Acta Bot. Croat. 80 (2), 191–198, 2021
DOI: 10.37427/botcro-2021-022

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Response of *Bacillus cereus* on *Zea mays* under different doses of zinc sulphate

Asim Shahzad[1,2*], Mingzhou Qin[1], Mudassar Nazir[2], Abdul Shakoor[3,4], Motsim Billah[5], Gul Zaib[6]

[1] Henan University, The College of Geography and Environment Science, Jinming ave, Kaifeng, China

[2] Mohi-ud-Din Islamic University Nerian Sharif, Department of Botany, Azad Jammu & Kashmir Pakistan

[3] Henan University, College of Environment and Planning, Jinming ave, Kaifeng, China

[4] Henan University, Laboratory of Geospatial Technology for the Middle and Lower Yellow River Regions, Ministry of Education Kaifeng 475004, Henan, China

[5] University of Haripur, Faculty of Applied Sciences, Khyber Pakhtunkhwa, Pakistan

[6] Institute of Epigenetics and Epigenomics, Yangzhou University, 48 East Wenhui Road, Yangzhou, Jiangsu, 225009, China

Abstract – Anthropogenic activities have added a large amount of heavy metals to the environment. Heavy metal contaminants affect the physiological and biological properties of soil and plant health. Zinc (Zn) is an essential micronutrient and it promotes plant growth and development but a higher concentration of the metal causes reduction in plant growth. The present study was aimed to evaluate the response of *Bacillus cereus* on maize plants at different concentrations of $ZnSO_4$ (20, 40 and 60 mg kg^{-1}) amended in the soil under pot experiment conditions. The experiment was conducted by using complete randomized design (CRD) with three replications. Higher doses of $ZnSO_4$ inhibited maize growth and nutrient uptake. However, inoculation of maize seeds with *Bacillus cereus* at 20 mg kg^{-1} concentration of $ZnSO_4$ increased seed germination about 39% and plant height by 15%. Moreover, 17% increase in leaf length and a 7% increase in leaf number were observed as compared to control at 20 mg kg^{-1} concentrations of $ZnSO_4$. Reductions in all growth parameters were observed with 60 mg kg^{-1} concentration of $ZnSO_4$. The Zn uptake was 75% higher in treatment T8 (uninoculated seeds with 60 mg kg^{-1} concentration of $ZnSO_4$) as compared to treatments which were inoculated and grown under different zinc concentrations. The results suggest that *Bacillus cereus* has good potential to remediate Zn from soil as well as to reduce the phyto-availibility and phytotoxicity of zinc.

Keywords: maize, nutrient availability, phytoextraction, phytotoxicity, plant microbial interaction, zinc

Introduction

Soil is a mixture of different components, organic matter (5%), minerals (45%), gases (25%), and liquids (25%). Besides this, several organisms which support and maintain life on Earth are also found in the soil. Soil provides several ecosystem services by maintaining biogeochemical cycles and protection against erosion (Tahat et al. 2020). Healthy soil acts as a dynamic living system that delivers multiple ecosystem services, such as plant productivity, sustaining water quality and controlling soil nutrient recycling decomposition. Soil health is closely associated with sustainable agriculture, because soil microorganism diversity and activity are the main components of the soil health (Tahat et al. 2020). Anthropogenic and industrial activities, facilities and products such as mining of natural resources, electroplating, smelting, fertilizers, pesticides, tanneries add a large number of contaminants to our natural environment. These contaminants affect the physiological and biological properties of soil (Arivalagan et al. 2014). Among different contaminants, heavy metals such arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), nickel (Ni), zinc (Zn), aluminum (Al) and manganese (Mn) are the most common (Ullah et al. 2015). Heavy metals have shown negative impacts on soil fertility and soil microbial community (Chu 2018). They are not soluble in water and are not read-

* Corresponding author e-mail: agron12@yahoo.com

ily degradable; therefore, they remain insoluble in the soil. Later on, they are assimilated by plant roots. In this way, heavy metals are transferred to different trophic levels of the ecosystem and contaminate the food chain (Tchounwou et al. 2012).

Zinc is an essential micronutrient which promotes plant growth and development (Hefferon 2019); however, higher concentration of Zn causes root blunt, cell wall thickening, reduction in cell division, cell elongation, and disruption in cell organelles and an increase in the number of the nucleoli (Glińska et al. 2016, Taghizadeh et al. 2018). Plants have different defense mechanisms or strategies for tolerance or detoxification, whenever they are exposed to heavy metal stress condition. In addition, microorganisms from soil assist plants to degrade a huge variety of contaminants. In contaminated soil, microorganisms can modify and adopt various mechanisms that convert toxic substances into a less toxic form (Jaiswal et al. 2017). These mechanisms include biosorption, bioaccumulation, biotransformation, and biomineralization. *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium* have the potential to detoxify contaminants in nature (Vergani et al. 2017). Some bacterial species such as *Bacillus* and *Pseudomonas* are generally used for the remediation of heavy metals from contaminated water and soil, as these bacterial strains have more metal binding affinities (Arivalagan et al. 2014, Ullah et al. 2015). Plant growth-promoting bacteria increase plant growth and development by increasing accessibility of nutrients (Bulgarelli et al. 2013), which contributes to plant health and increases their biomass and absorbing capacity to overcome stress (Weyens et al. 2013). *Rhizobia* have the potential to remove organic and metal contamination by degrading organic contaminants (Teng et al. 2015). Besides, bacteria have the ability to produce phytohormones such as auxins, cytokinins and gibberellins, which control all aspects of plant growth and development (Glick 2010). Plant growth-promoting bacteria enhance plant growth in stress conditions through various mechanisms including nitrogen (N) and phosphorus (P) solubilization, siderophore production and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production (Fatima et al. 2017).

