





OPEN

Bacillus pumilus induced tolerance of Maize (*Zea mays* L.) against Cadmium (Cd) stress

Asim Shahzad^{1,2}, Mingzhou Qin¹, Mahmood Elahie², Muhammad Naeem^{3,6}, Tasmia Bashir⁴, Humaira Yasmin⁵, Muhammad Younas⁶, Ahsan Areeb⁷, Muhammad Irfan⁷, Motsim Billah⁸, Abdul Shakoor¹ & Saman Zulfiqar⁹

Heavy metals contaminate the soil that alters the properties of soil and negatively affect plants growth. Using microorganism and plant can remove these pollutants from soil. The present investigation was designed to evaluate the induced effect of *Bacillus pumilus* on maize plant in Cadmium (Cd) contaminated soil. Three different concentrations of Cd (i.e. 0.25, 0.50 and 0.75 mg kg⁻¹) were applied in soil under which maize plants were grown. The germination percentage, shoot length, leaf length, number of leaves, root length, fresh weight and nutrient uptake by maize plant were determined. The experiment was conducted by using complete randomized design (CRD) with three replicates. The result indicated that germination percentage, Shoot length, leaf length, root length, number of leaves, and plant fresh weight were reduced by 37, 39, 39, 32 and 59% respectively at 0.75 mg kg⁻¹ of CdSO₄ concentration but when maize seeds inoculated with *Bacillus pumilus* significantly increased the germination percentage, shoot length, leaf length, number of leaves, plant fresh weight at different concentrations of CdSO₄. Moreover, the plant protein were significantly increased by 60% in T6 (0.25 mg kg⁻¹ of CdSO₄ + inoculated seed) and Peroxidase dismutase (POD) was also significantly higher by 346% in T6 (0.25 mg kg⁻¹ of CdSO₄ + inoculated seed), however, the Superoxide dismutase (SOD) was significantly higher in T5 (0.75 mg kg⁻¹ of CdSO₄ + uninoculated seed) and was 769% higher as compared to control. The Cd contents in *Bacillus pumilus* inoculated maize roots and shoots were decreased. The present investigations indicated that the inoculation of maize plant with *Bacillus pumilus* can help maize plants to withstand Cd stress but higher concentration of Cd can harm the plant. The *Bacillus pumilus* has good potential to remediate Cd from soil, and also have potential to reduce the phyto availability and toxicity of Cd.

Soil is composed of different components of solid, liquid and gases in the form of “organic, inorganic and mineral particles” which support plants and animals by providing or transferring energy in various ways in the environment¹. These resources are divided into two main groups i.e. renewable and non-renewable resources. Soil is non-renewable resource, which is formed due to weathering of rocks by fluctuations in climate, weather and organismic activities². At present time one of the main causes of pollution is heavy metals. These heavy metals remain untreated in soil and produce soil contamination which is very toxic for organisms. Heavy metals cannot be degraded by any process but it can be changed to less poisonous form³. These heavy metals cause various diseases to plants and animals by oxidative stress, their sources may be anthropogenic as well as natural⁴. Heavy metals pollution are foremost pollutant of our food particularly vegetables which is contaminated by absorbing heavy metals from polluted soil, water and air due to disposal of industries and urban waste. The elements that have density greater than 5 g cm⁻³ are called heavy metals⁵. The ingestion of heavy metals contaminated vegetables may lead to various long term lingering diseases like semphysema, bronchiolitis, and alveolitis, also

¹College of Geography and Environment, Henan University, Jinming Ave, Kaifeng, China. ²Department of Botany, Mohi-Ud-Din Islamic University, AJ&K, Pakistan. ³Faculty of Life Sciences, Department of Biology, University of Okara, Okara, Pakistan. ⁴Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan. ⁵Department of Biosciences, COMSATS University, Islamabad, Pakistan. ⁶Department of Biotechnology, Mohi-Ud-Din Islamic University, AJ&K, Pakistan. ⁷Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. ⁸Department of Health Sciences, Abasyn University Islamabad, Islamabad, Pakistan. ⁹Govt Sadiq College, Women University, Bahawalpur, Pakistan. ✉email: Agron12@yahoo.com; mzqin@vip.henu.edu.cn

short term disease like nervous, kidney, cardiovascular, and bone diseases⁶. Soil adulteration with heavy metals is a common problem for world which is alarming threat for human health⁷.

Cd is an unnecessary and greatly noxious heavy metal, which present in environment due to anthropogenic activities. Cd inhibit the plant to absorb important nutrients, in result plant growth is reduced which indicates Cd phytotoxicity⁸. Cd is non-amphoteric in nature and not properly dissolves in base solution⁹. The development of plant organs bears harmful effect of heavy metals like lead (Pb) and Cd which reduce biomass of various plant species¹⁰.

The plant species grown in contaminated soil having high concentration of pollutant reduce plant organ formation¹¹. The crop which are produced in contaminated soil, absorb contaminants in their tissues and are very toxic for living organisms when are used as food¹². Different plant species accumulate different types of heavy metals in their tissues from contaminate site¹³. Industrial pollutants contaminate water and play harmful impact on organisms. Uptake of toxic metals in plants effects variations in plant species, plants growth stage and translocation of metals¹⁴. These heavy metals damage molecular structure of plant and animals¹⁵. To eliminate contamination of non-degraded partials, phytoextraction is used which increase biomass and bio-concentration of plants¹⁶. There are different types of technologies used in present time to eliminate contaminants from polluted areas to reestablish natural condition. Phytoremediation is one of the best technology in which plant absorbs toxic substances from soil and water. Only selected plants are utilized for this purpose¹⁶. Phytoremediation is an ecofriendly technology to remove toxic metals¹⁷.

Numerous bacterial species are known that play vital role to tolerate plants under stress condition which can detoxify, transfer and collect heavy metals. Microorganisms and plants combine together against toxic effect of heavy metals by using rhizoremediation and phytoremediation mechanism. Microbes enhance the growth of plant in heavy metals stress¹⁸. Plant absorbs heavy metals in soil and transport from root to shoot via xylem tissue after physiological process accumulates into grains. Plants having different genotype and capacity to detoxify heavy metals stress¹⁹. Plant microbe's interaction decomposes various pollutants and increase plant development and growth²⁰. A bulk of enzymes from bacteria, have been reported to be concerned in the biodegradation of toxic organic pollutants and remove the soil contamination²¹.

Previous reports demonstrated that several species of *Bacillus* can beneficially promote growth and enzyme system which may help the plants to overcome the biotic stresses²². The application of several *Bacillus* strains in soil contaminated with heavy metals soil can help to reduce the harmful effects of heavy metals and enhances the plant growth. The *Bacillus* spp also have ability to accelerate the plant growth by increasing water uptake and reducing electrolyte leakage to mitigate Cd stress²³. *B. licheniformis* enhances Cu, Zn, Cd, Cr and Pb accumulation and distribution in plants grown in heavy metal-contaminated soil, which leads to reduced levels of toxic metals in soil²⁴. Similarly, higher concentration of Cd in soil reduce nutrient (P, Fe, Zn, and Mn) uptake in plants. *B. pumilus* is a promising plant growth promoting bacteria and previous reports²⁵ demonstrated that *B. pumilus* affected metal toxicity in tomato and rapeseed (*Brassica napus* L.) The application of *Bacillus* spp. alleviate stress effect by reducing lipid peroxidation and SOD activity and increasing amylase and protease to promote plant growth in heavy metal-polluted soil²⁶. Similarly, *Bacillus* spp. support plant tolerance against Zn and Cu stress by enhancing the activities of ROS scavenging enzymes, such as POD, SOD, CAT, APX, and DHAR²⁷. The regulation of antioxidants in cells inhibits oxidative stress damage and triggers plant growth-promoting substances to enable plants to adapt to metal stress. *Bacillus*-mediated plant tolerance against Ni and Cr stresses is achieved through the enhancement of photosynthetic pigments and leghemoglobin, which leads to increased crop yield²⁸. However, the effect of *B. pumilus* on Cd uptake by plants has received lesser attention. It is not clear whether plant physiological processes work independently or together with other mechanism like antioxidant system of plant under Cd stress. In this context, the present study was therefore performed to investigate the potential of *Bacillus pumilus* to induce growth and antioxidant enzymes of maize plants under Cd stress.

