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Effect of Sunflower and Amaranthus Culture and Application of Inoculants on Phytoremediation of the Soils Contaminated with Cadmium

[1]Babak Moteshare Zadeh, [2]Gholam Reza Savaghebi-Firozabadi,
[3]Hossein Ali Alikhani and [3]Hossein Mirseyed Hosseini

[1]Ph.D. Student in Soil Science Engineering, [2]Associate Professor and [3]Assistant Professors,
University College of Agriculture & Natural Resource, University of Tehran, Karaj, Iran

Abstract: Cadmium is a toxic element in plant and human nutrition which is added to the soil from different sources especially consumption of phosphate fertilizers with high cadmium concentration, application of industrial wastewaters, mine extraction operations and metal melting and leads to contamination of environment. Phytoremediation is an effective, economical and biocompatible method for remediation of contaminated soils. This research was executed in order to study effect of sunflower and amaranthus culture and inoculants of two native bacteria resistant to cadmium on phytoremediation of this metal. In a calcareous soil of Karaj region (Fine Loamy, Mixed Super Active Thermic Xeric Haplocambids) and in green house conditions, effect of culture of the two plants, sunflower and amaranthus and three levels of control inoculants (BO), *Bacillus mycoides M1* (B1), *Micrococcus roseus M2* (B2) and four levels of control cadmium concentration (0, 50, 100 and 200 mg/kg) was studied in a factorial experimental design with random blocks basic design with three replications. Concentration of cadmium, iron and zinc were measured in shoot and root as a function of dry material and photosynthetic chemical efficiency. The analysis of variance analysis showed that application of inoculant significantly ($P< 0.01$) increased phytoremediation efficiency and effect of amaranthus in cadmium phytoextraction was higher than that of sunflower. It seems that effect of sunflower on phytoremediation is generally through phytoestabilisation and effect of amaranthus is through phytoextraction process. Treatments of cadmium increased concentration of this element in plant and decreased photosynthetic quenching (Fv/Fm). It is recommended to do more studies in this field and under field conditions.

Key words: Pytoremediation · Soil pollution · Cadmium · Sunflower · Amaranthus

INTRODUCTION

With growth and development of industries, melting metals and consumption of chemical fertilizers, contamination of heavy metals have been turned into a serious environmental problem [1,2]. Contaminant heavy metals including cadmium, chromium, copper, mercury, lead and nickel are defined as elements with metal properties and atomic number higher than 20 and density higher than 6 g/cm3 [2,3]. When heavy metal ions are in higher levels in the environment, they are transmitted through phytoextraction by roots to shoots leading to disorder in metabolism of plant and fertility of soil and decrease in function of products [4,5]. In addition, a high concentration of heavy metals in soil reduces biological activity and fertility of soils leading to yield reduction [6]. As an unnecessary element, cadmium is considered a heavy and toxic metal for the plant and prevents the growth of root and shoots of the plants and is associated with agricultural products [2]. Concentration of cadmium in uncontaminated soils is usually less than 0.5 mg/kg, but it can reach 3 mg/kg depending on the type of parent materials. Higher values result from activities of human being such as mining, production and consumption of phosphate fertilizers and activated sludge. Concentration of cadmium in uncontaminated plant is between 0.05 and 2 mg/kg [7,8]. Annually, extent of entrance and accumulation of cadmium in agricultural soils increases and 40-70% of cadmium in agricultural products result directly from phosphate fertilizers and rock phosphate is considered as major source of heavy metal increase [9]. Tests showed that triple superphosphate fertilizers, zinc sulphate and rock phosphate consumed in US contained 150.3, 295.7 and 44.4 mg/kg of cadmium respectively and

