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ORIGINAL PAPER

Effect of bio-fertilizers on corn (*Zea mays* L.) growth characteristics in Cd-spiked soils

F. Rostami[1] · M. Heydari[1] · A. Golchin[1] · N. Khadem Moghdam Igdelou[1]

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Abstract

To investigate the effect of bio-fertilizers on contamination rate of plants to heavy metals, an experiment was carried out in factorial based on completely randomized design with three replications in research greenhouse of University of Zanjan in 2015. Treatments were: contamination levels of soil to cadmium (Cd) (0, 10, 25, 50 and 100 mg kg^{-1}) and inoculation with bio-fertilizers including phosphate-solubilizing bacteria *Pseudomonas putida*, mycorrhizal fungi *Funneliformis mosseae* and *Rhizophagus intraradices.* Measured characters include leaf chlorophyll index, plant height, shoot and root fresh and dry weight, phosphorus and potassium of shoot and root and Cd concentration in the plant. The results indicated that the use of bio-fertilizers increased the leaf chlorophyll index, plant height, shoot and root fresh and dry weight, phosphorus and potassium of shoot and root significantly in comparison with control. The treatment of *F. mosseae* and *P. putida* (M + P) could improve leaf chlorophyll index and plant height by 11.93 and 21.89% in comparison with control, respectively. The chlorophyll index significantly decreased with increasing contamination levels of soil to Cd. The simultaneous use of *F. mosseae* and *P. putida* (M + P) and Cd increased the shoot and root dry weight by 6 and 7% in comparison with treatment 100 mg kg^{-1}. In addition, the results indicated that inoculation of soil with bio-fertilizers can decrease the harmful effects of Cd on plants' growth and yield.

Keywords Mycorrhizal fungi · Phosphate-solubilizing bacteria · Cd · Contaminated soil

Introduction

An increase in activity of industrial factories, using the industrial wastewater in agriculture, population growth and developing urbanization with exacerbation of environmental contamination, has exposed the health and life of a living being, especially human, at serious risk. Soil contamination refers to an increase in concentration of dummy and natural chemicals in the soil profile (Gajewska et al. 2006). Soil, as a part of the environment receiving various contaminators such as wastewater and chemical contaminator like heavy metals, influences the health of human community (Ernest 1996). Heavy metals are the most toxic inorganic contaminator that is present in the soil naturally or enters into the soil as a result of the human activity (Ugolini et al. 2013). Heavy metals are dissoluble in the soil and can enter into human food chain via absorption by plants (Smith and Read 2008). These metals can make bound with human-required elements such as oxygen, sulfur and nitrogen that are found like S–S, S–H, O–H and COOH groups in structure of essential compounds of human body, such as enzymes and proteins, and cause disrupt in the enzymes activity and synthesis of essential compounds (Dalvand et al. 2015). Among heavy metals, Cd has special importance, because it is easily absorbed via root and its toxicity effects are 20 times more than other heavy metals (Mc Gerath et al. 2000; Han et al. 2011). Accumulation of Cd in agricultural farms and products has become an environmental problem. Plants are the most important transfer path of Cd to human food chain, and accumulation of Cd in agricultural products causes toxicity and makes torrid and chronic diseases. The concentration of Cd has been increased in agronomic and horticultural soil due to the extra application of phosphate fertilizers (Ghahramani 2008; Dalvand et al. 2015). Focusing on purifying the environment from Cd and lead is going to be developed because of their toxic and cancer effects. There are various

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✉ N. Khadem Moghdam Igdelou
Nader.khadem@znu.ac.ir

[1] Faculty of Agriculture, University of Zanjan, Zanjan, Iran

methods to improve contaminated soil from heavy metals that can prevent the negative effects of these metals on human health (Premsekhar and Rajashree 2009). Many of these methods are costly and affect the chemical and physical characters of soil negatively. Thus, using low-cost methods and compatible with the environment is necessary (Bolan et al. 2003). Today, using organic and bio-fertilizers is the acceptable strategy for increasing soil fertility and conservation of natural resources and environment (Permakhsar and Rajasheri 2009). So to achieve sustainable agriculture, the use of bio-fertilizers such as plant-growth-promoting rhizobacteria and useful microorganism is undeniable (Weber et al. 2018). The most important phosphate-solubilizing bacteria belong to pseudomonas and bacillus. Pseudomonas is the most important plant-growth-promoting bacteria that in addition to solubilizing the soil phosphate, with producing a noticeable amount of substances and growth-promoting hormones, specially auxin and gibberellin, influences the growth and yield of crops (Zahir et al. 2004). Mycorrhiza is the most important fungi in most undegraded soils. Around 70% of the biomass of soil microbial community is made of mycorrhiza mycelium. The term mycorrhiza is extracted from the Greek word myco meaning fungi and rhiza meaning root (Rejali et al. 2007). It expresses the coexistence relationship between plants root and mycorrhiza (Jeffries et al. 2003). Mycorrhizae are the microorganisms that make coexistence with various plants roots. The benefits of these fungi are as follows: increase the water absorption and help to decrease the environmental stress such as salinity and high concentration of heavy metals (Amanifar et al. 2011). Reactions between plants and useful microorganism in the rhizosphere can increase the biomass and plant tolerance to heavy metals (Glick 2010). The effect of microorganisms activities have been done on soil and rhizosphere characteristics and also metal bioavailability on absorption by root and shoot (Joner and Leyval 2001; Schnepf et al. 2011). Among rhizosphere microorganisms that play an important role in increasing the absorption of nutrient, PGPR such as potassium- and phosphate-solubilizing bacteria and nitrogen-fixing bacteria have become more prominent (Varvara et al. 2000; Weller et al. 2003). Today, phosphate-solubilizing microorganism is used as a bio-fertilizer and to increase agricultural products and conservation of soil health in a large area. The studies that were done on bio-fertilizer containing phosphate-solubilizing bacteria indicated that these bacteria decrease the negative effects of chemical fertilizers and conserve the environment.