Increased concentration of heavy metals inhibits normal plant functioning by disturbing protein structure; this alteration hampers metabolic processes (Hall 2002). The change in in protein structure can cause mutation in various physiological processes in plants such as photosynthesis, respiration, and enzymatic activities (Hossain et al. 2012). Plants have different defense mechanisms or strategies for tolerance or detoxification whenever they are exposed to elevated concentration of heavy metals. The first step in metal tolerance is inhibition or restriction of heavy metal entry into plant root (Viehweger 2014). Failure of the first step activates the mechanism of phytosequestration which permits heavy metals to enter in intracellular compartments (Patra et al. 2004), which produce different substances like phytochelatins (PCs), organic acid, polysaccharides and metallothionein (MTs), which are the two best-characterized metal-binding ligands in plant cells that help a plant to detoxify heavy metals (Dalvi and Bhalerao 2013). If all these strategies fail, plants activate their antioxidant defense mechanism which can help the plant by inducing reactive oxygen species (ROS), which inhibit most cellular processes at various levels of metabolism. ROS, being highly unstable, could play dual role (1) damaging cellular components and (2) act as an important secondary messenger for inducing plant defense system. Cells are equipped with enzymatic and non-enzymatic defense mechanisms to counteract this. (Sytar et al. 2013, Manara 2012).

Any plant used for phytoremediation should not be metal-tolerant, and must be fast growing with the potential to produce high biomass. Maize is mainly used in phytoextraction due to the fact that it is a common and rapidly growing crop, which possesses an extensive fibrous root system and can tolerate stress conditions (Farooq et al. 2015). Therefore, maize plants are suitable for the extraction of heavy metals from contaminated soil (Lu et al. 2015). There is insufficient information about the fate of bacterial inoculation in maize edible and non-edible parts to enhance maize tolerance in heavy metal soils (Shahzad et al. 2016). Henceforth, present investigation is aimed to evaluate the role of *Bacillus cereus* strain in enhancing the phytoextraction ability of maize plant under Zn contaminated soil conditions.

Materials and methods

Bacterial strain

Bacillus cereus (Accession no. KR232400) was selected on the basis of its plant growth promoting potential (Shahzad et al. 2020). The strain was obtained from phytohormone laboratory at Quaid-i-Azam University, Pakistan. Bacterial strain was previously identified by 16S rRNA gene sequencing (Shahzad et al. 2016).

Preparation of heavy metal solution

Three different concentrations of Zn solution (20, 40 and 60 mg kg^{-1}) for maize plant treatments were prepared in pure distilled water by dissolving zinc sulfate ($ZnSO_4$). We selected these three concentrations on the basis of previous scientific data (Long et al. 2003, Yang et al. 2005). Pure distilled water was used as the control for the experiment. 200 mL of each solution was added in 1 kg of potted soil.

Preparation of inoculum

For the preparation of inoculum, five ml of nutrient broth medium (Oxoid, UK) was autoclaved in test tubes for cultivation of bacterial strain. Bacteria were incubated in a shaker incubator (EXCELLA E24 Germany) at 37 °C and 150 rpm for 48–72 h. Thereafter, the culture was centrifuged at 3000 rpm for 10 min. The pellet was re-suspended in autoclaved distilled water and the optical density (OD) was adjusted to 0.100 at 660 nm with UV-VIS spectrophotometer (UV-VIS Double Beam Spectrophotometer, Model NO:

AE-S90-2D A & E Lab (UK) Co., Ltd.). Bacterial density was 10^6 cells mL^{-1} at optical density of 0.10 at 660 nm. The optical density was adjusted by adding about 200 ml sterile water to pure bacterial pellets.

Preparation of treatment applications and seed inoculation

Kashmir Gold variety maize (*Zea mays* L) seeds were collected from the National Agricultural Research Centre, Islamabad, Pakistan. The seeds were surface sterilized by washing with 95% ethanol, following by soaking in 10% Clorox for 2–3 minutes. The seeds were washed successively 2–3 times in autoclaved distilled water.

For experiment preparation, seeds were soaked in the *B. cereus* inoculum for 2–3 hrs, after that about 10 seeds were sown in each pot. Different concentrations of Zn solutions (100, 200 and 300 mg L^{-1}) and distilled water was added to each pot. Eight different treatments with three replicates were made. Detailed explanation of treatments is given in Table 1.

Parameter measured

The percentage germination was noted after seven day of sowing. Maize seedlings were uprooted after 21 days of sowing, after which root length (cm), plant height (cm), leaf length (cm), number of leaves and fresh plant weight parameters were recorded. Each treatment contained three replicates and 5 plants from each replicate were measured.

