal transport of important nutrients in plant plummet the production of plant biomass and general plunge in plant growth (Duscan., 2000).

Yuan and Gao., (2015) reported that *Bacillus pumilus* have property to enhance the nutrient uptake and have interactive relation with symbiotic microorganism which directly have positive impact on plant growth parameters for instance leaf fresh weight and height.

Single leaf area (cm^2)

The ANOVA illustrated that single leaf area of lettuce 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 non-significant.

The collected data illustrated that single leaf area (cm^2) recorded for lettuce ranged from 29.6-50.6 cm^2. Maximum single leaf area 49.0 cm^2 was recorded in lettuce grown on garden soil with no chromite mining soil and minimum single leaf area was recorded (37.7cm^2) in lettuce grown on soil with highest chromite concentration (7.14%). Additionally, non-inoculated lettuce resulted in lower leaf area (37.3 cm^2) however; the inoculated lettuce treated with sequence C-1 SSK-8, C-1 PWK-7 and C-2 PMW-8had highest single leaf area (45cm^2).

Survival percentage:

The ANOVA illustrated that survival percentage for lettuce 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 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 lettuce 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 lettuce were grown on garden soil (figure 3).

It is evident through previous research work done on 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 plant grown on metalliferous environment. Plant growth regulating bacteria decrease 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).

Table 3 . Effect of mining soil and *Bacillus pumilus* strains on mean value and F-value for lettuce growth parameters.

Growth parameters	Mean value		F-value	
	Mining soil	***Bacillus pumilus* strain**	**Mining soil**	***Bacillus pumilus* strain**

DGS	317.556	46.5	464.72	68.05
GP	4130.41	4.19	233.82	0.24
FLW (g)	109.204	22.533	235.54	48.6
NLP	84.1875	74.5208	108.43	95.98
SLA (cm2)	269.243	148.187	74.42	40.96
SP	1769.58	122.81	934.09	64.82

DGS: Days to germination of seeds.

GP: Germination percentage.

FLW: Fresh leaf weight

NLP: Number of leaves per plant

SLA: Single leaf area

SP: Survival percentage

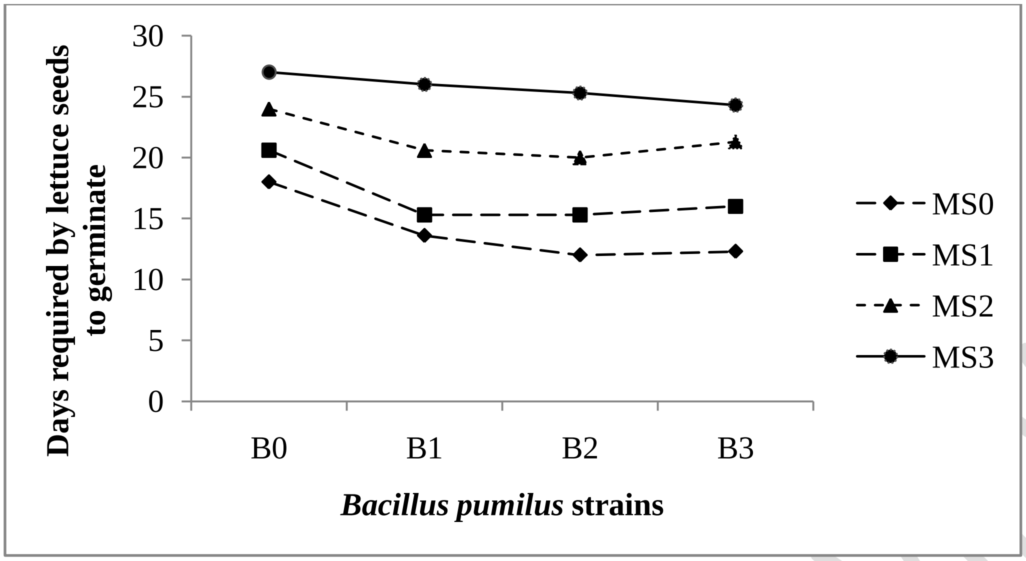


Figure 1: Effect of *Bacillus pumilus* strains and various concentration of chromite mine soil on days required by lettuce seeds to germinate.