Due to Iran's development in industry and technology and, of course, the increase in waste by-products of factories and mines and their entry into agricultural lands, it is possible to spread pollution, so be aware of the level of pollution of Iran soils and it seems necessary to take action to eliminate them. Zanjan Province, with its numerous mines and factories, is a prone area for polluting agricultural soils with heavy elements such as cadmium and lead, so the purpose of this research is investigating the effect of mycorrhiza and phosphate-solubilizing bacteria on *Zea Mays* growth and Cd absorption in contaminated soil.

Purposes

1. Investigation of the effect of *Pseudomonas putida* bacteria and *Funneliformis mosseae* and *Rhizophagus intraradices* mycorrhizal fungi on the uptake of cadmium in spiked soil.
2. Comparison of the ability of *P. putida* bacteria and mycorrhizal fungi of *F. mosseae* and *R. intraradices* in terms of effects on the growth of corn and the absorption of heavy metals in soils contaminated with various levels of cadmium.
3. Comparison of the effect of separate consumption of *P. putida* bacteria and mycorrhizal fungi on corn growth and cadmium and lead uptake from contaminated soils.

Materials and methods

To investigate the effect of bio-fertilizer on *Z. mays* growth under soil contamination with Cd, a factorial experiment was conducted based on the completely randomized design (CRD) in three replications. The factors were: levels of soil contamination with Cd (0, 10, 25, 50 and 100 mg kg^{-1}) (Abbaspour et al. 2010) and inoculation with bio-fertilizers containing no inoculation (C), inoculation with phosphate-solubilizing bacteria *P. putida* (P), inoculation with *F. mosseae* (M), inoculation with *mycorrhiza F. mosseae* + phosphate-solubilizing bacteria (M + P), inoculation with mycorrhiza *R. intraradices* (I) and inoculation with mycorrhiza *R. intraradices* + phosphate-solubilizing bacteria (I + P). This experiment was done in the greenhouse of the soil science department at University of Zanjan in 2015. The bacteria used in this study are *P. putida* species, a heterotroph bacterium which was used in liquid form with 5×10^8 population from the Water and Soil Research Institute, Karaj, Iran. Also, the mycorrhiza fungus contains two species: *F. mosseae* and *R. intraradices* from the microbial collections of the Soil and Water Research Institute, Karaj, Iran, with a population equal to 115 active fungal organs per gram. After the sampling of different soils, the subjected soil was provided from 0 to 20 depth of research farm. After air drying, the soil was passed through a sieve of 2 mm. Then, some of the chemical and physical characters of soil were measured (Table 1). Soil texture was determined with hydrometer method (Gee and Bauder 1986), and soil pH was measured with the pH meter of model Metrohm 691 (Nelson

1982). Electrical conductivity was measured with conductor model WTW Series inoLab (Rhoades 1982), and organic carbon was measured with Walkley–Black method (Nelson and Sommers 1986). Also, Cd was measured with DTPA and atom absorption set model Varian SpectrAA 20 (Walingh et al. 1998). To contaminate soil samples with Cd, the proper amount of Cd sulfate was dissolved in deionized water and was sprayed onto soil samples. The amount of deionized water was so high that the soil moisture could reach FC. The contaminated soils (in the amount of 4 kg) were transferred into plastic pots, and to reach relative balance they were kept in greenhouse at 25–28 °C. Wetting and drying cycles (to reach FC to air drying) were imposed on them. After one month, two types of microorganisms (fungi and bacteria) were added to contaminated and non-contaminated soils. The environment of the fungi was solid, so after adding this medium to the treatments, the same amount was added to the bacterial treatment in a double-sterile autoclave. Consumption of solid medium is containing 50 g of fungus per pot. The number of bacteria per plant was 2 cc. (The number of germs per cc was 5×10^8.) Four *Z. mays* seeds (maxima cultivar) were sown in each pot. After emergence, the number of plants was decreased to 3 with thinning. Plants were grown in the greenhouse at 25–28 °C and FC for 75 days. The moisture of pots was controlled through weighting. Due to soil fertility and to prevent the unwanted reaction, only urea fertilizer with water was equally given to pots. After passing 75 days (completing the stage of vegetative growth and before entering reproductive growth), shoot and root were harvested. The studied characters were: phosphorus of root (Pr) and shoot (Ps) and potassium of root (Kr) and shoot (Ks), Cd concentration of root (Cdr) and shoot (Cds), root dry weight (RDW), root fresh weight (RFW), shoot dry weight (SDW), shoot fresh weight (SFW), plant height and chlorophyll index (Chl). Also, SAS software (version 9/1) and comparison of averages were used by Duncan's multidimensional test at the probability level of 0.05 to analyze the data.