Soil nutrient analyses

Determination of macro- and micro-nutrients (Na, Ca, Mg, K, P, N, Fe, Cu, Cr, Co, Zn, and Mn) was carried out by following the ammonium bicarbonate and diethylenetriamine pentaacetic acid (DTPA) method as described by Soltanpour and Schwab (1977). The 0.005 M ammonium bicarbonate and (DTPA) solution was prepared by adding 1.97 g of DTPA to 800 mL of distilled water. About 2 mL of ammonium hydroxide (NH_4OH) was added to facilitate dilution and to prevent effervescence due to ammonium bicarbonate addition. When DTPA was dissolved completely, 79.06 g of ammonium bicarbonate (NH_4HCO_3) was added.

Tab. 1. Kashmir Gold variety *Zea mays* seeds were inoculated (+) or not (–) with *Bacillus cereus*, germinated and grown in soil supplemented with 20, 40 or 60 mg kg^{-1} ZnSO4. Eight treatments (T) were applied.

Treatments	*Bacillus cereus* inoculation	$ZnSO_4$ (mg kg^{-1})
T1	–	0
T2	+	0
T3	+	20
T4	+	40
T5	+	60
T6	–	20
T7	–	40
T8	–	60

The pH of the solution was adjusted to 7.6 by adding 2 mL of ammonium hydroxide. Final volume was adjusted to 1 L with the addition of distilled water. For the analysis of different nutrient, 10 g of soil sample was mixed with 15 mL of distilled water. The suspension was stirred for 30 minutes on a magnetic stirrer. After stirring step, suspension was filtered. Two mL of filtrate was removed and 18 mL of distilled water was added for nutrient analysis.

Plant nutrient analysis

The perchloric-acid digestion scheme was used to determine the content of various nutrients in maize plants (Allen et al. 1974). A solution of nitric acid, sulfuric acid, and perchloric acid in the ratio 5:1:0.1 (6.5 mL) was added to 0.25 g of plant material in a 50 mL flask and the mixture was heated on the hot plate in the fume hood to complete the digestion process. The formation of white fumes in the mixture confirmed the completion of digestion of the plant extract. Thereafter, distilled water (few drops) was poured into the flask and the mixture was allowed to cool. The digested plant extract samples were transferred to the volumetric flasks (50 mL) and the volume was raised up to 50 mL by the addition of distilled water. The samples were filtered with Whatman no.42 filter paper and were stored for further element analysis. The concentration of different elements/nutrients present in the plant samples were measured by atomic absorption spectrophotometer (Shimadzu AA-670 Germany).

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. Five plants from each replicate were measured.

Results

Effects on seed germination

Percentage germination of maize seeds has shown variations among inoculated and uninoculated seeds (Fig. 1a). Maximum seed germination (%) was observed in *B. cereus*-inoculated seeds in combination with 20 mg kg^{-1} $ZnSO_4$ (treatment T3), which was significantly higher than control (39%) and the majority of the treatments. High germination percentage was also recorded in *B. cereus*-inoculated seeds in combination with 40 mg kg^{-1} $ZnSO_4$ (treatment T4), which was significantly higher than the treatment of uninoculated seeds with 60 mg kg^{-1} $ZnSO_4$ (treatment T8), where the lowest values were obtained (germination reduction of 9% as compared to control).

Effects on root length, leaf length and number of leaves

The root length was only mildly affected by investigated treatments (Fig. 1b). The maximum increase in the root length (15%) was observed in *B. cereus* inoculated seeds in