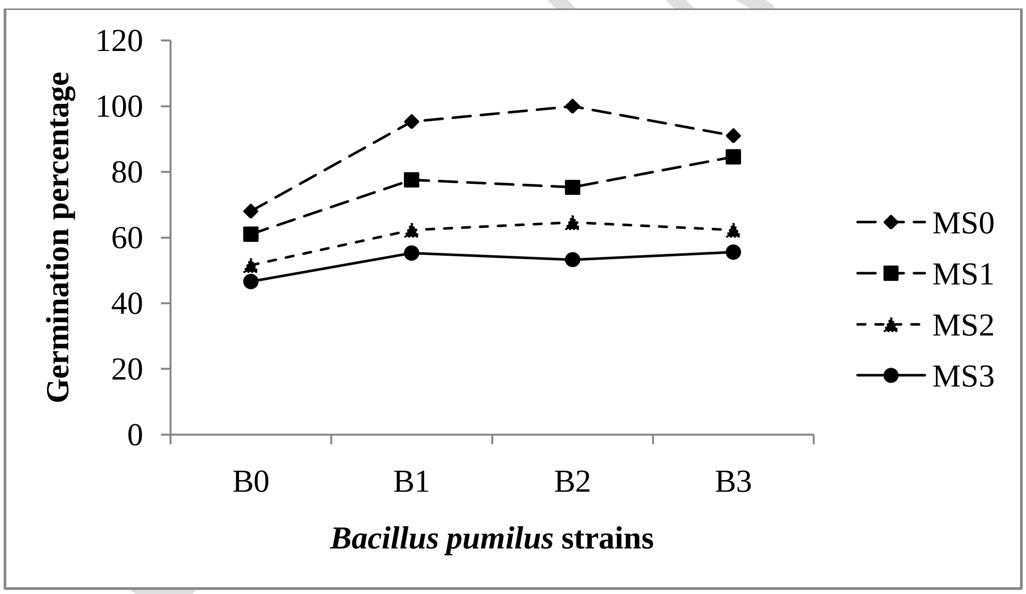


Figure 2: Effect of *Bacillus pumilus* strains and various concentration of chromite mine soil on germination percentage of lettuce seeds.

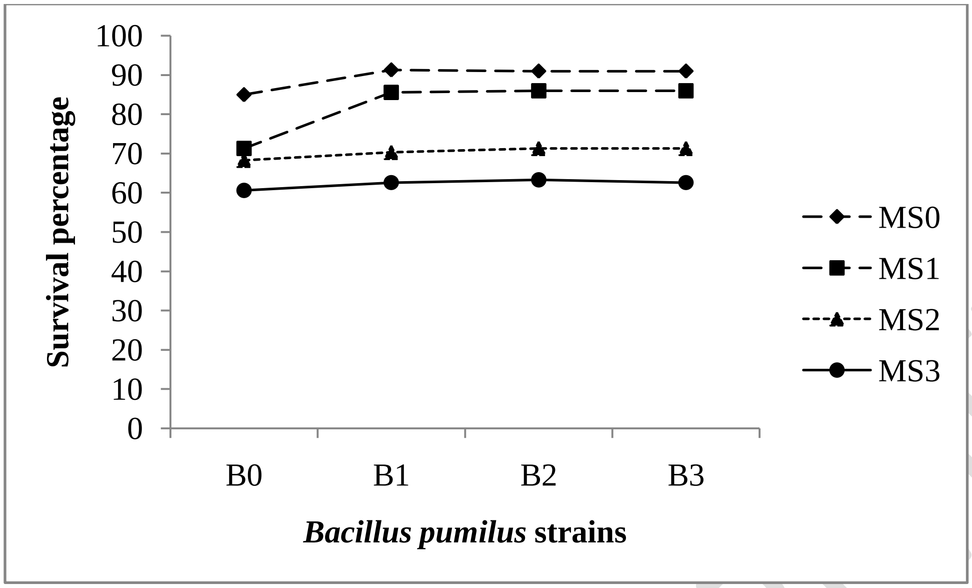


Figure 3: Effect of *Bacillus pumilus* strains and various concentration of chromite mining soil on survival percentage of lettuce.