Results and discussion

Cd of root and shoot

Application of mycorrhizal and bacterial fungi reduced the concentration of Cd of shoot in mays significantly ($P \leq 0.01$) (Table 2). The highest concentration of Cd in the shoot was observed in the treatment without inoculation or control, and the lowest in inoculation with mycorrhizal *R. intraradices* + phosphate-solubilizing bacteria (I + P) (Table 3). Also, application of mycorrhizal fungi and the bacterium significantly increased root Cd concentrations ($P \leq 0.01$) (Table 2). The highest Cd concentration of root was obtained

Table 1 Main properties of the soils selected for this study on the toxicity of Cd to different soil organisms and processes

Extractable Cd (mg kg^{-1})	Total Cd (mg kg^{-1})	Avail. manganese (mg kg^{-1})	Avail. (mg kg^{-1})	Avail. iron (mg kg^{-1})	Potassium (mg kg^{-1})	Phosphorus (mg kg^{-1})	EC (dS m^{-1})	pH	Organic carbon (%)	Lime (%)	soil texture
0.11	2	4.72	0.5	4.5	230	14	0.25	7.75	1.1	14.7	Clay loam

Table 2 Analysis of variance of simple and cross-effects of experimental treatments on the fresh and dry weight of shoot and root, chlorophyll leaf index, altitude, phosphorus and potassium of root and aerial parts of *Z. mays*

S.O.V	df	Cds	Cdr	Ks	Kr	Phs	Phr	Height	Chl	Drw	Dsw	Frw	Fsw
MS													
Cd	4	30,709.81**	497,780.8**	3.16**	1.3**	0.03**	0.05**	795.48**	13.54**	0.7**	28.32**	38.83**	850.19**
B	5	1981.65**	22,184.18**	1.94**	0.2**	0.02**	0.03**	109.99**	1.73**	0.18**	1.10**	5.47**	10.98**
B × Cd	20	342.11**	3199.73**	0.42**	0.11**	0.003**	0.005**	9.03*	0.3 ns	0.03**	0.4**	0.51 ns	5.84 ns
E	60	58.66	132.76	0.03	0.017	0.0001	0.0002	4.56	0.38	0.01	0.07	0.63	0.61
CV		15.37	5.91	7.29	12.80	7.96	6.74	5.32	7.94	17.64	13.71	12.41	8.02

from inoculation with *R. intraradices* + phosphate-solubilizing bacteria (I + P) and the lowest concentration obtained in control treatment. This treatment increased root Cd by 84.99% compared to control (without inoculation) (Table 3). By studying the effects of mycorrhizal fungi on the ability of plants in different plants in soils contaminated with heavy lead and Cd, it was found that mycorrhiza increases the absorption of these elements in the root (Adewole et al. 2010; Kungu et al. 2010; Andrade et al. 2004). Heydari et al. (2020) investigated the effect of inoculation with mycorrhizal fungi and phosphorus-solubilizing bacteria on the absorption of heavy elements by *Zea Mays*. The results showed that in plants inoculated with fungi and bacteria, the absorption of heavy metals such as lead and Cd increased in the root compared to the control. Inoculation with mycorrhizal fungi increased the absorption of heavy elements in soybean plants in soils contaminated with these elements, and these elements were less transmitted to the shoot and accumulated more in the root (Andrade et al. 2004). Therefore, more accumulation of Cd in the root in inoculation with mycorrhizal fungi *R. intraradices* + phosphate-solubilizing bacterium caused less Cd to be transferred to the shoot (Table 3). With increasing levels of phosphorus in the presence of Cd, the Cd of the shoot decreased, possibly due to the formation of insoluble compounds of these two elements (Oskuei et al. 2008). A decrease in the transfer of Cd from the root to the shoot can be due to the non-displacement of the element in the cell wall or the Cd binding to the organic compounds in the root (Lia and Zhiwei 2005). On the other hand, the fungal heifers are able to keep the metal in itself and not to pass it into the plant system, reducing the toxicity of heavy metals (Horst 2004). With increasing levels of soil contamination with Cd, the concentration of Cd in the shoot and root of corn increased significantly ($P \leq 0.01$) (Table 2). The highest concentration of Cd in the aerial part was found to be 4377.127 mg kg^{-1} and in the root at 105.57 mg kg^{-1} and 100 mg kg^{-1}, and the lowest concentration of Cd was obtained in both parts from the level of 0 mg kg^{-1} (control without pollution) of soil (Table 3). According to researchers, for plants that were exposed to Cd for 24 h, the element penetrates the plant cells and causes plant physiological and genetic damage, resulting in a decrease in plant growth and weight (Rostami et al. 2020; Varvara et al. 2000). Also, Cd in plants decreased the plant weight by decreasing the water use efficiency, reducing transpiration, reducing the concentration of nutrients in the plant and reducing plant resistance to diseases and pests (Vassilev et al. 2002). Interactions of bio-fertilizer and different levels of Cd on soil

Table 3 Results of comparison of the effects of bio-fertilizer and different levels of Cd on the fresh and dry weight of shoot and root, leaf chlorophyll index and plant height