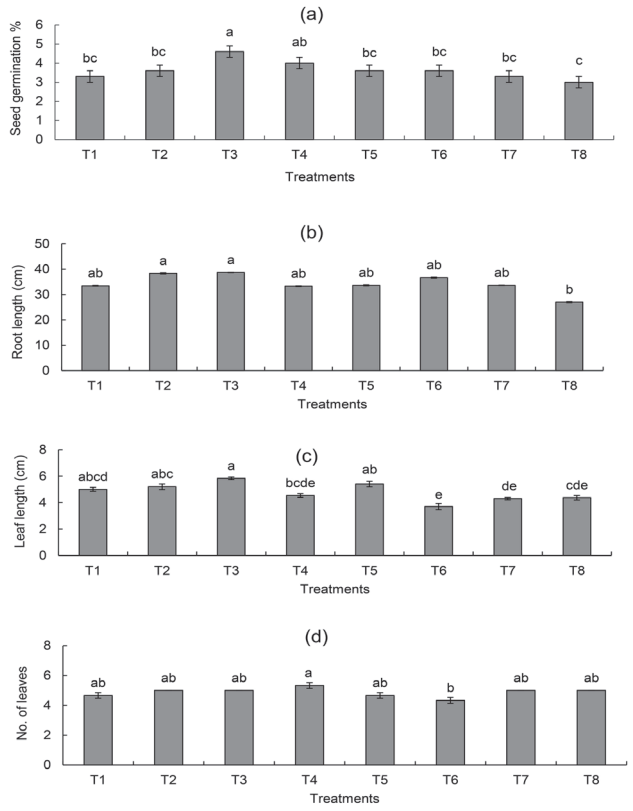

Fig. 1. Effect of zinc on Kashmir Gold variety *Zea mays* seed germination % (a), root length (b), leaf length (c) and number of leaves (d). Plants were measured at 21 day after sowing. Results, expressed as means ± standard error (SE) of 3 replicates and each replica contains 10 plants. Results were compared by using compare means with completely randomized design (CRD). Treatments sharing a common letter (a>b>c) are similar, otherwise differ significantly at $P < 0.05$. T1 – control, T2 – *Bacillus cereus*-inoculated seeds, T3 – *B. cereus*-inoculated seed + 20 mg kg^{-1} $ZnSO_4$, T4 – *B. cereus*-inoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T5 – *B. cereus*-inoculated seeds + 60 mg kg^{-1} $ZnSO_4$, T6 – uninoculated seeds + 20 mg kg^{-1} Zn SO_4, T7 – uninoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T8 – uninoculated seeds + 60 mg kg^{-1} $ZnSO_4$.

combination with 20 mg kg^{-1} $ZnSO_4$ (treatment T3), although it was not significant compared to control. Similar increase in root length (14%) was also recorded for *B. cereus*-inoculated seed (treatment T2 treatments). On the contrary, the 19 % reduction in root length was observed in the treatment of uninoculated seeds exposed to60 mg kg^{-1} $ZnSO_4$ (treatment T8), which was not significant compared to control, but was significantly reduced in comparison to treatments T2 and T3. Other treatments exhibited values similar to those of control

Maximum increase in leaf length (17%) was observed in *B. cereus*-inoculated seeds in combination with 20 mg kg^{-1} $ZnSO_4$ (treatment T3), although it was not significant as compared to control and other treatments of inoculated seeds; however, it was significantly higher in comparison to result obtained for uninoculated seeds (Fig. 1c). Namely, all treatments of uninoculated seeds resulted in reduction in leaf length, which was particularly after exposure to 20 mg kg^{-1} $ZnSO_4$ (treatment T6).

Maximum number of leaves (1% increase compared to control) was observed in *B. cereus*-inoculated seeds in combination with 40 mg kg^{-1} $ZnSO_4$ (treatment T4), although this value was not significantly different as compared to the majority of the treatments; the only exception was the treatment of uninoculated seeds exposed to 20 mg kg^{-1} $ZnSO_4$ (treatment T6), which exhibited the lowest value (7% decrease compared to control) (Fig. 1d).

Effects on plant height, fresh weight and zinc uptake

The inoculation of maize seeds with *B. cereus* significantly increased the fresh weight and height of maize plants (Fig. 2). A significantly higher increase in fresh weight was observed upon exposure of inoculated seeds to 20 mg kg^{-1} $ZnSO_4$ (treatment T3, 80% increase) and 40 mg kg^{-1} $ZnSO_4$ (treatment T4, 62% increased) in comparison to control and the treatments of uninoculated seeds. On the contrary, a significant reduction in plant fresh weight as compared to control was observed in all treatments of uninoculated seeds, with the maximum reduction of 27% recorded upon exposure to 60 mg kg^{-1} $ZnSO_4$ (treatment T8) (Fig. 2b).

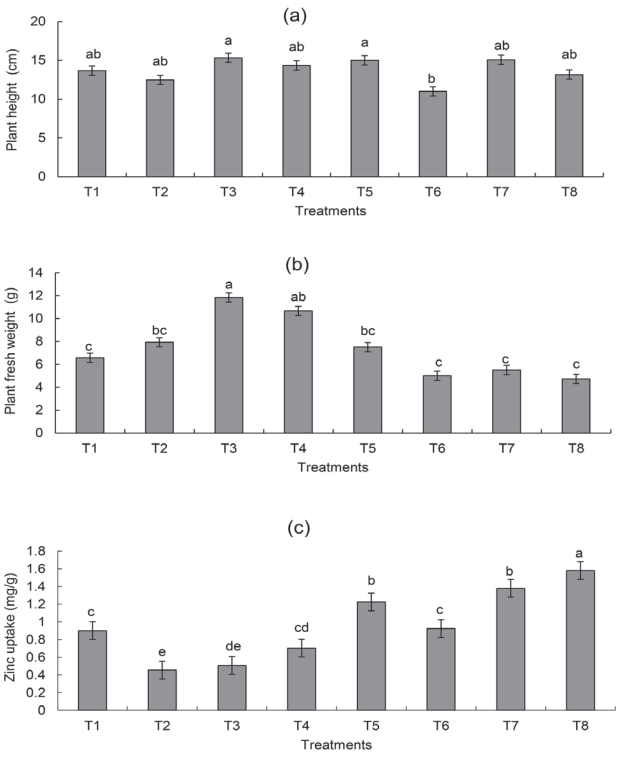

Fig. 2. Effect of zinc on Kashmir Gold variety *Zea mays* plant height (a), plant fresh weight (b) zinc uptake (c). Plants were measured at 21 day after sowing. Results, expressed as means ± standard error (SE) of 3 replicates and each replica contains 10 plants. Results were compared by using compare means with completely randomized design (CRD). Different letters (a> b> c) indicate treatments sharing a common letter are similar but otherwise differ significantly at $P < 0.05$. T1 – control, T2 – *B. cereus*-inoculated seeds, T3 – *B. cereus*-inoculated seed + 20 mg kg^{-1} $ZnSO_4$, T4 – *B. cereus*-inoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T5 – *B. cereus*-inoculated seeds + 60 mg kg^{-1} $ZnSO_4$, T6 – uninoculated seeds + 20 mg kg^{-1} Zn, T7 – uninoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T8 – uninoculated seeds + 60 mg kg^{-1} $ZnSO_4$.