Concentration of chromium (mgkg^{-1}) in lettuce plant

The effect of *Bacillus pumilus* stains and chromite mining soil on concentration of chromium in lettuce plant and soil on both mean value and F-value is given in table 4. 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 lettuce plant 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 lettuce 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 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 1) and can be consumed by human. Furthermore, Cr concentration 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).

Chromium is considered as one of the most commonly occurring heavy metal and become part of environment when industrial effluents are discharged. Leafy vegetables have highest heavy metal bio-accumulation (Qureshi *et al*., 2016). It was reported that Cr negatively affect the microorganism however, certain Cr toxicity resistant bacteria such as Bacillus sp. can be utilized to plunge its effects (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). 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 1) .

Chromium is most commonly occurring heavy metal in environment, have more deleterious impact on living organism and the main source of Cr is either through discharge of industrial effluents or excessive use of pesticides. It was documented that Enterobacter and Bacillus possess potential to utilize hexavalent Cr from soil and dissolve them using soluble enzyme (Upadhyay*et al*., 2017). Ahemad .,(2015) reported that Bacillus sp. have potential to increase immobilization of Cr or converting hexavalent Cr which is quite toxic into derivatives which are not highly toxic to environment. Consequently, chick pea was inoculated with Bacillus species

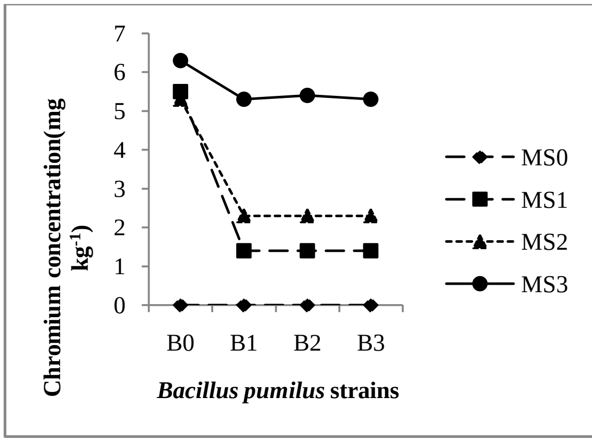
PSB10 and it stimulated the growth parameters and reduces the Cr content in root and plant (Wani and Khan., 2010).

Table 4. Effect of mining soil and *Bacillus pumilus* strains on mean heavy metal accumulation in lettuce and soil.

Heavy metal (mgkg^{-1})	Mean value		F-value	
	Mining soil	***Bacillus pumilus* strain**	**Mining soil**	***Bacillus pumilus* strain**
In lettuce plants				
Cr	12.3364	63.4231	160.33	824.27
In soil				
Cr	1.1006	32.465	18.04	532.09

Cr: Chromium

Effect of chromite mining soil and *Bacillus pumilus* strains on heavy metal concentration (mg kg^{-1}) in soil and lettuce.



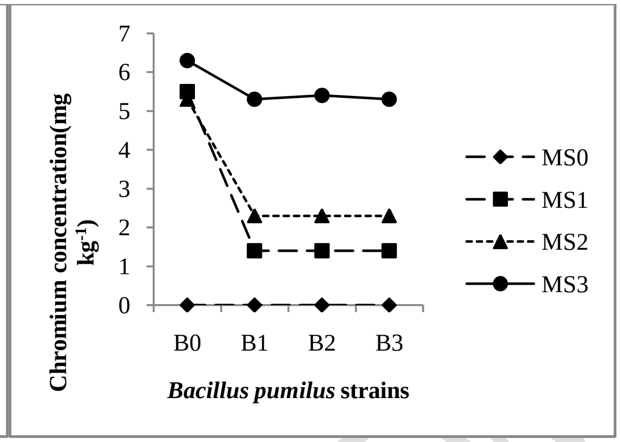


Figure 4: Effect of *Bacillus pumilus* strains and mining soil on chromium (mg kg^{-1}) accumulation in lettuce plant.

Figure 5: Effect of mining soil and *Bacillus pumilus* strains on chromium concentration (mg kg^{-1}) in soil.

Conclusion:

After collecting the data following conclusions are drawn from results:

- Chromite mine soil contains cadmium (2.3mgkg^{-1}).
- Chromite mining soil when used for growing lettuce significantly inhibited the growth and survival of lettuce.
- Lettuce 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 lettuce.
- Lettuce 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.