BioF	Height	Chl	drw	dsw	Frw	fsw	Phr	Phs	Kr	Ks	Cds	Cdr
C	34.89^{d}	7.29^{c}	0.56^{c}	1.71^{d}	5.60^{d}	8.53^{c}	0.16^{f}	0.11^{e}	0.79^{b}	1.9^{d}	68.9^{a}	133.36^{e}
P	39.9^{c}	8.06^{ab}	0.62^{bc}	1.9 ^{cd}	6.05^{Dc}	9.34^{b}	0.25^{b}	0.17^{c}	1.10^{a}	2.73^{a}	55.56^{b}	181.14^{d}
M	40.8^{bc}	7.6^{bc}	0.69^{b}	1.9^{c}	6.32^{c}	9.56^{b}	0.23^{c}	0.13^{d}	1.03^{a}	2.03^{d}	52.33^{b}	188.92 ^{cd}
M + P	42.53^{a}	8.16^{a}	0.82^{a}	2.18^{b}	6.99^{ab}	10.56^{a}	0.29^{a}	0.20^{a}	1.80^{a}	2.71^{ab}	40^{c}	221.15^{b}
I	40.46^{bc}	7.75^{ac}	0.69^{b}	2.09^{bc}	6.43^{bc}	6.94^{b}	0.18^{e}	0.12^{de}	1.09^{a}	2.56^{c}	43.34^{c}	197.81^{c}
I + P	42.06^{ab}	8.09^{ab}	0.85^{a}	2.5^{a}	7.25^{a}	10.89^{a}	0.20^{d}	0.18^{b}	1^{a}	2.59^{bc}	38.89^{c}	246.71^{a}
Mg kg^{-1}) Cd levels)												
0	50.97^{a}	9.03^{a}	1^{a}	4.28^{a}	8.37^{a}	21.72^{a}	0.30^{a}	0.22^{a}	1.39^{a}	3.06^{a}	0.01^{e}	0.02^{e}
50	41.55^{b}	8.08^{b}	0.78^{b}	1.75^{b}	7.45^{b}	9.21^{b}	0.24^{b}	0.17^{b}	1.17^{b}	2.55^{b}	25.00^{d}	109.28^{d}
100	37.91^{c}	7.88^{bc}	0.68^{c}	1.49^{c}	6.26^{c}	7.08^{c}	0.22^{c}	0.13^{c}	0.96^{c}	2.27^{c}	44.45^{c}	149.10^{c}
200	35.94^{d}	7.49^{c}	0.58^{d}	1.32^{de}	5.23^{d}	5.74^{d}	0.18^{d}	0.13^{c}	0.83^{d}	2.32^{c}	74.08^{b}	287.09^{b}
400	34.23^{e}	6.65^{d}	0.49^{e}	1.03^{e}	4.89^{d}	5.04^{e}	0.15^{e}	0.11^{d}	0.72^{e}	1.92^{e}	105.57^{a}	428.78^{a}

Averages with at least one common alphabet do not have a statistically significant difference and Cd concentrations of the shoot and root of *Z. mays* were significant ($P \leq 0.01$) (Table 2). The highest concentration of Cd in the shoot was obtained from inoculum treatment, i.e., 100 mg kg^{-1}, and the lowest concentration was obtained from application of inoculation with mycorrhizal fungi *R. intraradices* + phosphate-solubilizing bacteria as well as inoculation with fungi *F. mosseae* + bacteria phosphate solubilization, i.e., 10 mg kg^{-1} (Table 3). The highest root Cd concentrations were obtained from inoculation with mycorrhizal *R. intraradices* + phosphate-solubilizing bacteria and inoculation with fungi + phosphate-solubilizing bacteria, i.e., 100 mg kg^{-1}, and the lowest ones were obtained from the treatment control (without inoculation) from the level of 0 mg kg^{-1} (Table 3).

The phosphorus of root and shoot

The highest amount of phosphorus in the shoot and root was treatment inoculation with *F. mosseae* + phosphate-solubilizing bacteria (M + P) (Table 3). This bio-fertilizer increased the amount of phosphorus in the shoot and root by 81.81 and 81.25%, respectively, compared to non-inoculated (C) treatments. Root growth stimulation has been reported during phosphorus deficiency in some herbaceous species such as clover (Rostami et al. 2020; Nadian et al. 1996) and Soybean (Nurlaeny et al. 1996). Increasing root length is a response to phosphorus deficiency (Hall 2002; Marschner 1995). Because inadequate soil phosphorus causes the formation of the drainage area around the root, the first positive effect of root extension is root expansion to a more phosphorus region (Marschner 1995). Plants that were inoculated with *Azotobacter* and mycorrhizal fungi were more developed in comparison with the treatments that were inoculated with each of these two bio-fertilizers and were rich in phosphorus storage (Mohandas 1987). The presence of mycorrhizal fungi in mays growth area has led to the 115% increase in phosphorus uptake (Sharifi et al. 2007). The reason for this was the increase in plant root absorption due to the development of mycorrhizal *mycelia mycelium* external mycelia in the soil and also increased phosphorus absorption by the mycorrhizal fungus hypha. *Pseudomonas* bacteria increase the solubility of insoluble phosphorus through mechanisms such as secretion of organic acids such as oxalic acid and citric acid. The produced organic acids cause an increase in available phosphorus by decreasing the acidity of the rhizosphere and chelating the aluminum ion in acid soils and calcium ions in the limestone soils (Henry et al. 2008). With increasing levels of Cd, the amount of phosphorus in the shoot and root was significantly decreased ($P \leq 0.01$) (Table 2). The highest amount of phosphorus in the shoot and root was obtained from control treatment; however, the lowest level was 100 mg kg^{-1} (Table 3). 100 mg kg^{-1} soil reduced the concentration of root and shoot phosphorus by 50% in comparison with control treatment. With increasing levels of Cd, the amount of phosphorus in the plant decreases, which is probably due to the formation of insoluble compounds such as Cd phosphate (Zhang et al. 2010). These results are consistent with the findings of other researchers (Christie et al. 2004) that with increasing concentration of Cd in the soil, the concentration of plant phosphorus decreased.