Exposure of inoculated seeds to $ZnSO_4$ slightly improved the plant height (treatments T3, T4 and T5) as compared to control; the maximum value (12% increase as compared to control) was obtained upon exposure to 20 mg kg^{-1} Zn (treatment T3). On the other hand, exposure of uninoculated seeds to the same Zn concertation (20 mg kg^{-1}) resulted in a reduction in plant height up to 19% (Fig. 2a).

Zinc uptake was reduced in the majority of the treatments with inoculated seeds as compared to control; significantly for treatments T2 and T3 (exposure to 100 mg L^{-1} $ZnSO_4$), and notably for treatment T4 (exposure to 49 mg kg^{-1}); reduced Zn uptake was 49%, 44% and 22% in T2, T3 and T4 respectively (Fig. 2c). However, the exposure of inoculated seeds to the highest tested Zn concentration (treatment T5) resulted in a significantly higher Zn uptake as compared to control. Among treatments with uninoculated seeds, exposure to 20 mg kg^{-1} (treatment T6) resulted in a value similar to that of control, while upon treatment with 40 and 60 mg kg^{-1} Zn (treatments T7 and T8, respectively) Zn uptake was significantly higher; the maximum increase (75% as compared to control) was observed in treatment T8 (Fig. 2c).

Nutrients status of soil and accumulation of nutrients by maize plant

Different nutrients including Ca, Mg, K, Na, Fe, Cd, Cu, Cr, Zn, Co and Ni were detected in soil samples (Tab. 2). The plant nutrient status (Tab. 3) showed that the Cu content decreased in majority of the treatments as compared to control; the only exceptions were treatments T3 and T8. The Mn content was significantly elevated in the treatment of plants from inoculated seeds exposed to 20 mg kg^{-1} $ZnSO_4$ (treatment T3), while the exposure of plants from uninoculated seeds to 40 mg kg^{-1} $ZnSO_4$ (treatment T7) resulted

Tab. 2. Analysis of soil micro and macro nutrients before addition of $ZnSO_4$ and sowing of *Zea mays* measured in mg kg^{-1}. Results are expressed as means ± standard error (SE) of three soil samples.

Nutrient	Soil content (mg kg^{-1})
Ca	19.6 ± 1.11
Mg	2.46 ± 0.23
K	1.67 ± 0.21
Na	22.39 ± 0.66
Fe	3.18 ± 0.12
Cd	4.39 ± 0.19
Cu	11.20 ± 0.44
Cr	10.5 ± 0.12
Zn	8.9 ± 0.11
Co	0.19 ± 0.02
Ni	2.23 ± 0.15
Mn	6.22 ± 0.27

Tab. 3. Accumulation of micro and macro nutrients by Kashmir Gold variety *Zea mays* plants. Plants were measured at 21 day after sowing. Results, expressed as means ± standard error (SE) of three replicates and each replica contains 5 plants. Results were compared by using compare means with completely randomized design (CRD). Treatments sharing a common letter (A>B>C) are similar, otherwise differ significantly at P <0.05. T1 – control, T2 – *Bacillus cereus*-inoculated seeds, T3 – *B. cereus*-inoculated seed + 20 mg kg^{-1} $ZnSO_4$, T4 – *B. cereus*-inoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T5 – *B. cereus*-inoculated seeds + 60 mg kg^{-1} $ZnSO_4$, T6 – uninoculated seeds + 20 mg kg^{-1} Zn, T7 – uninoculated seeds + 40 mg kg^{-1} $ZnSO_4$, T8 – uninoculated seeds + 60 mg kg^{-1} $ZnSO_4$.