Corresponding Author: Hossein Mirseyed Hosseini, Soil Sci. Dept., Soil and Water Facult. Univ. of Tehran, Karaj, Iran

consumption of these fertilizers increased extent of cadmium available in soil and agricultural products [10]. With regard to emergence of heavy metals contamination in environment, remediation of contaminated places is a serious challenge. These compounds are not degraded and removed and their remediation depends on their removal from the environment incurring high expenses. In addition, during metals removal stage, one should use chemicals or physicochemical material preventing fertilization of soil and having negative effect on ecosystem and biodiversity [11]. Phytoremediation is an effective, cheap and biocompatible method with considerable dynamic capability [12-14]. Phytoremediation is a technology for use of plants for extraction, sequester or detoxification of contaminants through physical, chemical and biological methods [15-17]. Some plants which are called "hyperaccumulator" physiologically have suitable and usable potential for contaminated soils phytoremediation purposes. So far, 400 plant species from 45 families have been reported to be hyperaccumulator [18]. Among these the most hyperaccumulator plants are for nickel and the least are for cadmium with only one species known to be effective [19]. Hyperaccumulators absorb metals in their shoots more than 100 times more than natural plants do [20]. Phytoremediation of heavy metals and other inorganic material can be in the following forms:

Phytoextraction: Is extraction and concentration of metals from soil in shoot of the plant.

Rhizofiltration: Is the use of roots of plants for removal of metals through water flow.

Phytostabilisation: Is the use of plants for decrease in expansion of metal contamination in the environment and finally.

Phytovolatilization: Which is extraction and release of elements to atmosphere in the form of gas compounds is important for mercury and arsenic [21]. The plants used in phytoremediation should have the ability for high metals toxicity resistant in their biomass and tolerate high concentration of these in their own shoot [2]. In order to expand application of phytoremediation with regard to low biomass of hyperaccumulators, in different researches, agricultural plants with high biomass such as sunflower, maize, canola, chick pea, wheat, cabbage, oat, barley and Indian mustard have been used as the replacement options [22-28]. The first findings for use of plants in soil remediation were related to removal of nickel from the soil with indian mustard and canola [21]. Zinc phytoextraction with oat, barley and Indian mustard were also studied and the results showed that adding EDTA to the soil significantly led to accumulation of zinc in *indian mustard*. It was also shown that barley had potential of phytoremediation though this potential was not more than Indian mustard [29]. In another research, zinc and cadmium absorption in *Zea Maize* and *Thlaspi caerulescens* was studied and necessity of phytoremediation expansion was emphasized with regard to its economic value [30]. In a study in spain, effect of sunflower on absorption of microelements and heavy metals including lead, arsenic, cadmium and zinc on role of sunflower as suitable option for phytoremediation was emphasized, in addition it was indicated that this plant has low potential for phytoextraction, other researchers reported that sunflower was a suitable plant for zinc contaminated soils remediation [31,32]. In addition to agricultural plants with high biomass, identification of native plants around mines is one of pioneering solutions in phytoremediation. In a research, 17 plant species including gramine, tree, shrub and grass which contained different and high concentration of lead, cadmium, copper and zinc were identified and reported around the mine [33]. In Slovenia, Thlapsi praecox Wulf plant containing zinc with concentration of 14590 mg/kg, cadmium with concentration of 5960 mg/kg and lead with concentration of 3500 mg/kg was identified and introduced as Hyperaccumulator of zinc and cadmium [34]. Production of low biomass in hyperaccumulators and sensetively of plants root to high densities of metals led to many researches on possibility to use microorganisms in order to expand use of phytoremediation and economization of this method [2,35]. Researches showed that some of the effective and plant growth promoting rhizobacteria (PGPR) imposing mechanisms can increase absorption of metal in the soil [2]. Metal absorption may be increased with bacteria by production of siderophore releasing iron and allowing movement of other heavy metals in soil [36]. Some of the bacteria producing Acc-Deaminase which prevents from ethylene stress, decrease effects of heavy metals in plant tissues. Thus, sinergisty of plant and bacterium can increase efficiency of use of phytoremediation [21]. Generally, with regard to low growth speed and production of low biomass in hyperaccumulators and necessity of economization of metals phytoremediation on one hand and expansion of heavy metals contamination in agricultural products on the other hand emphasize the need for study on translation of these metals to food chain. Thus, study on agricultural plants with high biomass (sunflower) and study and identification of native plant species and nonagricultural plants (amaranthus) and hyperaccumulators of heavy metals are very important in each region. In the present research, by emphasizing on the purposes like study on the bacteria called PGPR group in native bacteria separated from the soils around the lead and zinc mine in Haft Emarat, Arak, effects of application of inoculants of two strains resistant to metals, on function and absorption of mineral nutrition and cadmium in the root and shoot of sunflower and amaranthus were studied and in order to achieve the above objectives, two laboratory and greenhouse experiments were executed.