The potassium of root and shoot

The amount of potassium in the shoot and root reduced with increasing levels of Cd ($P \leq 0.01$) (Table 2). The highest amount of potassium of shoot was obtained from control treatment, and the lowest level was 100 mg kg^{-1}. In the root, the highest amount of potassium was obtained from control treatment and the lowest level was 100 mg Cd per kg of soil (Table 3). Treatment with 100 mg kg^{-1} reduced the potassium root and shoot concentrations to 48% and 37.2%, respectively, compared to control (Table 3).

Inoculation with bio-fertilizers increased the potassium concentration of the shoot and root significantly ($P \leq 0.01$) (Table 2). The highest concentration of potassium in the shoot was obtained from phosphate-solubilizing bacteria (P). This bio-fertilizer increased the potassium concentration of shoot by 43.68% compared to the control sample (control without inoculation) (Table 3). The highest root potassium content was obtained from the treatment of mycorrhizal fungi *R. intraradices* and mycorrhizal fungus *F. mosseae* and also phosphate-solubilizing bacteria (I + P and M + P). Phosphorus-solubilizing bacteria by decomposition of phosphorus, iron and potassium minerals release elements such as potassium, calcium, and magnesium. Potassium and calcium levels in leaf and root of triangular orange leaves (*Poncirus trifoliate*) were inoculated with the mycorrhizal fungus which was more than their amount in roots and leaves of plants without fungi (Wu et al. 2006). Positive results of the effect of phosphate-solubilizing bacteria on potassium absorption may have different reasons. First, producing plant hormones cause the development of the root system and increase the absorption level, and second, producing proton and siderophores cause the release of potassium from potassium minerals. The soil environment is also leached from the alkaline to acidic by the action of mycorrhizal fungi (Henry et al. 2008), which leads to more solubility or low solubility of elements such as potassium, and the concentration of this element increases in the soil solution (Goyanrajlo et al. 2005).

Plant height

Plant height was increased significantly under the effect of inoculation with bio-fertilizers (Table 2). Bacteria caused an increase in cell division and cell to prolong growth as a result, they increase the plant height. Accordingly, the highest plant height was measured in treatment M + P, whereas the lowest plant height was obtained in the control treatment (Table 2). Treatment M + P increases the plant height (21.89%) in comparison with C (Fig. 1). Due to that, gibberellin increases cell division and cell prolong growth, especially the length of internode of the stem. On the other hand, inoculation with PGPR likely caused a decrease in ethylene. Raj et al. (2004) indicated that inoculation with P.S.B caused an increase in plant height in *Jasminum sambac*. Yusefirad and Esmaeil (2010) stated that the inoculation plant with mycorrhiza was excellent significantly due to the height in comparison with un-inoculated plants. Gupta et al. (2002) reported that coexistence of mycorrhiza with menthol mint root increased water and nutrient absorption and caused improvement in growth parameter like plant height. Heydari et al. (2020) reported an increase in *Z. mays* plant height under inoculation with *pseudomonas*. Ansari et al. (2014) indicated that inoculation with mycorrhiza caused an increase in *lobsters* height. Plant height in the *Z. mays* was significantly decreased ($P \leq 0.01$) with increasing Cd (Table 2). The highest plant height was obtained in control treatment, whereas the lowest was observed in treatment 100 mg/kg Cd (Fig. 1). Treatment 100 mg/kg Cd decreased the plant height (48%) in comparison with C. Wu et al. (2006) reported that the plant height of barley was decreased because of Cd concentration. Yaghubzade et al. (2011) indicated that the effect of Cd levels on morphologic traits was significant and the highest plant height was related to C and the lowest was related to treatment 100 mg kg^{-1} Cd.

The interaction of bio-fertilizer and Cd levels on plant height was significant ($P \leq 0.05$) (Table 1). The highest plant height belonged to treatment M + P and level 0 mg kg^{-1} Cd. The lowest plant height was observed in C and level 100 mg kg^{-1} Cd (Table 4). Although bio-fertilizer could not play the positive and noticeable role on plant height in Cd critical concentration (100 mg kg^{-1} Cd), it could increase plant height slightly (5%) in concentration 500 mg kg^{-1} Cd (Table 4). The results of previous researches declare that bio-fertilizers do not have suitable performance in the critical concentration of heavy metals (Wu et al. 2009). These results are predictable, considering the dangerous and harmful effects of heavy metals (Cd), but at low concentration of Cd (50 mg kg^{-1}), bio-fertilizer influenced the plant height.