Materials and method

Preparation of heavy metal solution. Three different concentration of cadmium sulphate (CdSO_4) solution (0.25, 0.50 and 0.75 mg mL^{-1}) were prepared for different treatment in pure distilled water by dissolving the cadmium sulfate (CdSO_4). The different concentrations of CdSO_4 were selected on the basis of the previous scientific data²⁹. Pure distilled water was used as control for the experiment. 100 mL of each solution was added in 1 kg of potted soil.

Preparation of bacterial inoculum. The *Bacillus Pumilus* (Acc KF859972) used in this study was taken from phytohormone Lab Quaid-i-Azam University, Islamabad, Pakistan, on the basis of its plant growth indorsing latent³⁰. For the preparation of inoculum, the nutrient broth was purchased from OXOID-UK. The nutrient broth was sterilized at 121 °C for 20 min. The isolated strain was inoculated in nutrient broth and incubated in shaker incubator (EXCELLA E24 Germany) at 150 rpm for 48–72 h. After that, the culture was centrifuged for 10 min at 3000 rpm. The pellet was again suspended in double distilled water and optical density (O.D) was adjusted to 0.100 at 660 nm with UV-VIS spectrophotometer. The inoculum was prepared by culture of bacterial strain having O.D 0.100 at 660 nm and bacterial density (10^6 cells/ml).

Seed inoculation. Maize (*Zea mays* L.) seeds (KASHMIR GOLD) was obtained from NARC (National Agricultural Research Centre) Islamabad, Pakistan. The seeds were washed with ethanol (95%) for surface sterilization, following by soaking in 10% Chlorox for 2–3 min and subsequently the seeds were washed successively 2–3 times with autoclaved distilled water³¹. Moreover all the methods were performed in accordance with the relevant guidelines given by the national agriculture research center for the cultivation of maize plants.

Treatments	<i>Bacillus pumilus</i> inoculation	CdSO ₄ (mg kg ⁻¹)
T1	–	0
T2	+	0
T3	+	0.25
T4	+	0.50
T5	+	0.75
T6	–	0.25
T7	–	0.50
T8	–	0.75

Table 1. Preparation of treatment applications.

Preparation of treatment applications. The seeds were dipped in the inoculum for two to 2–3 h. Then three different solutions of Cd sulphate prepared (i.e. 0.25, 0.50 and 0.75 mg kg⁻¹). Eight different treatments with three replicates (**three Pots per treatment**) were made and five seeds of maize were sown in each pot (Table 1). For further analysis plants were harvested after 28 days of sowing.

Parameter measured. The germination percentage was observed after four day of sowing whereas, maize were harvested after 28 day of sowing. In order to remove non-aggregated soil, seedlings were slightly shaken. The following parameters were studied³². Shoot and root lengths were measured from the root initiation up to the tip of the longest shoot and root. It was measured in centimeters³³. Leaf size was measured in cm, from node to tip of the leaf³⁴. Root length was measured from the junction of root and stem towards the tip of the longest root. It was measured in centimeters³⁵. After harvesting plants from the pots, they were shaken to remove extra soil other than aggregates, the weight measured in grams³⁶. Each plant of each replicate was measured and their mean value was used to compare the treatments.

Leaf proline. Proline content of maize plant leaves was determined by the method of³⁷ about 0.5 g of fresh maize leaves were used to determine the proline content.

$$\text{Proline} = K \times \text{Dilution factor} \times \text{optical density/weight of sample}$$

where the *K* value is 19.6.

Peroxidase dismutase assay. The POD activity of maize leaves was measured by the method of³⁸ about 1 g of fresh maize leaves were used to determine the POD enzyme.

Superoxide dismutase assay. The SOD activity of maize leaves was measured by the method of³⁹. The activity of SOD was expressed as units/100 g fresh weight. About 0.2 g of fresh maize leaves were used to determine the SOD enzyme.

Plant nutrient analysis. The per chloric-acid digestion method was used to determined presence of the nutrients in the plant organs like root leaves and shoot⁴⁰. Maize leaves (0.25 g) were used for nutrient analysis.

“Cations in plants= (ppm in extract – blank) × A × dilution factor”

“WA = Total volume of extract (mL)”.

“W = Weight of dry plants.

Statistical analysis. The experiment was conducted in a completely randomized design (CRD) by using Statistic 8.1.1. (<https://statistix.informer.com/8.1/>). The results are the compare means and standard error of means of three replicates of a treatment.

Results

The experiment was carried out in pots with complete randomize design (CRD) and plants were harvested after 28th day of seed sowing and results were analyzed. Different parameter were observed i.e. fresh biomass, root length, shoot length, leaf size and number of leaves, Cd contents in roots, shoots and seeds germination .

Effect of cadmium (Cd) on maize seed germination percentage. The germination percentage was significantly increased with the inoculation of *Bacillus pumillus* (T2), however the inhibition in germination was observed at all concentration of Cd as compared to the control. About 39% reduction in seed germination percentage was observed in T5 (0.75 mg kg⁻¹ CdSO₄ + uninoculated seed) as compared to control. While inoculation of *Bacillus pumillus* in the presence of Cd increased the germination percentage however this increase was non-significant (Fig. 1a). The germination percentage was increased in *Bacillus pumillus* inoculated seeds