Reference

Achankzai, A. K. H., Z. A. Bazai and S. A. Kayani. 2011. Accumulation of heavy metals by lettuce (*Lactuca sativa* L) irrigated with different levels of wastewater of Quetta city. Pak. J. Bot. 43(6): 2953-2960.

Ali, R., M. Habib, S. N. Kakavand, Z. Zahid, N. Zahra, R. Sharif and M. Hasanuzzaman. 2020. Phytoremediation of cadmium: physiological, biochemical, and molecular mechanisms. Bio. 9(7): 177-179.

Ahemad, M. 2015. Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria. J. Genet. Eng. Biotechnol. 13(1): 51-58.

Ahmad, I., M. J. Akhtar., S. Mehmood., K. Akhter., M. Tahir., M. F. Saeed., M. B. Hussain, M.B. and S. Hussain. 2018. Combined application of compost and Bacillus sp. CIK-512 ameliorated the lead toxicity in radish by regulating the homeostasis of antioxidants and lead. Ecotoxicol. Environ. Saf.148: 805-812.

Bashir, M. A., M. Ahmad and M. A. Anjum. 2003. Effect of different growing media on growth of rooted Jojoba cuttings. Int. J. Agri. Bio. 9(1): 147-151.

Boamponsem, G. A., M. Kumi, and I. Debrah.2012. Heavy metals accumulation in cabbage, lettuce and carrot irrigated with wastewater from Nagodi mining site in Ghana.Int. J. Sci. Res. 1(11): 124-129.

Dasgupta, S., S. Sengupta., S. Saha, A. Sarkar and K. C. Anantha. 2021. Approaches in advanced soil elemental extractability: catapulting future soil–plant nutrition research. In: Rakshit, A., S. K. Singh., P. C. Abhilash and A. Biswas. (eds) Soil Science: Fundamentals to Recent Advances. Springer, Singapore.

Ducsay, L., 2000. The influence of phytotoxic effects of As, Cd, Pb on biomass production, their accumulation and chlorophyll content. Acta III. Int. Conf.Inorganic substances in living environment, SPU,Nitra: 34-38.

FAO (2011) Food balance sheet, Rome. National Institute of Health National Nutrition Survey, Islamabad.

Hayes, W. J., R. T. Chaudhry, R. T. Buckney and A. G. Khan. 2003. Phytoaccumulation of trace metals at the Sunny Corner mine, New South Wales, with remediation strategy. Aust. J. Ecotoxicology. 9(1): 69–82. .

Islam, F., T. Yasmeen., Q. Ali., S. Ali., M.S. Arif., S. Hussain and H. Rizvi. 2014. Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotox. Environ. Safe. 104. 285-293.

Jan, M. T., P. Shah, P. A. Hollington, M.J. Khan and Q. Sohail. 2009. Agriculture research: Design and analysis, Department of Agronomy, Agriculture University Peshawar, Pakistan.

Jean, S. Y., Y. O. Bernard and Y. D. A. Cyrill. 2015. Heavy metals contamination in*Lactuca sativa* (Lettuce) from two agricultural sites of Abidjan. Int.J. Pure Appl. Sci. 27(2): 59-64.

Khan, A., X. Q. Zhao., M. Javed., K.S. Khan., A. Bano.,R. F. Shen and S. Masood. 2016. *Bacillus pumilus* enhances tolerance in rice (*Oryza sativa* L.) to combined stresses of NaCl and high boron due to limited uptake of Na+. Environ. Exp. Bot. 124: 120-129.

Lee, S. W., S. H. Lee., K. Balaraju., K. S. Park., K. W. Nam., J. W. Park and K. Park. 2014. Growth promotion and induced disease suppression of four vegetable crops by a selected plant growth-promoting rhizobacteria (PGPR) strain *Bacillus subtilis* 21-1 under two different soil conditions. Acta PhysiolPlant. 36: 1353–1362.

Lucas, G., J. A. Probanza, A. Ramos, B. R. Palomino and M. G. Manero. 2004. Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. Agronmie. 24: 169–176.

Masood, S., X. Q. Zhao and R. F. Shena. 2020. *Bacillus pumilus* promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization. Sci. Hortic. 272: 1-9.