Chlorophyll content

Using various bio-fertilizers increases the chlorophyll index significantly (Table 2). Maximum chlorophyll content was obtained in M + P, and minimum chlorophyll content was obtained in C (Table 3). The increase in chlorophyll index is due to the fact that mycorrhiza increases the sugar content and hormones such as cytokine and gibberellin (Demir 2004). The increase in hormone level, especially cytokine, can be affected by transfer of effective ion in regulating and rising chlorophyll content. On the other hand, the increase in chlorophyll content can be caused by phosphor absorption from the soil by the plant (Smith and Rid 2008). The similar result was reported by Vivas et al. (2003) that mycorrhiza and P.S.B increase stomata conductivity and chlorophyll content. The increase in chlorophyll content in plants under inoculation with mycorrhiza was reported by Singh and Mir (1998). According to the result of variance analyses, the significant decrease in chlorophyll index coincides with an increase in Cd concentration (Table 2). The chlorophyll index was decreased linearly by increasing Cd concentration, so that the lowest chlorophyll content was obtained in the concentration of 100 mg kg^{-1} Cd and the highest was observed in C (Table 4). The decrease in chlorophyll index is probably due to inhibition of delta aminolevulinic acid biosynthesis and protochlorophyll reductase formation (Rostami et al. 2020; Perasad and Strazalka 1999), in other

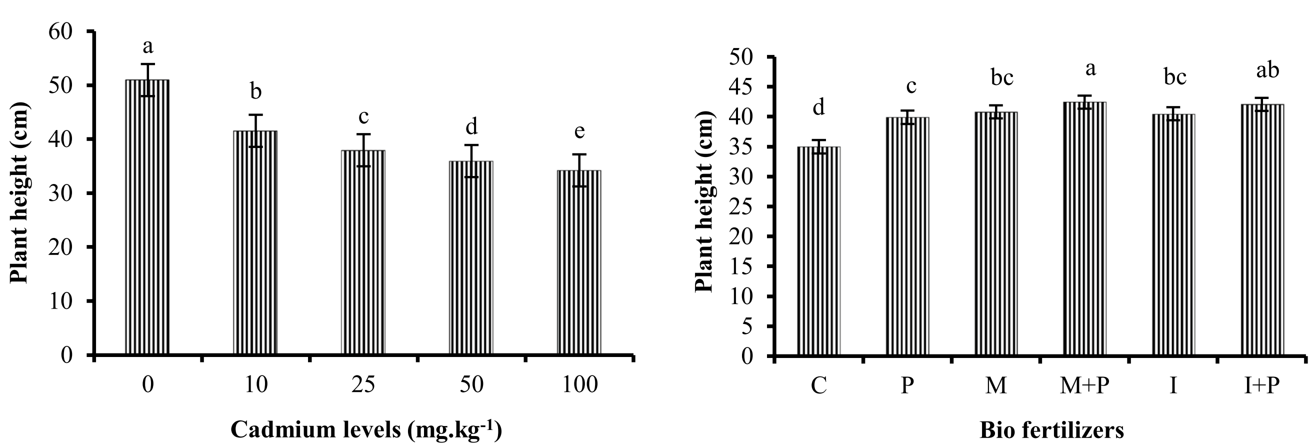

Fig. 1 Effects of bio-fertilizers (right) and Cd (left) on plant height of *Z. mays*

Table 4 Interaction effects of different levels of Cd and bio-fertilizer on the dry weight of root and shoot and plant height of *Z. mays*