MATERIALS AND METHODS

Soil Sampling and Analysis: First, compound soil was sampled from depth of 0-30 cm of soil from the Campus of Agriculture and Natural Resources of University of Tehran located in Karaj with coordinates of latitude of northern 35°48' 35" and longitude of eastern 50° 58' 18" and 1315.5 meters above sea level. The soil was classified as *Xeric Haplocambids, Fine Loamy, Mixed, Super Active thermic*. Samples were air dried and passed through 2-mm sieve and mixed uniformly. Physical and chemical properties and concentration of elements in samples were measured. Measurement of total nitrogen in the soil by Kjeldal method, available phosphorus by Olsen method, available potassium by normal ammonium acetate, moisture content (SP), pH and electrical conductivity determined by Rods, [38], percentage of equivalent calcium carbonate (calcimeteric method) [39], percentage of organic carbon by Walkly and Black method [40] and soil tissue in hydrometric method were determined [41]. Available concentration of zinc, lead and cadmium were measured by DTPA method [42] and with Atomic Absorption Spectrometry (AAS). Results are given in table 1.

Table 1: Physical and chemical properties of soil used in greenhouse culture before adding cadmium

Characteristic	Quntity	Characteristic	Quntity
Soil texture	Loam	Total nitrogen (%)	0.08
Clay (%)	25.00	Available Phosphate (mg/kg)	17.10
Silt (%)	36.00	Available Potassium(mg/kg)	247.00
Sand (%)	39.00	SO_4 (meq/l)	40.60
pH	7.90	Fe(mg/kg)*	4.28
EC(dS/m)	4.31	Cu(mg/kg)*	4.061
$CaCO_3$%	8.90	Mn(mg/kg)*	8.244
OC%	0.84	Zn(mg/kg)*	0.812
% SP	35.60	Pb(mg/kg)*	2.023
CO_3(meq/l)	0.40	Cd(mg/kg)*	0.10
CEC($Cmolkg^{-1}$)	26.00	Cl(mg/kg)	0.084

* DTPA-Extractable

Greenhouse Test: The soil taken from Campus farmland was passed through 4-mm sieve after air drying and threshing. In order to create cadmium pollution in soils of pots, cadmium chloride was used. concentration of cadmium in soil for greenhouse test included control treatment 0, 50,100 and 200 mg Cd /kg and three levels of inoculants B0 (control), (*Bacillus mycoides M1*) B1 and (*Micrococcus Roseus M2*) B2 and the test plants were sunflower and amaranthus. In each pot, some of necessary food elements including 1/3 of nitrogen, phosphorus and potassium consistent with weight of the soils were weighed and were added to each pot equally. Pots were made of poly ethylene material and weighted about 280 g each wit diameter of 15.5 and height of 18 cm. In each pot 3500 g of sieved soil were added (in cadmium treatments, consistent with type of cadmium treatment, 50, 100 and 200 mg Cd /kg of cadmium was added and soil was mixed uniformly and poured in the pot). Pots were irrigated based on weight loss and up to 70%±10 of FC moisture content with distilled water. Transfer of germinated seeds from the bottle to pots and their inoculation with inoculants (5×10^8 $cfuml^{-1}$) were done on 26 July 2007. After germination, seedlings were thinned to two plants in each pot and grown for 70 days. Fertilizers containing micro and macro elements were applied to the plants based on soil test results to have optimal conditions for plant growth and reaching suitable limits of phytoremediation. Pots were placed in growth room with lighting of 14 hours and temperature of 24°C to 28°C and light intensity of 20000 lux for 70 days. For measuring photosynthetic efficiency, Handy PEA device, model RF 232 was used and Fv/Fm parameter or photosynthetic photochemical quenching was assessed in different treatments[43].