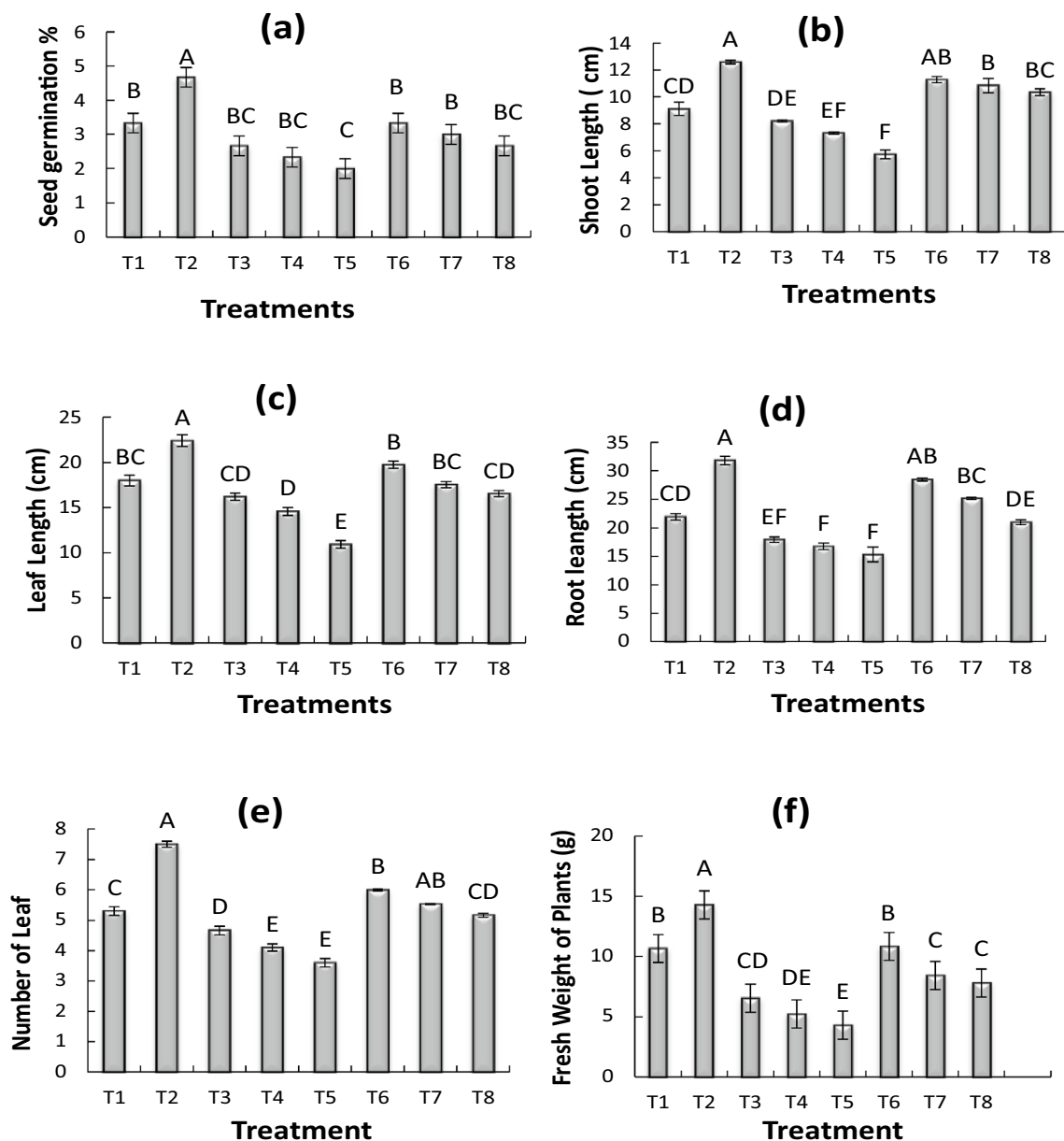


Figure 1. Effect of Cadmium (Cd) on Maize Seed Germination % (a), shoot length (b), leaf length (c), root length (d), no. of leaves (e), fresh weight (f). All treatments sharing common letter with similar bar pattern are similar otherwise differ significantly at $p < 0.05$. T1 = control, T2 = inoculated seed, T3 = 0.25 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T4 = 0.50 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T5 = 0.75 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T6 = 0.25 mg CdSO₄ 100 mL⁻¹ + Inoculated seed, T7 = 0.50 mg CdSO₄ 100 mL⁻¹ + Inoculated seed, T8 = 0.75 mg CdSO₄ 100 mL⁻¹ + Inoculated seed.

as compared to control and uninoculated seeds. The maximum seed germination was observed in T2 (*Bacillus pumillus* inoculated seed) which was 40% higher than control.

Effect of cadmium (Cd) on maize shoot length (cm). The *Bacillus pumillus* inoculation (T2) significantly induced shoot length of maize plant as compared to control (T1) but cadmium (Cd) inhibited the shoot length and maximum reduction (37%) in shoot length was observed in (T5) however *Bacillus pumillus* inoculation significantly increased the shoot length (Fig. 1b). The maximum shoot length was observed in treatment T2 (*Bacillus pumillus* inoculated Seeds.) which was 39% higher than control while 37% reduction in shoot length was observed in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed) as compared to control. Cd also affected leaf length of maize plants. However, the inoculation of maize seeds with *B. pumillus* significantly enhanced the leaf length of maize plant at different concentration of Cd as compared to control and uninoculated maize plants. Moreover, 40% increase in leaf length was observed in T2 (*Bacillus pumillus* inoculated seed) as compared to control while, seeds showed 39% reduction in leaf length in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed) as

compared to control (Fig. 1c). The Fig. 1d shows the root length of maize plant affected by Cd; however, the root length of inoculated maize seeds with *Bacillus pumilus* significantly increased when grown at different concentrations of Cd. The maximum (40%) root length was observed in T2 (*Bacillus pumilus* + seed) which were 40% higher than control and uninoculated plants, as compared to control. The reduction in root length was observed at different concentration of Cd and about 39% reduction in root length was observed in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed) as compared to control, however inoculation of *Bacillus pumilus* significantly enhanced the root length at different concentrations of Cd. The Cd affected the number of leaves in maize plants (Fig. 1e), while inoculation of maize seeds with *Bacillus pumilus* enhanced the number of leaves in maize plants at different concentrations of Cd. The number of leaves were increased by 42% in T2 (*Bacillus pumilus* inoculated Seed) as compared to control and uninoculated seeds, while uninoculated seeds T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed) showed 32% reduction in number of leaves as compared to control. The result presented in Fig. 1 shows the Cd affected fresh weight of maize plants. However, inoculation of maize seeds with *Bacillus pumilus* notably enhanced the fresh biomass of maize plant at different concentration of Cd. The maximum (34%) plant fresh weight was observed in T2 (*Bacillus pumilus* inoculated seed) as compared to control, while 59% reduction in plant fresh biomass was observed in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed) as compared to control.

Effect of cadmium (Cd) on plant protein. Though a reduction in the protein content was observed in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed), and T8 (0.75 mg CdSO₄ kg⁻¹ + inoculated seed) where heavy metals concentration was higher. But, the protein content percentage was significantly higher in T6 (0.25 mg CdSO₄ kg⁻¹ + inoculated seed) by 60%. However, the lower concentration of Cd in (Cd) T3 (0.25 mg CdSO₄ kg⁻¹ + uninoculated seed) also triggered the protein content in maize cultivar by 36% compared to control. In the presence of *Bacillus pumilus* T2 and T7 (0.50 mg CdSO₄ kg⁻¹ + inoculated seed), showed an increase of 36 and 16% (Fig. 2a).

Effect of cadmium (Cd) on peroxide dismutase (POD) enzyme. To scrutinize the effect of various concentrations of Cd, the antioxidant activities (POD and SOD) were determined. Results exhibited that treatment T6 (0.25 mg CdSO₄ kg⁻¹ + inoculated seed), T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated seed), T7 (0.50 mg CdSO₄ kg⁻¹ + inoculated seed), T8 (0.75 mg CdSO₄ kg⁻¹ + inoculated seed), and T3 (0.25 mg CdSO₄ kg⁻¹ + uninoculated seed) showed a significant increase of 346, 246, 213, 106 and 106% as compared to control respectively. On the other hand, a reduction of 13% in POD activity was observed in treatment T2 (*Bacillus pumilus*) (Fig. 2b).