Bio-fertilizer	Cd level	Cds	Cdr	Kr	Ks	Phr	Phs	DWR (g)	DSW (g)	Height (cm)
	0	0.00^{n}	0.00^{l}	1.17^{c-f}	2.36^{g-i}	0.20^{hi}	0.17^{f-h}	0.71^{ci}	2.98^{f}	45.33^{bc}
	10	38.89^{i-k}	77.79^{k}	1.05^{e-h}	2.25^{hi}	0.17^{j-m}	0.13^{i}	0.64^{ek}	1.59^{gh}	35.33^{hk}
C	25	72.23^{ef}	105.57^{j}	0.70^{l-o}	2.19^{h-j}	0.19^{h-k}	0.12^{ij}	0.58^{fk}	1.42^{gh}	32.5^{jk}
	50	105.57^{b}	194.48^{gh}	0.62^{m-p}	1.78^{lm}	0.15^{m}	0.08^{lm}	0.48^{ik}	1.33^{gh}	31^{k}
	100	127.80^{a}	288.97^{e}	0.42^{p}	1.01^{o}	0.10^{n}	0.04^{n}	0.42 k	1.23 h	30.73 k
	0	0.00^{n}	0.00^{l}	1.01^{f-j}	3.26^{bc}	0.40^{b}	0.23^{c}	0.72^{ci}	3.56d	52.5^{ab}
	10	22.22^{lm}	77.79^{k}	1.31^{b-d}	2.89^{de}	0.26^{e}	0.16^{gh}	0.71^{ci}	1.56^{gh}	40^{em}
P	25	38.89^{h-j}	127.80^{i}	1.13^{c-g}	2.72^{ef}	0.26^{e}	0.16^{gh}	0.68^{dj}	1.52^{gh}	37.66^{fj}
	50	88.90^{cd}	261.16^{f}	1.01^{f-j}	2.48^{f-h}	0.19^{h-j}	0.12^{ij}	0.53^{gk}	1.52^{gh}	34.16^{ik}
	100	127.80^{a}	438.97^{b}	1.05^{e-i}	2.32^{g-i}	0.12^{n}	0.18^{fg}	0.49^{ik}	1.18^{h}	35.16^{hk}
	0	0.00^{n}	0.00^{l}	1.48^{b}	2.91^{de}	0.26^{de}	0.19^{ef}	0.90^{bd}	4.17^{c}	52^{ab}
	10	22.22^{k-m}	83.35^{k}	1.17^{c-f}	2.21^{h-j}	0.25^{ef}	0.12^{ij}	0.76^{bg}	1.56^{gh}	40.33^{dg}
M	25	55.56^{gh}	127.80^{i}	1.01^{c-f}	1.91^{j-m}	0.26^{e}	0.08^{lm}	0.67^{dj}	1.43^{gh}	39.16^{ei}
	50	77.79^{de}	216.61^{e}	0.93^{f-l}	1.68^{mn}	0.18^{i-l}	0.18^{ef}	0.59^{ek}	1.24^{h}	38.5^{fi}
	100	100.02^{bc}	450.09^{b}	0.56^{op}	1.44^{n}	0.17^{j-m}	0.06^{mn}	0.52^{gk}	1.24^{h}	34^{ik}
	0	0.00^{n}	0.00^{l}	1.91^{a}	3.28^{bc}	0.44^{a}	0.32^{a}	1.31a	4.83b	54^{a}
	10	16.67^{m}	144.47^{i}	1.09^{e-h}	2.60^{e-g}	0.36^{c}	0.25^{b}	0.83^{be}	1.62^{gh}	40.33^{dg}
M + P	25	33.34^{j-l}	188.92^{gh}	0.99^{f-j}	2.17^{h-k}	0.25^{ef}	0.16^{gh}	0.72^{ci}	1.53^{g}h	39^{ei}
	50	61.12^{fg}	300.06^{e}	0.82^{i-m}	3.66^{a}	0.23^{fg}	0.12^{ij}	0.65^{ej}	1.51^{gh}	37^{fj}
	100	88.90^{cd}	472.32^{a}	0.58^{n-p}	1.85^{k-m}	0.19^{h-j}	0.15^{h}	0.61^{ek}	1.41^{gh}	34.33^{ik}
	0	0.00^{n}	0.00^{l}	1.50^{b}	3.11^{cd}	0.20^{hi}	0.16^{gh}	0.99^{b}	4.44^{bc}	51.5^{ab}
	10	27.78^{k-m}	94.46^{jk}	1.34^{bc}	2.72^{ef}	0.19^{i-l}	0.13^{i}	0.80bf	1.84^{gh}	41.16^{df}
I	25	44.45^{h-j}	138.91^{i}	0.91^{g-l}	2.46^{f-h}	0.19^{i-l}	0.11^{jk}	0.69di	1.42^{gh}	38.16^{fi}
	50	50.01^{hi}	322.28^{d}	0.74^{l-o}	2.25^{hi}	0.15^{m}	0.11^{jk}	0.55^{gk}	1.38^{gh}	36^{fk}
	100	94.46^{bc}	433.42^{b}	0.95^{f-l}	2.25^{hi}	0.16^{k-m}	0.08^{l}	0.43^{jk}	1.39^{gh}	35.5^{hk}
	0	0.00^{n}	0.00^{l}	1.27^{b-e}	3.44^{ab}	0.29^{d}	0.22^{cd}	1.41^{a}	5.73^{a}	50.5^{ab}
	10	16.67^{m}	177.81^{h}	1.07^{e-i}	2.62^{e-g}	0.22^{gh}	0.21^{de}	0.94^{bc}	2.16^{f}	44.16^{cd}
I + P	25	22.22^{lm}	205.59^{g}	1.03^{e-i}	2.17^{h-k}	0.16^{lm}	0.17^{f-h}	0.74^{ch}	1.59^{gh}	41^{dg}
	50	61.12^{fg}	361.18^{c}	0.87^{h-m}	2.03^{i-l}	0.19^{i-k}	0.18^{ef}	0.68cj	1.55^{gh}	39^{ei}
	100	94.46^{bc}	488.96^{a}	0.78^{j-o}	2.66^{ef}	0.15^{m}	0.13^{ij}	0.50^{hk}	1.47^{gh}	35^{gk}

The meanings of at least one common alphabet are not statistically significant in terms of meaning

words, by increasing Cd concentration in plant. The biosynthesis of the protochlorophyl reductase enzyme is prevented. This enzyme is the key enzyme in the pathway of chlorophyll formation that leads to a decrease in chlorophyll index. Cadmium has a direct and negative effect on chlorophyll index. In the critical concentration of Cd in the soil, the chlorophyll content was decreased by 35.7% in comparison with C (Table 4). This percentage of decrease in chlorophyll index has been reported until 50% in other research papers (Kabata-Pendias and Pendias 2010) that is affirmation on the achieved result in this research.

Shoot and root fresh and dry weight

The main effects of bio-fertilizers and soil contamination to Cd on the shoot and root fresh and dry weight were significant ($P \leq 0.01$). Also, co-application of bio-fertilizers and Cd on shoot and root dry weight was significant ($P \leq 0.01$). The highest shoot fresh and dry weight was observed in I + P (*R. intraradices* + *P. putida*) treatment, and the lowest was observed at control treatment. Inoculation with bio-fertilizers increased the shoot fresh and dry weight (27.66 and 46.19%), consequently in comparison with C. According to reports, inoculation of soybean seeds with *Pseudomonas* causes an increase in the dry matter in the shoot.