Nutrient	Nutrient concentration (mg kg^{-1})							
	T1	T2	T3	T4	T5	T6	T7	T8
Cu	0.33 ± 0.01 AB	0.15 ± 0.001 D	0.38 ± 0.02 A	0.20 ± 0.001 D	0.30 ± 0.02 BC	0.21 ± 0.02 CD	0.16 ± 0.02 D	0.2±0.01 D
Mn	0.12 ± 0.01 BC	0.14 ± 0.01 B	0.2 ± 0.02 A	0.07 ± 0.003 CD	0.09 ± 0.01 BCD	0.06 ± 0.01 CD	0.05 ± 0.01 D	0.09± 0.01 CD
Ni	0.02 ± 0.001 B	0.008 ± 0.0002 D	0.03 ± 0.002 A	0.013 ± 3.33 BC	0.007 ± 0.001 D	0.007 ± 0.0004 D	0.009 ±0.002 CD	0.009 ± 0.0003 CD
Co	0.10 ± 0.001 B	0.11 ± 0.002 A	0.03 ± 0.001 F	0.05 ± 0.002 DE	0.03 ± 0.0001 F	0.04 ± 3.85 EF	0.05 ± 0.005 CD	0.06 ± 0.001 C
Na	11.3 ± 0.34 E	16.1 ± 0.7CD E	13.3 ± 1.0 DE	29.19 ± 0.5 AB	33.8 ± 0.52 A	35.9 ± 0.9 A	23.6 ± 3.6 BC	19.1 ± 0.2 CD
Cr	0.20 ± 0.01 BC	0.3 ± 0.02 AB	0.4 ± 0.02 A	0.32 ± 0.05 A	0.20 ± 0.014 BC	0.12 ± 0.003 C	0.13 ± 0.02 C	0.1 ± 0.03 C
Fe	30.3 ± 0.1 C	34.1 ± 0.8 BC	43.9 ± 0.3 ABC	47.01 ± 0.5 AB	49.9 ± 2.03 A	28.1 ± 8.17 ABC	11.8 ± 1.5 D	10.1 ± 0.31 D
Ca	13.1 ± 0.2 E	31.4 ± 0.6 C	39.82 ± 0.4 B	49.60 ± 1.2 A	37.4 ± 0.7 B	18.7 ± 0.1 D	17.8 ± 0.9 D	14.07 ± 0.3 E
Mg	7.10 ± 0.6 E	29.3 ± 0.4 BC	35.23 ± 0.9 A	30.9 ± 0.3 ABC	32.5 ± 1.2A B	25.7 ± 1.2 C	14.5 ± 1.5 D	13.4 ± 0.4 D
K	34.7 ± 0.5 B	58.3 ± 4.1 A	61.34 ± 2.01 A	56.20 ± 1.1 A	47.3 ± 0.8 AB	30.07 ± 4.7 B	10.4 ± 4.3 C	6.7±0.7 C

in significantly lower Mn content as compared to control. Reduced Ni and Co contents were recorded in the majority of treatments as compared to control. Seed inoculation with *B. cereus* in general decreased the Na content as compared to values obtained in uninoculated plants, since the higher values were obtained in treatments of plants from uninoculated seeds (treatments T6, T7 and T8) as compared to treatments of plants from inoculated seeds to the corresponding Zn concentrations. Seed inoculation with *B. cereus* increased Cr content in plants as compared to control as well as to the treatments of plants from uninoculated seeds upon exposure to 20 and 40 mg kg^{-1} $ZnSO_4$ (treatments T3 and T4) as compared to treatments T6 and T7, respectively). Fe content was significantly elevated in plants from inoculated seeds upon exposure to 40 and 60 mg kg^{-1} $ZnSO_4$ compared to control as well as to the exposure of plants from uninoculated seeds to the same Zn concentration. Seed inoculation with *B. cereus* in general increased the Ca and Mg uptake in all investigated treatments as compared to control, with higher values obtained in plants from inoculated seeds compared to the uninoculated ones. K content was significantly higher in plants from inoculated seeds as compared to the control as well as to plants from uninoculated seeds exposed to the corresponding Zn concentrations.

Discussion

Zinc is a trace element that performs a vital role in plant growth and development. However, at higher concentrations, it reduces seed germination, length of seedlings and stem as well as contents of chlorophyll, carotenoid, sugar, and amino acids (Todeschini et al. 2011, Glińska et al. 2016). In this study, the effects of zinc toxicity on plant height, leaf length, number of leaves, root length, fresh weight, and nutrient content was observed. Our results indicated that high Zn concentrations inhibited seed germination as compared to the control. Previous studies found a negative impact of Zn on plant growth parameters. Current findings are in agreement with previous studies (Islam et al. 2014, Glińska et al. 2016, Boi et al. 2020), in these studies, they reported that increased heavy metal concentration causes decrease in plant biomass and growth. Nonetheless, maize inoculated with *B. cereus* showed increases in seed germination, plant height and fresh weight as well as nutrient uptake as compared to control and uninoculated plants. Enhanced root growth results due to inoculation of *B. cereus* under higher concentrations of zinc and thus a positive impact of *B. cereus* on root growth was observed, which may be due to the stress tolerance capacity of *B. cereus* (Hassan 2018). Improvement in plant root development under zinc concentration resulted in an increase in nutrient uptake, which increased seed germination, plant height, leaf length, number of leaves, root length and plant fresh weight. Inoculated seeds showed an increase in leaf growth and number of leaves as compared to un-inoculated plants grown in the soil supplemented with zinc.