Effect of cadmium (Cd) on superoxide dismutase (SOD) enzyme. The maximum antioxidant (SOD) activity was observed at a higher concentration of Cd specifically in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated Seed). The significant percentage increase of SOD was 769% when compared with control. Likewise, compared with control all other treatments showed a significant increase of SOD enzymatic activity in the presence of *Bacillus pumilus* in T8 (0.75 mg CdSO₄ kg⁻¹ + inoculated Seed), T6 (0.25 mg CdSO₄ kg⁻¹ + inoculated seed), T7 (0.50 mg CdSO₄ kg⁻¹ + inoculated seed) and in uninoculated treatments; T4 (0.5 mg CdSO₄ kg⁻¹ + uninoculated Seed), T3 (0.25 mg CdSO₄ kg⁻¹ + uninoculated Seed) by 437, 338, 287, 220, and 125%. While treatment T2 (*Bacillus pumilus*) showed the least increase with 43% higher than control (T1) (Fig. 2c).

Accumulation of cadmium (Cd) in maize roots (mg/g). There was variation in the accumulation of Cd contents in maize roots which was observed in all the treatment as shown in Fig. 3a. The maximum 45% Cd uptake was found in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated Seed) as compared to control. However, the Cd accumulation was reduced in all treatments when inoculated with *Bacillus pumilus* as compared to uninoculated seeds. The minimum 21% Cd contents in maize plant were observed in T6 (0.25 mg CdSO₄ kg⁻¹ + inoculated Seed).

Accumulation of cadmium (Cd) by maize leaves (mg/g). The Fig. 3b showed variation in Cd contents in maize plant leaves in all the treatment, however the maximum 90% Cd uptake was found in T5 (0.75 mg CdSO₄ kg⁻¹ + uninoculated Seed) as compared to control. While Cd concentration was reduced in all treatments when inoculated with *Bacillus pumilus* as compared to control and uninoculated seeds. (The minimum 45% Cd contents in maize plants observed in T6 (0.25 mg CdSO₄ kg⁻¹ + inoculated Seed).

Accumulation of micro and macro nutrients by maize plants. The results presented in Table 2 showed Cu content increased in all the treatments when inoculated with *Bacillus pumilus* as compared to uninoculated plants. The maximum Cu content (10.14C) was observed in T3 and minimum in T5 (0.2D). The Mn content showed variations in inoculated and uninoculated seeds, and maximum content of Mn (7.9A) was recorded in T3 while minimum content of Mn was observed in T7 (1.16D). Inoculation decreases Na content in plant as compared to uninoculated plants. Maximum Na content observed in T3 (6.11A) and minimum was reported in T8 (0.48E) Fe content showed variation in inoculated seeds as compared to uninoculated seeds. Maximum Fe content was reported in T1 (2.89A) while minimum Fe was in T7 (0.54D). Inoculation of *Bacillus pumilus* increases Ca content in plants while uninoculated plants have low Ca content. Maximum Ca content found in T3 (6.81A) and minimum were found in T8 (0.60E) Mg and K content also showed variation in inoculated and uninoculated plants. Maximum Mg concentration was observed in T2 (1.36A) while minimum concentration was recorded in T5 (0.62D), and maximum K content was noted in T2 (2.72A) and minimum in T8 (0.29D).

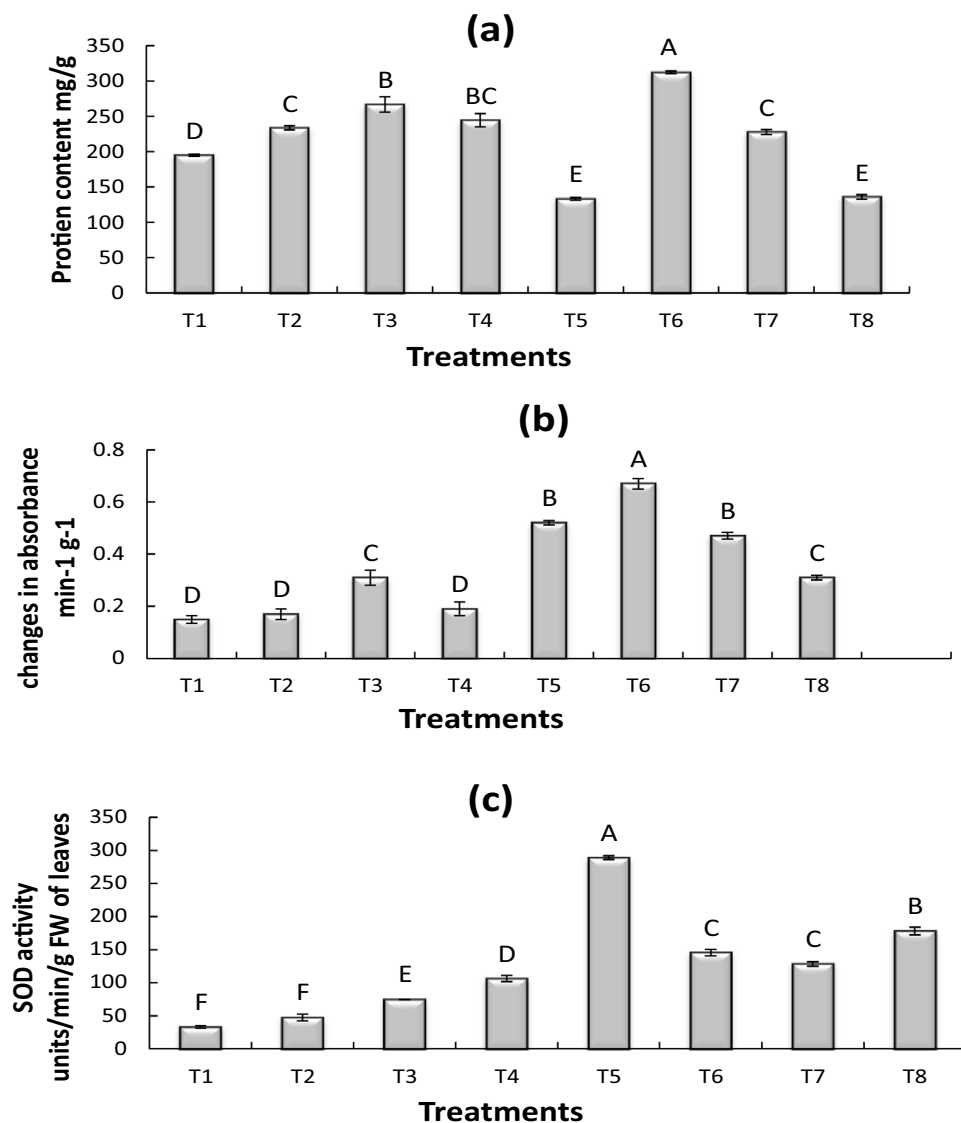


Figure 2. Effect of Cadmium (Cd) on maize protein (a), antioxidant enzymes peroxidase dismutase (b) and super oxidase dismutase (c). All treatments sharing common letter with similar bar pattern are similar otherwise differ significantly at $p < 0.05$. T1 = control, T2 = inoculated seed, T3 = 0.25 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T4 = B = 0.50 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T5 = 0.75 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T6 = 0.25 mg CdSO₄ 100 mL⁻¹ + Inoculated seed, T7 = 0. CdSO₄ 100 mL⁻¹ + Inoculated seed, T8 = 0.75 mg CdSO₄ 100 mL⁻¹ + Inoculated seed.