An increase in shoot dry weight by *mycorrhizal arbuscular* was reported in Chinese chive and pepper. Luo et al. (2005) showed that application of phosphate solubilization bacteria increases the shoot dry weight in the bean. Also, the highest root fresh and dry weight was gained by application of bio-fertilizer, I + P and M + P, whereas the lowest was gained in C. application of bio-fertilizer, I + P, and M + P increased the root dry weight (51.78 and 46.42%) and the root fresh weight (29.46% and 24.66%) in comparison with C. Gamalero et al. (2004) revealed that shoot and root fresh and dry weight were increased under inoculation with *Pseudomonas* and *mycorrhiza arbuscular*. Luo et al. (2005) indicated that inoculation with *mycorrhizal fungi* caused a dry matter increase in comparison with control treatment. In a study, the effect of phosphate-solubilizing bacteria on the yield of *Hyssopus officinalis* was observed that these bacteria caused an increase in the shoot and root dry weight (Kochaki et al. 2008).

By rising the Cd levels, shoot and root fresh and dry weight decreased significantly (Table 2). The highest shoot and root fresh and dry weight was obtained by C treatment, and the lowest was obtained in treatment Cd 100 mg kg^{-1} (Table 3). Cadmium toxicity reduces photosynthesis and respiration, which is a reason for reduced growth. Metabolism of carbohydrate and colorization lead to a decrease in growth and biomass. So, treatment 100 mg kg^{-1} decreased the shoot fresh and dry weight (76.79 and 75.93%) in comparison with C (Table 3). Also, the highest and lowest root fresh and dry weights were related to control and 100 mg kg^{-1} of Cd, respectively. The treatment 100 mg kg^{-1} Cd decreased the root dry weight to 51/17% and root fresh weight to 71/11% in comparison with C (Table 3). Because of that, Cd prevents the meristem division and growth (Pal et al. 2006). The plant growth decreased in the presence of Cd ion and so shoot and root dry weight also decreased. By increasing Cd levels, dry weight of shoot in rice decreased, because the increase in Cd concentration caused the shoot fresh and dry weight to decrease (Shirazi et al. 2013). Bio-fertilizer and Cd levels interaction was significant on shoot dry weight ($P \leq 0.01$) (Table 2). The highest shoot dry weight was observed in I + P and Cd 0 mg kg^{-1} treatment, whereas the lowest shoot dry weight was observed in C and Cd 100 mg kg^{-1} (Table 4). The concentration of 100 mg kg^{-1} Cd can be considered critical concentration for plants because its natural yield and normal condition of the plant were not expected. But simultaneous applying of bio-fertilizers and Cd to pots could increase shoot and root dry weight by 6 and 7% in comparison with Cd treatment (Table 4). These results can detect the role of bio-fertilizer in neutralizing the harmful effects of heavy metals on crops.

Conclusion

High concentrations of heavy elements (Cd) used in this study were toxic for *Z. mays* plant and could have side effects on measured parameters. The results showed that in the concentration of 100 mg kg^{-1} of Cd, the measured parameters such as fresh weight of shoot (76.79%), shoot dry weight (75.93%), fresh weight of root (71.11%), root dry weight (51.7%), leaf chlorophyll index (37.5%), plant height (48%), shoot and root phosphorus (50%), root potassium (48%) and potassium of shoot (37.2%) decreased in comparison with control (no Cd). Soil inoculation with mycorrhizal fungi and bacteria in the absence of Cd element improved the growth indices and plant yield. Accordingly, *F. mosseae* + phosphate-solubilizing bacteria (I + P) inoculated with mycorrhizal fungus (I + P) could increase shoot fresh weight (27.66%), dry weight of shoot (46.19%), leaf chlorophyll index (93.9%), plant height (21.88%), root phosphorus (81.25%) and shoot phosphorus (81.81%), compared to the control treatment (without inoculation). Also, phosphate-solubilizing bacteria could increase root potassium by 68.63% compared to non-inoculated (control) treatment. Today, soil pollution is an important environmental issue that needs to be addressed. Soil pollution significantly reduces the quality of the environment and threatens human health. Heavy metals are considered to be an important environmental problem due to their toxicity, cumulative effects and long shelf life in the environment, carcinogenicity and non-degradability. The use of organic and biological fertilizers is an acceptable solution to increase soil fertility and preserve natural resources and the environment. Therefore, in order to achieve sustainable agriculture on the one hand and preserve natural resources and the environment on the other hand, the use of bio-fertilizers and the removal of heavy metals from the soil are undeniable.

The application of mycorrhizal fungi and bacteria to soils containing Cd could decrease the harmful effects of this element. In simultaneous application, *F. mosseae* + phosphate-solubilizing bacteria (M + P) and Cd were significant ($P \leq 0.05$) and plant height (5%), root dry weight (6%) and shoot dry weight (7%) were increased in comparison with the conditions of 100 mg kg^{-1} of Cd. The results of this study clearly showed that the application of critical concentration of Cd (100 mg kg^{-1}) is distinctly severe and harmful for plants. In these conditions, the application of bio-fertilizers will not be able to completely neutralize these hazards. However, in lower concentrations of heavy metal Cd, bio-fertilizers can decrease the harmful effects of these heavy metals on the shoot and root organs of the plant. It seems that in future research, the use of higher concentrations of bio-fertilizers and greater diversity of bio-fertilizers can reduce the damage of high concentrations of heavy metals such as cadmium to crops.

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