We argue that gibberellins play a vital role in plant growth development. Scientific data suggests that *B. cereus* helps the plant by producing different plant promoting hormones i.e gibberellic acid (GA). Therefore, the increase in some plant growth parameters due to inoculation of *B. cereus* might be because of production of certain plant promoting enzymes, which can stimulate seed germination by enhancing growth ability (Finch-Savage and Leubner-Metzger 2006, Tuan et al. 2018). Gibberellins increase leaf area by suppressing peroxidase secretion (de Souza and MacAdam 2001), and induce cell mitotic division (Takatsuka and Umeda 2014, Fonouni-Farde et al. 2019). Moreover, some researchers suggested that *B. cereus* induces production of indole acetic acid (IAA), which may help to increase root length, plant height, and leaf area (Shafi et al. 2017). Henceforth, phytohormones produced by *B. cereus* might have played vital role in maize growth. Zn uptake decreased in *B. cereus*-inoculated maize plants. Common logic suggests that microbes including *Bacillus* species have ability to convert Zn into smaller, inaccessible and immoveable form in the soil (Saravanan et al. 2004, Li et al. 2016). Another explanation for reduced Zn uptake is production of metal binding proteins in the rhizosphere. *Bacillus cereus* produces metal binding proteins to counter heavy metal toxicity and these proteins have ability to restrict phytoavailability of heavy metals (Islam et al. 2014). We also demonstrated the accumulation of macro and micronutrients in maize plant owing to *B. cereus*. *Bacillus cereus* showed increase uptake of Cu, Mn, Ni, Na, Cr, Fe, Ca, and Mg in maize plant. Tiwari et al. (2013) also demonstrated that *Bacillus* species can enhance the phytoavailbility of nutrients Fe, Cd, Pb, Cr, Ni, Cu.

Conclusion

It is concluded from the present investigation that maize seeds inoculated with *B. cereus,* in the presence of 20 mg kg^{-1} of zinc showed significant increases in seed germination, plant height, leaf length, number of leaves; however, higher concentrations of zinc in uninoculated seeds indicated reduction in plant growth. Furthermore, the uptake of zinc in maize is decreased in the presence of *B. cereus* in the soil, which reduces the mobility of zinc leading to lower zinc accumulation in the maize plant. Our study demonstrated the positive impact of *B. cereus* on maize growth at different levels of Zinc toxicity. The present investigation revealed that *B. cereus* inoculation can be used as bio-fertilizer in Zinc-stressed soil.

References

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C., 1974: Chemical analysis of ecological materials. Blackwell Scientific, Oxford.

Arivalagan, P., Singaraj, D., Haridass, V., Kaliannan, T., 2014: Removal of cadmium from aqueous solution by batch studies using *Bacillus cereus*. Ecological Engineering 71, 728–735.

Boi, M.E., Porceddu, M., Cappai, G., De Giudici, G., Bacchetta, G., 2020: Effects of zinc and lead on seed germination of *He-*

lichrysum microphyllum subsp. *tyrrhenicum*, a metal-tolerant plant. International Journal of Environmental Science and Technology 17, 1917–1928.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P., 2013: Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology 64, 807–838.

Chu, D., 2018: Effects of heavy metals on soil microbial community. IOP Conference Series Earth and Environmental Sciences 113, 012009.

Dalvi, A.A., Bhalerao, S.A., 2013: Response of plants towards heavy metal toxicity: an overview of avoidance, tolerance and uptake mechanisms. Annals of Plant Sciences 2, 362–368.

de Souza, I.R.P., MacAdam, J.W., 2001: Gibberellic acid and dwarfism effects on the growth dynamics of B73 maize (*Zea mays* L.) leaf blades: a transient increase in apoplastic peroxidase activity precedes cessation of cell elongation. Journal of Experimental Botany 52, 1673–1682.

Farooq, M., Hussain, M., Wakeel, A., Siddique, K.H.M., 2015: Salt stress in maize: effects, resistance mechanisms, and management. A review. Agronomy of Sustainable Development 35, 461–481.

Fatima, K., Imran, A., Saeed, M., Afzal, M., 2017: Plant-bacteria synergism: An innovative approach for the remediation of crude oil-contaminated soils. Soil & Environment 36, 93–113.

Finch-Savage, W.E., Leubner-Metzger, G., 2006: Seed dormancy and the control of germination. New Phytologist 171, 501–523.

Fonouni-Farde, C., Miasssod, A., Laffont, C., Morin, H., Bendahmne, A., Diet, A., Frugier, F., 2019: Gibberellins negatively regulate the development of *Medicago truncatula* root system. Scientific Reports 9, 2335.

Glick, B.R., 2010: Using soil bacteria to facilitate phytoremediation. Biotechnology Advances 28, 367–374.

Glińska, S., Gapińska, M., Michlewska, S., Skiba, E., Kubicki, J., 2016: Analysis of *Triticum aestivum* seedling response to the excess of zinc. Protoplasma 253, 367–377.

Hall, J.L., 2002: Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53, 1–11.

Hassan, T., 2018: *Bacillus cereus*: A competent plant growth promoting *Bacterium* of saline sodic field. Pakistan Journal of Botany 50, 1029–1037.

Hefferon, K., 2019: Biotechnological approaches for generating zinc-enriched crops to combat malnutrition. Nutrients 11, 253.

Hossain, M.A., Piyatida, P., da Silva, J.A.T., Fujita, M., 2012: Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany 2012, 37.

Islam, F., Yasmeen, T., Riaz, M., Arif, M.S., Ali, S., Raza, S.H., 2014: Proteus mirabilis alleviates zinc toxicity by preventing oxidative stress in maize (*Zea mays*) plants. Ecotoxicology and Environmental Safety 110, 143–152.