Discussion

The Cd contaminant adversely affects plants and animals directly and indirectly however, trace amount of Cd in soil did not harm plants¹². Cd enters into soil in different anthropogenic activities as well as by natural process. Heavy metals present in soil and air remain untreated and enters plant body through dust and moisture contents, which first impacts seeds, and roots of plants, afterwards damages shoots and leaves respectively⁴¹. It is obvious that germination or growth of plants is increased in inoculated treatments and the growth of maize plant affected by high concentration of Cd, however the effect is minimized by inoculating with *Bacillus pumilus*. During the present study, the germination was improved with the inoculation of *Bacillus pumilus* when grown over Cd and these findings are in agreement with⁴², who reported that the inoculation of plant seeds with microorganism species like *Pseudomonas*, *Pasteurella*, *Salmonella*, *Bacillus* and *Burkholderia* have the ability to resist. The result finding are also supported by⁴³, who reported that Cd toxicity has decreased seed germination percentage.

The removal of heavy metals contaminants from contaminated site, the combined application of plant and microbe is a successful method as compared to the use of plant or bacteria separately⁴⁴. The higher concentration of lead (Pb) reduces the flower production⁴⁵. In the present study, Cd affected the maize plant in the same way. The higher concentration of Cd can cause plant toxicity and reduction in growth through interference with mineral and Cd absorption, and movement of necessary elements⁸. The findings of present study are in accordance

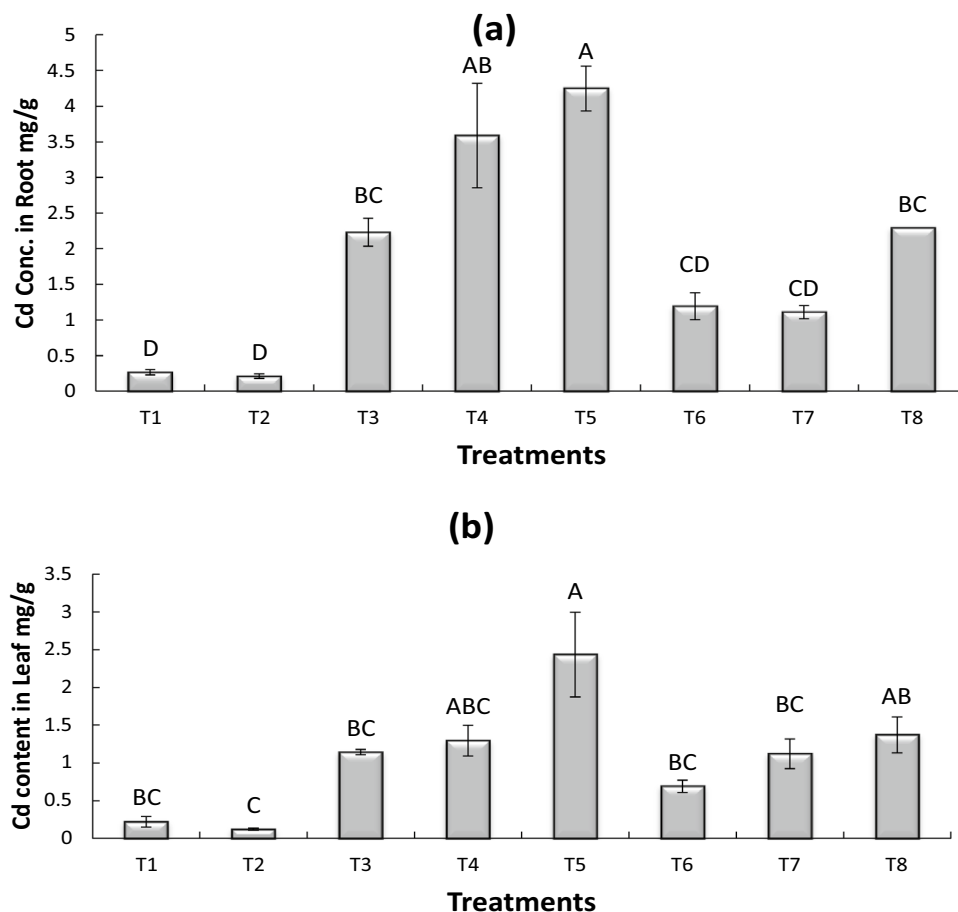


Figure 3. Accumulation of Cadmium (Cd) in maize roots (a) and maize leaves (b). All treatments sharing common letter with similar bar pattern are similar otherwise differ significantly at $p < 0.05$. T1 = control, T2 = inoculated seed, T3 = 0.25 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T4 = 0.50 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T5 = 0.75 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T6 = 0.25 mg CdSO₄ 100 mL⁻¹ + Inoculated seed, T7 = 0. CdSO₄ 100 mL⁻¹ + Inoculated seed, T8 = 0.75 mg CdSO₄ 100 mL⁻¹ + Inoculated seed.

Nutrients	Nutrient concentration							
	T1	T2	T3	T4	T5	T6	T7	T8
Cu (mg/g)	4.33 ± 0.35C	6.84 ± 0.171B	4.33 ± 0.37C	2.66 ± 0.151D	1.98 ± 0.13BD	2.66 ± 0.29CD	2.37 ± 0.28D	2.04 ± 0.26D
Mn (mg/g)	3.28 ± 0.016C	6.40 ± 0.26B	10.47 ± 0.22A	3.33 ± 0.07C	1.78 ± 0.07BD	1.62 ± 0.18D	1.16 ± 0.04D	1.62 ± 0.21D
Na (g/Kg)	1.57 ± 0.03D	5.13 ± 0.18B	6.11 ± 1.21A	2.56 ± 0.19C	2.27 ± 0.17C	0.90 ± 0.04E	0.896 ± 3.03E	0.49 ± 0.5E
K (mg/g)	2.62 ± 0.14A	2.72 ± 0.15A	1.44 ± 0.03B	1.03 ± 0.01BC	0.38 ± 0.01D	0.41 ± 0.02D	0.83 ± 0.22CD	0.29 ± 0.02 D
Fe (mg/g)	2.89 ± 0.24A	1.65 ± 0.15BC	1.41 ± 0.15BCD	0.70 ± 0.14BCD	1.15 ± 2.26BCD	2.04 ± 8.23AB	0.54 ± 1.19D	1.29 ± 0.20BCD
Ca (g/Kg)	1.68 ± 0.12D	4.53 ± 0.14B	6.81 ± 0.13A	5.26 ± 0.020B	2.49 ± 0.13C	2.36 ± 0.19DCD	2.47 ± 0.20C	0.607 ± 0.14E
Mg (g/Kg)	1.18 ± 0.01AB	1.36 ± 0.142A	0.75 ± 0.13BCD	1.11 ± 0.020ABC	0.62 ± 0.019CD	1.066 ± 0.020ABCD	0.56 ± 0.0135D	0.66 ± 0.54CD

Table 2. Accumulation of micro and macro nutrients by maize plants. All treatments sharing common letter are similar otherwise differ significantly at $p < 0.05$. T1 = control, T2 = inoculated seed, T3 = 0.25 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T4 = 0.50 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T5 = 0.75 mg CdSO₄ 100 mL⁻¹ + uninoculated seed, T6 = 0.25 mg CdSO₄ 100 mL⁻¹, + Inoculated seed, T7 = 0. CdSO₄ 100 mL⁻¹ + Inoculated seed, T8 = 0.75 mg CdSO₄ 100 mL⁻¹ + Inoculated seed.

with these results. The Cd concentration reduced the plant growth and prompted phytochelatin (PC), Cd destructively lowers plant growth because it is non-essential element⁴⁶. Inoculation of seeds with *Bacillus pumilus* also enhanced plant growth, this increase in plant length might be due to the production of phytohormones⁴⁷. It is also reported that the growth in bacterial inoculated seeds with different Cd concentrations showed significant leaf growth, which showed that bacterial inoculation can promote the tolerant capacity of plants which are in

agreement with our findings in which the seed inoculated with *Bacillus pumilus* showed better leaf growth under Cd stress⁴⁸. Cd transported from soil to all parts of plants tissue, damages the tissues in various ways, so size of contaminated leaf stunted. Likewise⁴⁹, reported visual symptoms of chlorosis and necrosis in tomato plant when applied up to 25 and 50 μM of CdCl_2 . We also got same result when 75 mg dose of Cd on maize plant caused wilting in uninoculated treatment but inoculated treatment did not showed these symptoms because *Bacillus pumilus* inhibit toxic symptoms by providing tolerance ability.