Miljakovic, D., J. Marinkovic and S. Balesevic-Tubic. 2020. The significance of Bacillus spp. in disease suppression and growth promotion of field and vegetable crops. 8(7): 1037.

Minaxi., L. Nain., R. C. Yadav and J. Saxena. 2012. Characterization of multifaceted Bacillus sp. RM-2 for its use as plant growth promoting bio-inoculant for crops grown in semi-arid deserts. Appl. Soil. Ecol. 59: 124-135.

Ministry of national food and security and research. 2020. Fruit, vegetable and condiments statistics of Pakistan 2018-2019. http://www.mnfsr.gov.pk/frm.

Musa, D. M., Y. Garba and M. D. Sani. 2017. Potential of lettuce (*Latuca sativa*) for phytoremediation of Cd, Cu, Cr and Pb in contaminated soil along river salanta. Agr. Plant. Sci. 3(2): 258-269.

Naaz, S and S. N. Pandey. 2010. Effects of industrial waste water on heavy metal accumulation, growth and biochemical responses of lettuce (*Lactuca sativa* L.). J. Environ. Biol. 31(3): 273.

Onakpa, M. M., A. A. Njan and O. C. Kalu. 2018. A review of heavy metal contamination of food crops in Nigeria.Ann. Glob. Health. 84(3): 488–494.

Oya, T., A. L. Neopmoceno, N. Neumaier, J. R. B. Farias, S. Tobita and O. Ito. 2004. Drought tolerance characteristics of Brazilian soybean cultivars. J. Plant Prod. Sci. 7(3): 129-137.

Pink, D. A. C and E. M. Keane. 2012. Lettuce: *Lactuca sativa* L. Chapter.40. p. 543-571. In:Kallo. G and B.O. Bergh (eds). Genetic improvement of vegetable crops. Pergamon press. UK.

Qureshi, A.S., M. I. Hussain., S. Ismail and Q. M. Khan. 2016. Evaluating heavy metal accumulation and potential health risks in vegetables irrigated with treated wastewater. Chemosphere. 163: 54-61.

Sethy, S.K. and S. Ghosh.2013. Effect of heavy metals on germination of seeds. J. Nat. Sci. Biol Med. 4(2): 272.

Shahzad,A., M.Qin., M. Elahia., M. Naeem., T. Bashir., H. Yasmin., M. Younas., A. Areeb., M. Irfan., M. Billah., A. Shakor and S. Zulfiqar. 2021. *Bacillus pumilus* induced tolerance of Maize (*Zea mays* L.) against cadmium stress. Sci. Rep. 11(1): 1-11.

Sulaiman, F. R., N. H. Ibrahim and N. S. Ismail. 2020.Heavy metal (As, Cd, and Pb) concentration in selected leafy vegetables from Jengka, Malaysia, and potential health risks.Appl. Science. 2: 1430-1432.

Tiwari, S. and C. Lata. 2018. Heavy metal stress, signaling, and tolerance due to plant-associated microbes: an overview. Front. Plant Sci. 9: 452.

Torresdey, J. L. G., J. R. P. Videa, G. D. L. Rosa and J. G. Parsons. 2005. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord. Chem. 249: 1797–1810.

Upadhyay, N., K. Vishwakarma., J. Singh., M. Mishra., R. Kumar., R. Rani., R. K. Mishra., D. K. Chauhan., D.K. Tripathi and S. Sharma. 2017. Tolerance and reduction of chromium (VI) by Bacillus sp. MNU16 isolated from contaminated coal mining soil. Front. Plant Sci. 8: 778.

Wani, P.A. and M. S. Khan. 2010. Bacillus species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. Food. Chem. Toxicol. 48(11): 3262-3267.

Waseem, A., J. Arshad, F. Iqbal, A. Sajjad, Z. Mehmood and G. Murtaza. 2014. Pollution status of Pakistan: A retrospective review on heavy metal contamination of water, soil, and vegetables. Biomed. Res. Int. 2014: 29-31.

Yuan, Y. and M. Gao. 2015. Genomic analysis of a ginger pathogen *Bacillus pumilus* providing the understanding to the pathogenesis and the novel control strategy. Sci. Rep. 5(1): 1-9.