Jaiswal, D.K., Verma, J.P., Yadav, J., 2017: Microbe induced degradation of pesticides in agricultural soils. In: Singh, S. (ed.), Microbe-induced degradation of pesticides, 167–189. Environmental Science and Engineering. Springer, Cham.

Li, X., Peng, W., Jia, Y., Lu, L., Fan, W., 2016: Bioremediation of lead contaminated soil with Rhodobacter sphaeroides. Chemosphere 156, 228–235.

Long, X.X., Yang, X.E., Ni, W.Z., Ye, Z.Q., He, Z.L., Calvert, D.V., Stoffella, J.P., 2003: Assessing zinc thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. Communications in Soil Science and Plant Analysis 34, 1421–1434.

Lu, J., Wilson, P.C., Wen, X., Jin, Q., Wu, J., 2015: Harmful chemicals in the environment: Measurement, fate and remediation. Journal of Chemistry 723749, 2.

Manara, A., 2012: Plant responses to heavy metal toxicity. In: Furini, A. (ed.), Plants and heavy metals, 27–53. Springer, Dordrecht, Netherlands.

Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., 2004: Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany 52, 199–223.

Saravanan, V.S., Subramoniam, S.R., Raj, S.A., 2004: Assessing in vitro solubilization potential of different zinc solubilizing bacterial (zsb) isolates. Brazilian Journal of Microbiology 35, 121–125.

Shafi, J., Tian, H., Ji, M., 2017: *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnology and Biotechnological Equipment 31, 446–459.

Shahzad, A., Bano, A., Siddiqui, S., 2020: Effect of bacterial consortium on alfalfa (*Medicago sativa* L.) plant nutrient uptake and antioxidant enzymes at different levels of oily sludge. Israel Journal of Plant Sciences 1, 1–13.

Shahzad, A., Saddiqui, S., Bano, A., 2016: The response of maize (*Zea mays* L.) plant assisted with bacterial consortium and fertilizer under oily sludge. International Journal of Phytoremediation 18, 521–526.

Soltanpour, P., Schwab, A.A., 1977: New test for simultaneous extraction of macro- and micro-nutrients in alkaline soils. Communication in Soil Science and Plant Analysis 8, 195–207.

Sytar, O., Kumar, A., Latowski, D., 2013: Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiologiae Plantarum 35, 985–999.

Taghizadeh, M., Solgi, M., Karimi, M., Khoshbin, S., 2018: Heavy metals effects on *Brassica oleracea* and elements accumulation by salicylic acid. Archives of Hygiene Sciences 7, 1–11.

Tahat, M., Alananbeh M., Othman, A., Leskovar, D., 2020: Soil health and sustainable agriculture. Sustainability 12, 4859.

Takatsuka, H., Umeda, M., 2014: Hormonal control of cell division and elongation along differentiation trajectories in roots. Journal of Experimental Botany 65, 2633–643.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D. J., 2012: Heavy metal toxicity and the environment. Experentia Supplementum, 133–164.

Teng, Y., Wang, X., Li, L., Li, Z., Luo, Y., 2015: Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. Frontiers in Plant Science 6, 32.

Tiwari, S., Singh, S.N., Garg, S.K., 2013: Induced phytoremediation of metals from fly ash mediated by plant growth promoting rhizobacteria. Journal of Environmental Biology 34, 717–727.

Todeschini, V., Lingua, G., D'Agostino, G., Carniato, F., Roccotiello, E., Berta, G., 2011: Effects of high zinc concentration on poplar leaves: A morphological and biochemical study. Environmental and Experimental Botany 71, 50–56.

Tuan, P.A., Kumar, R., Rehal, P.K., Toora, P.K., Ayele, B.T., 2018: Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. Frontiers in Plant Science 9, 668.

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S., Yang, X., 2015: Phytoremediation of heavy metals assisted by plant growth

promoting (PGP) bacteria: A review. Environmental and Experimental Botany 117, 28–40.

Vergani, L., Mapelli, F., Zanardini, E., Terzaghi, E., Di Guardo, A., Morosini, C., Raspa, G., Borin, S., 2017: Phyto-rhizoremediation of polychlorinated biphenyl contaminated soils: An outlook on plant-microbe beneficial interactions. Science of the Total Environment 575, 1395–1406.

Viehweger, K., 2014: How plants cope with heavy metals. Botanical Studies 55, 35.

Weyens, N., Schellingen, K., Beckers, B., Janseen, J., Ceulemans, R., van der Lelie, D., Taghavi, S., Carleer, R., Vangronsveld, J., 2013: Potential of willow and its genetically engineered associated bacteria to remediate mixed Cd and toluene contamination. Journal of Soils and Sediments 13, 176–188.

Yang, X.E., Ye, H.B., Long, X.X., He, B., He, Z.L., Stoffella, P.J., Calvert, D.V., 2005: Uptake and accumulation of cadmium and zinc by *Sedum alfredii* Hance at different Cd/Zn supply levels. Journal of Plant Nutrition 27, 1963–1977.