Root is the first organ of plant which is affected by Cd and Cd adversely affects the root length. The study of⁵⁰, showed similar findings which showed decreased root length in the presence of Cd without any inoculation, because Cd destroyed the protein structure however root length showed better growth when inoculated with *Bacillus pumilus* and accumulation of Cd in roots of *Barlay* plant was 25% more than stem which inhibited the normal growth of plant root^{51,52}. The effects of heavy metals depend on type of environment and toxic substances uptake by plants. Greater the toxic substance in soil will cause reduction in plants growth⁵³ also confirmed our finding that in high level of Cd the maize plant showed reduced growth.

The Cd stress in maize plant produce free radicals which damage membrane and cause leakage of electrolyte⁵⁰, therefore number of leaves decreased in Cd stress⁵⁴ reported soybean plant change its physiology as well as morphology like number, shape and size of leaf against Cd is agreement of our present finding in which the inoculation *Bacillus pumilus* significantly change the structure of bacterial community which enhance growth as compare to control after 15 days of experiment⁵⁵ confirmed our findings that *Bacillus pumilus* promote the tolerance capacity of plants.

In this study *Bacillus pumilus* also enhanced plant fresh weight by producing phytohormones like IAA and GA⁵⁶. These hormones increase the plant root and shoot length, and leaf volume which promote fresh weight of maize plant. The *Bacillus* species also responsible for bioavailability of macro and micro nutrients from soil⁵⁷ have beneficial effect on plant fresh weight.

Root secretions have vital function in altering metal bioavailability, these secretions have various compounds that combine with metals and restrict their movement in soil. These rhizo secretions also provide essential elements to microbial communities that enhance their growth and survival ability. Root secretions have different enzymes and protons that make the soil acidic and increase the heavy metal bioavailability⁵⁸.

Maize plant accumulate Cd in shoots and inhibit the growth of shoot by damaging cell membrane which remove ions from damage site Cd⁵⁹. Result presented in this experiment shows that Cd uptake by maize plant decrease in all treatments that were inoculated with *Bacillus pumillus* as compared to control and stressed plants. The reduction in Cd uptake was observed in plants that were inoculated with *Bacillus pumilus* and highest Cd uptake was observed in uninoculated plants. *Bacillus pumilus* converts Cd in to unavailable form in soil, and also reduces its toxicity. Previous studies also supported these results that inoculation with *Bacillus* species reduces Cd bioavailability^{60–62}.

The plant possess a well-organized antioxidant defense system. The accumulation of Cd toxicity was observed in maize cultivar with various treatments with *B. pumillus* and without *B. pumillus* inoculation in order to discern their ability to tolerate different concentration levels of Cd. The present study revealed that antioxidant activities (POD and SOD) stimulated at the higher concentration of Cd. The higher Cd concentrations in maize cause an increase in enzymatic activities because of the activation of enzymes that are already present in plants^{63–66}. Comparable changes in the enzymatic activities under different concentrations of heavy metals specifically Cd toxicity have been reported earlier^{67,68}. However, some of the studies are in deviation with our results reporting a decrease in SOD activity under the higher concentration of Cd level^{69–71}. The deviation in results could ensue due to the difference in the time duration of Cd stress applied, the intensity of Cd, and specifically plant stage and cultivar. Moreover, no significant increase was observed in maize plants treated with *Bacillus pumilus*⁷².

Present study depicted an increased SOD and POD activity at higher concentrations suggesting that both of these enzymes act simultaneously to avert the formation of OH ions and remove H_2O_2 ^{71,73}. Therefore, the increased enzymatic (particularly SOD) activity at a higher concentration of Cd is considered a good indication for defensive mechanism stimulation⁷⁴. In addition to this, it was observed in a study that the SOD activity was higher at the lower concentration of Cd in soil (20–25 mg/kg), normal when the concentration ranges between 50 and 75 mg/kg Cd in the soil and start to decrease when the soil Cd toxicity levels reached to 100 mg/kg⁷¹. The decrease in the enzymatic activity perhaps might be attributed to inhibition caused by accelerating H_2O_2 ^{75,76}. Thus, heavy metal stress causes an induction of SOD and POD enzymes which in return provides protection and membrane integrity.

It is a known phenomenon that Cd stress leads to the denaturation of proteins. The present study validated the phenomenon that with the gradual increase in the Cd toxicity level the protein content started to decrease. The results are in agreement with the preceding studies demonstrating the reduction of protein content in maize due to Cd stress^{77–79}.

Heavy metals like aluminum, nickel, lead, and Cd accumulate in root of plants and effect metabolisms of plant by reducing cell elongation and new cell formation⁸⁰, so plant cannot promote their growth. Similarly in our present study plants treated with Cd showed stunted growth and accumulates maximum Cd in their roots⁸¹ also reported that most plant species like cucumber, rice, maize and etc. hold chief Cd concentration in their roots which reduced the plant growth by disturbing their metabolic activity. Cd. Soil polluted with Cd impacts roots of plants directly which disturb roots to uptake essential nutrients for metabolic activities of plants. However different plant species have tolerance capacity against specific heavy metals⁸².

Conclusion

In conclusion, Cd adversely affects the growth of maize (*Zea mays*) plant; however inoculation of maize seeds with *Bacillus pumilus* promoted the tolerance to Cd toxicity. The application of *Bacillus pumilus* (T2) showed significant affect than all other treatments in germination, plant height, leaf length, number of leaves and fresh weight. Higher Cd concentration in soil inhibited plant growth, while the inoculation of *Bacillus pumilus* significantly reduced the adverse effect of Cd in all the treatments. Treatment T6 was significantly different from all other treatments under Cd stress. Furthermore, the uptake of Cd in maize is decreased in the presence of *Bacillus pumilus* in soil which reduced the mobility of Cd leading to less Cd accumulation in maize plant. However 0.75 mg/100 ml of Cd was toxic to maize plant but the inoculation of maize seed with *Bacillus pumilus* was effective to reduce Cd toxicity and uptake (T5 and T8). The present investigation reveals that *Bacillus pumilus* inoculation can be used as bio-fertilizer in different level of Cd stress soil.

Received: 27 May 2021; Accepted: 19 July 2021

Published online: 25 August 2021

References

- Vinita, S. *et al.* Potential application of antioxidants. *J. Pharm. Res.* **7**, 828–835 (2013).
- Berendse, F., van Ruijven, J., Jongejans, E. & Keesstra, S. Loss of plant species diversity reduces soil erosion resistance. *Ecosystems* **18**, 881–888 (2015).
- Ayangbenro, A. S. & Babalola, O. O. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int. J. Environ. Res. Public Health* **14**, 94 (2017).
- Rai, A., Fulekar, J. & Fulekar, M. in *Plant Microbiome Paradigm* 163–174 (Springer, 2020).
- Shabir, R. *et al.* Cadmium tolerance and phytoremediation potential of acacia (*Acacia nilotica* L.) under salinity stress. *Int. J. Phytoremed.* **20**, 739–746 (2018).
- Venu, V., Jothamani, P., Krishnamoorthy, S., Prasanthrajan, M. & Kalpana, P. Characterization of heavy metal contamination in mulberry cultivated soils of Erode district in Tamil Nadu. *J. Pharmacogn. Phytochem.* **8**, 730–733 (2019).
- Fereidoun, H. *et al.* The effect of long-term exposure to particulate pollution on the lung function of Teheranian and Zanjanian students. *Pak. J. Physiol.* **3**, 4–7 (2007).
- Karcz, W. & Kurtyka, R. Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. *Biol. Plant.* **51**, 713 (2007).
- Borsari, M. Cadmium: coordination chemistry. In *Encyclopedia of Inorganic and Bioinorganic Chemistry*, 1–16 (2011).
- Cimrin, K. M., Turan, M. & Kapur, B. Effect of elemental sulphur on heavy metals solubility and remediation by plants in calcareous soils. *Fresenius Environ. Bull.* **16**, 1113–1120 (2007).
- Opeolu, B. O., Bamgbose, O., Arowolo, T. & Adetunji, M. Utilisation of biomaterials as adsorbents for heavy metals' removal from aqueous matrices. (2011).
- Jolly, Y. N., Islam, A. & Akbar, S. Transfer of metals from soil to vegetables and possible health risk assessment. *Springerplus* **2**, 1–8 (2013).
- Incrocci, L. *et al.* in *International Symposium on XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010)* 927. 115–122.
- Mansoor, S. *et al.* Biochar as a tool for effective management of drought and heavy metal toxicity. *Chemosphere*, 129458 (2020).
- Ghoneim, M. M. *et al.* Removal of cadmium from aqueous solution using marine green algae, *Ulva lactuca*. *Egypt. J. Aquat. Res.* **40**, 235–242 (2014).
- Cherian, S., Ryu, S. B. & Cornish, K. Natural rubber biosynthesis in plants, the rubber transferase complex, and metabolic engineering progress and prospects. *Plant Biotechnol. J.* **17**, 2041–2061 (2019).
- Rahman, Z. & Singh, V. P. The relative impact of toxic heavy metals (THMs)(arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environ. Monit. Assess.* **191**, 1–21 (2019).
- Pathania, D. & Srivastava, A. Advances in nanoparticles tailored lignocellulosic biochars for removal of heavy metals with special reference to cadmium (II) and chromium (VI). *Environ. Sustain.* 1–14 (2020).
- Das, R. & Jayalekshmy, V. Mechanism of heavy metal tolerance and improvement of tolerance in crop plants. *J. Glob. Biosci.* **4**, 2678–2698 (2015).
- Truyens, S., Weyens, N., Cuypers, A. & Vangronsveld, J. Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environ. Microbiol. Rep.* **7**, 40–50 (2015).
- Karigar, C. S. & Rao, S. S. Role of microbial enzymes in the bioremediation of pollutants: a review. *Enzyme Res.* **2011** (2011).
- Macoy, D. M., Kim, W.-Y., Lee, S. Y. & Kim, M. G. Biotic stress related functions of hydroxycinnamic acid amide in plants. *J. Plant Biol.* **58**, 156–163 (2015).
- Ahmad, I. *et al.* Cadmium-tolerant bacteria induce metal stress tolerance in cereals. *Environ. Sci. Pollut. Res.* **21**, 11054–11065 (2014).
- Tauqeer, H. M. *et al.* Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. *Eco-toxicol. Environ. Saf.* **126**, 138–146 (2016).
- Masood, S., Zhao, X. Q. & Shen, R. F. *Bacillus pumilus* promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization. *Sci. Horticult.* **272**, 109581 (2020).
- Pandey, S., Ghosh, P. K., Ghosh, S., De, T. K. & Maiti, T. K. Role of heavy metal resistant *Ochrobactrum* sp. and *Bacillus* spp. strains in bioremediation of a rice cultivar and their PGPR like activities. *J. Microbiol.* **51**, 11–17 (2013).
- Gururani, M. A. *et al.* Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *J. Plant Growth Regul.* **32**, 245–258 (2013).
- Jamil, M. *et al.* Role of *Bacillus licheniformis* in phytoremediation of nickel contaminated soil cultivated with rice. *Int. J. Phytorem.* **16**, 554–571 (2014).
- Sayari, A., Hamoudi, S. & Yang, Y. Applications of pore-expanded mesoporous silica. 1. Removal of heavy metal cations and organic pollutants from wastewater. *Chem. Mater.* **17**, 212–216 (2005).
- Shahzad, R., Khan, A. L., Bilal, S., Asaf, S. & Lee, I.-J. Plant growth-promoting endophytic bacteria versus pathogenic infections: an example of *Bacillus amyloliquefaciens* RWL-1 and *Fusarium oxysporum* f. sp. lycopersici in tomato. *PeerJ* **5**, e3107 (2017).
- Lindsey III, B. E., Rivero, L., Calhoun, C. S., Grotewold, E. & Brkljacic, J. Standardized method for high-throughput sterilization of *Arabidopsis* seeds. *JoVE J. Vis. Exp.* e56587 (2017).
- Mo, Y. *et al.* Regulation of plant growth, photosynthesis, antioxidation and osmosis by an arbuscular mycorrhizal fungus in watermelon seedlings under well-watered and drought conditions. *Front. Plant Sci.* **7**, 644 (2016).

33. Vernay, P. *et al.* Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. *Chemosphere* **72**, 763–771 (2008).
34. Badshah, L., Hussain, F. & Sher, Z. Floristic inventory, ecological characteristics and biological spectrum of plants of Parachinar, Kurram agency, Pakistan. *Pak. J. Bot.* **48**, 1547–1558 (2016).
35. Jean, L. *et al.* Effect of citric acid and EDTA on chromium and nickel uptake and translocation by *Datura innoxia*. *Environ. Pollut.* **153**, 555–563 (2008).
36. Pan, F. *et al.* Enhanced Cd extraction of oilseed rape (*Brassica napus*) by plant growth-promoting bacteria isolated from Cd hyperaccumulator *Sedum alfredii* Hance. *Int. J. Phytorem.* **19**, 281–289 (2017).
37. Bates, L. S., Waldren, R. P. & Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* **39**, 205–207 (1973).
38. Van Assche, F., Cardinaels, C. & Clijsters, H. Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. *Environ. Pollut.* **52**, 103–115 (1988).
39. Beauchamp, C. & Fridovich, I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **44**, 276–287 (1971).
40. Chapin, F. S. & Van Cleve, K. in *Plant physiological ecology* 185–207 (Springer, 2000).
41. Anjum, N. A. *et al.* Transport phenomena of nanoparticles in plants and animals/humans. *Environ. Res.* **151**, 233–243 (2016).
42. Baudhdh, K. & Singh, R. P. Cadmium tolerance and its phytoremediation by two oil yielding plants *Ricinus communis* (L.) and *Brassica juncea* (L.) from the contaminated soil. *Int. J. Phytoremed.* **14**, 772–785 (2012).
43. Anjum, S. A. *et al.* Alteration in growth, leaf gas exchange, and photosynthetic pigments of maize plants under combined cadmium and arsenic stress. *Water Air Soil Pollut.* **228**, 1–12 (2017).
44. Tara, N. *et al.* On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater. *J. Clean. Prod.* **217**, 541–548 (2019).
45. Alegbeleye, O. O., Opeolu, B. O. & Jackson, V. A. Polycyclic aromatic hydrocarbons: a critical review of environmental occurrence and bioremediation. *Environ. Manag.* **60**, 758–783 (2017).
46. Idrus, F. A., Basri, M. M., Rahim, K. A. A., AbdRahim, N. S. & Chong, M. D. Concentrations of cadmium, copper, and zinc in *Macrobrachium rosenbergii* (giant freshwater prawn) from natural environment. *Bull. Environ. Contam. Toxicol.* **100**, 350–355 (2018).
47. Ryu, R. J. & Patten, C. L. Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. *J. Bacteriol.* **190**, 7200–7208 (2008).
48. Sandalio, L., Dalurzo, H., Gomez, M., Romero-Puertas, M. & Del Rio, L. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **52**, 2115–2126 (2001).
49. Fu, L.-J. *et al.* Systemic induction and role of mitochondrial alternative oxidase and nitric oxide in a compatible tomato–tobacco mosaic virus interaction. *Mol. Plant Microbe Interact.* **23**, 39–48 (2010).
50. Ahmad, P. *et al.* Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS ONE* **10**, e0114571 (2015).
51. Tamás, M. J., Fauvet, B., Christen, P. & Goloubinoff, P. Misfolding and aggregation of nascent proteins: a novel mode of toxic cadmium action in vivo. *Curr. Genet.* **64**, 177–181 (2018).
52. Tiryakioglu, M., Eker, S., Ozkutu, F., Husted, S. & Cakmak, I. Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. *J. Trace Elem. Med Biol.* **20**, 181–189 (2006).
53. Barceló, J. & Poschenrieder, C. Hyperaccumulation of trace elements: from uptake and tolerance mechanisms to litter decomposition; selenium as an example. *Plant Soil* **341**, 31–35 (2011).
54. Weryszko-Chmielewska, E. & Chwil, M. Lead-induced histological and ultrastructural changes in the leaves of soybean (*Glycine max* (L.) Merr.). *Soil Sci. Plant Nutr.* **51**, 203–212 (2005).
55. Herschkovitz, Y., Lerner, A., Davidov, Y., Okon, Y. & Jurkevitch, E. *Azospirillum brasilense* does not affect population structure of specific rhizobacterial communities of inoculated maize (*Zea mays*). *Environ. Microbiol.* **7**, 1847–1852 (2005).
56. Shafi, J., Tian, H. & Ji, M. Bacillus species as versatile weapons for plant pathogens: a review. *Biotechnol. Biotechnol. Equip.* **31**, 446–459 (2017).
57. Shahzad, R. *et al.* Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. *Plant Physiol. Biochem.* **106**, 236–243 (2016).
58. Ma, Y., Oliveira, R. S., Freitas, H. & Zhang, C. Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. *Front. Plant Sci.* **7**, 918 (2016).
59. Poschenrieder, C., Cabot, C., Martos, S., Gallego, B. & Barceló, J. Do toxic ions induce hormesis in plants?. *Plant Sci.* **212**, 15–25 (2013).
60. Ahemad, M. & Khan, M. S. Evaluation of plant-growth-promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. *Ann. Microbiol.* **62**, 1531–1540 (2012).
61. Della Puppa, L., Komárek, M., Bordas, F., Bollinger, J.-C. & Joussein, E. Adsorption of copper, cadmium, lead and zinc onto a synthetic manganese oxide. *J. Colloid Interface Sci.* **399**, 99–106 (2013).
62. Choppala, G. *et al.* Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. *Crit. Rev. Plant Sci.* **33**, 374–391 (2014).
63. Van Assche, F. & Clijsters, H. Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **13**, 195–206 (1990).
64. Lagriffoul, A., Mocquot, B., Mench, M. & Vangronsveld, J. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). *Plant Soil* **200**, 241–250 (1998).
65. Anjum, S. A., Ashraf, U., Khan, I., Saleem, M. F. & Wang, L. C. Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes and yield formation in maize. *Pak. J. Agric. Sci.* **53**, 751–757 (2016).
66. Guo, J. *et al.* Cadmium stress increases antioxidant enzyme activities and decreases endogenous hormone concentrations more in Cd-tolerant than Cd-sensitive wheat varieties. *Ecotoxicol. Environ. Saf.* **172**, 380–387 (2019).
67. Ekmekçi, Y., Tanyolac, D. & Ayhan, B. Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. *J. Plant Physiol.* **165**, 600–611 (2008).
68. Sun, Y., Zhou, Q. & Diao, C. Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L. *Biores. Technol.* **99**, 1103–1110 (2008).
69. Lin, R. *et al.* Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere* **69**, 89–98 (2007).
70. Ci, D., Jiang, D., Dai, T., Jing, Q. & Cao, W. Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. *Chemosphere* **77**, 1620–1625 (2009).
71. Xu, D. *et al.* Cadmium adsorption on plant-and manure-derived biochar and biochar-amended sandy soils: impact of bulk and surface properties. *Chemosphere* **111**, 320–326 (2014).
72. Hayat, K. *et al.* Plant growth promotion and enhanced uptake of Cd by combinatorial application of *Bacillus pumilus* and EDTA on *Zea mays* L. *Int. J. Phytorem.* **22**, 1372–1384 (2020).
73. Liu, K., Yuan, C., Chen, Y., Li, H. & Liu, J. Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Sci. Hort.* **176**, 45–53 (2014).
74. El Dakak, R. A. & Hassan, I. A. The alleviative effects of salicylic acid on physiological indices and defense mechanisms of maize (*Zea mays* L. Giza 2) stressed with cadmium. *Environ. Proces.* **7**, 873–884 (2020).

75. Aravind, P. & Prasad, M. N. V. Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: a free floating freshwater macrophyte. *Plant Physiol. Biochem.* **41**, 391–397 (2003).
76. Luo, X.-S. *et al.* Source identification and apportionment of heavy metals in urban soil profiles. *Chemosphere* **127**, 152–157 (2015).
77. Pál, M., Leskó, K., Janda, T., Páldi, E. & Szalai, G. Cadmium-induced changes in the membrane lipid composition of maize plants. *Cereal Res. Commun.* **35**, 1631–1642 (2007).
78. Wang, C. Q. & Song, H. Calcium protects *Trifolium repens* L. seedlings against cadmium stress. *Plant Cell Rep.* **28**, 1341–1349 (2009).
79. Hussain, A. *et al.* Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. *Environ. Pollut.* **242**, 1518–1526 (2018).
80. Song, X.-Q. *et al.* Disruption of secondary wall cellulose biosynthesis alters cadmium translocation and tolerance in rice plants. *Mol. Plant* **6**, 768–780 (2013).
81. Dresler, S., Wójcik, M., Bednarek, W., Hanaka, A. & Tukiendorf, A. The effect of silicon on maize growth under cadmium stress. *Russ. J. Plant Physiol.* **62**, 86–92 (2015).
82. Tsunemitsu, Y. *et al.* A member of cation diffusion facilitator family, MTP11, is required for manganese tolerance and high fertility in rice. *Planta* **248**, 231–241 (2018).

Author contributions

A.S., M.Q., M.E., M.N. and T.B. were involved in practice contribution of different experiments and authors A.S., M.Q., M.E. were key writers of the manuscript. H.Y. and M.Y. helped in antioxidant analysis while A.A., M.I., M.B. and A.S. helped in statistical analysis and also in preparation of figures while S.Z. helped in writing results and almost all the authors reviewed and contributed at every step.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.S. or M.Q.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021