Isolation and Purification of Native Resistant Bacteria from Soil: After study of status of the regions contaminated with heavy metals, Arak lead and zinc mine in Markazi Province located 46 Km south west of Arak at coordinates of 33° 45' and 34° latitude and 49° 30' and 49° 45' longitude at 2150 M above sea level. 19 samples of soil were taken from different regions around the mine from depth of 0-30 cm randomly and more than 130 bacteria were isolated from the soils[44] and finally, two strains of bacterium resistant to heavy metals (Cadmium, Lead, Zinc and Nickel) were isolated from the bacteria by performing biochemical tests in laboratory (microbiology section of Razi Serum and Vaccine Production Institute) on the basis of Bergeys Manual [45]. Two strains of *Bacillus mycoides M1* and *Micrococcus roseus M2* were identified and used in greenhouse test.

Biological Tests: These tests were done on bacteria before performance of greenhouse tests and included:

Siderophore production power by CAS-Agar method [46], ACC Deaminase enzyme production power quality test by Penroz and Gilck, [47], test of resistance to salinity [48].

Plant Harvesting and Analysis: After 70 days from culture, at the beginning of reproductive period and on 4 Oct. 2007, shoot and root of each plant from place of crown were cut and after washing with distilled water and measurement of wet weight, they were placed in the special packets and dried in oven at 70°C. After recording the samples dry weight, they were milled with mixer or steel blade and concentration of cadmium, iron and zinc were measured by wet digestion method with nitric acid and perchloric acid (3:1) using ICP-OES, model CAP-6500 for metal analysis [33]. Translocation factor indicating hyperaccumulation of heavy metals in harvested plant parts was obtained by dividing metal concentration in shoot by its concentration in the root [49]. Ratios higher than 1 indicates higher concentration of metal in shoot and are one of the factors which indicates suitability of the plant for use in phytoremediation. Statistical analysis of data in the form of factorial experimental design with random basic design in three replications was done with SAS software and comparison of means was done with LSD test at 1% level and graphs were drawn with Excel software.

RESULTS AND DISCUSSION

Physico-Chemical Properties of Soil: The soil used in greenhouse test table 1 was selected based on metal toxicity limits and study of sources and doubling concentration of each treatment in comparison with the previous treatment, contaminated with cadmium at rates (0, 50, 100 and 200 mg Cd /kg) [50,51]. Results of the analysis showed that the intended soil have suitable physical and chemical properties for greenhouse culture. In addition, no contamination with heavy metals was observed in soil.

Cadmium Concentration in Shoots and Roots: The results obtained from plant analysis showed that concentration of cadmium in shoots of amaranthus in all treatments was more than that of sunflower and there was significant difference between B2Cd100 and B0Cd200 statistically ($P<0.01$). In sunflower, with increase in concentration of cadmium in soil, concentration of this element in shoot also increased. The highest cadmium concentration in amaranthus was observed in B2Cd100, B1Cd200 and B1Cd100 treatments respectively. This increase was consistent with Cd50, Cd100 and Cd200 treatments. The highest cadmium concentration in shoot of sunflower was in B1Cd200, B1Cd100 and B2Cd200 treatments and B1Cd200 was different significantly from other treatments Figure 1. In comparison between two plants, control treatments and Cd50 had the least amount of cadmium. Consumption of both inoculants had better effect on increase in concentration of cadmium in shoots of amaranthus in comparison with control. Effects of consumption of both inoculants in sunflower associated with cadmium metal in shoot were not observed the same as those in amaranthus.

Results obtained from cadmium concentration in root of sunflower and amaranthus showed that in all applied treatments, increased concentration of cadmium was higher in sunflower than that in amaranthus. There was significant difference in cadmium concentration between all treatments of sunflower and amaranthus except B2Cd50. The highest amount of cadmium was observed in root of sunflower, in B1Cd200, B1Cd100, B2Cd100 treatments respectively. The obtained data showed that in amaranthus, the highest cadmium concentration was observed in B1Cd100, B1Cd200 and B2Cd200. In B0Cd200, due to emergence of toxic effects of cadmium, amount of cadmium decreased in root in comparison to B0Cd100 treatment. In terms of inoculants, results showed that consumption of inoculants of Bacillus bacterium in both plants was more effective than that of Micrococcus and with increase in concentration of cadmium in treatments, consumption of inoculants led to an increase in concentration of cadmium in root and reduced toxic effects of cadmium.

It is concluded that amaranthus had suitable ability for phytoremediation by phytoextraction method, transmitting more cadmium from root to shoot and sunflower in response to cadmium was

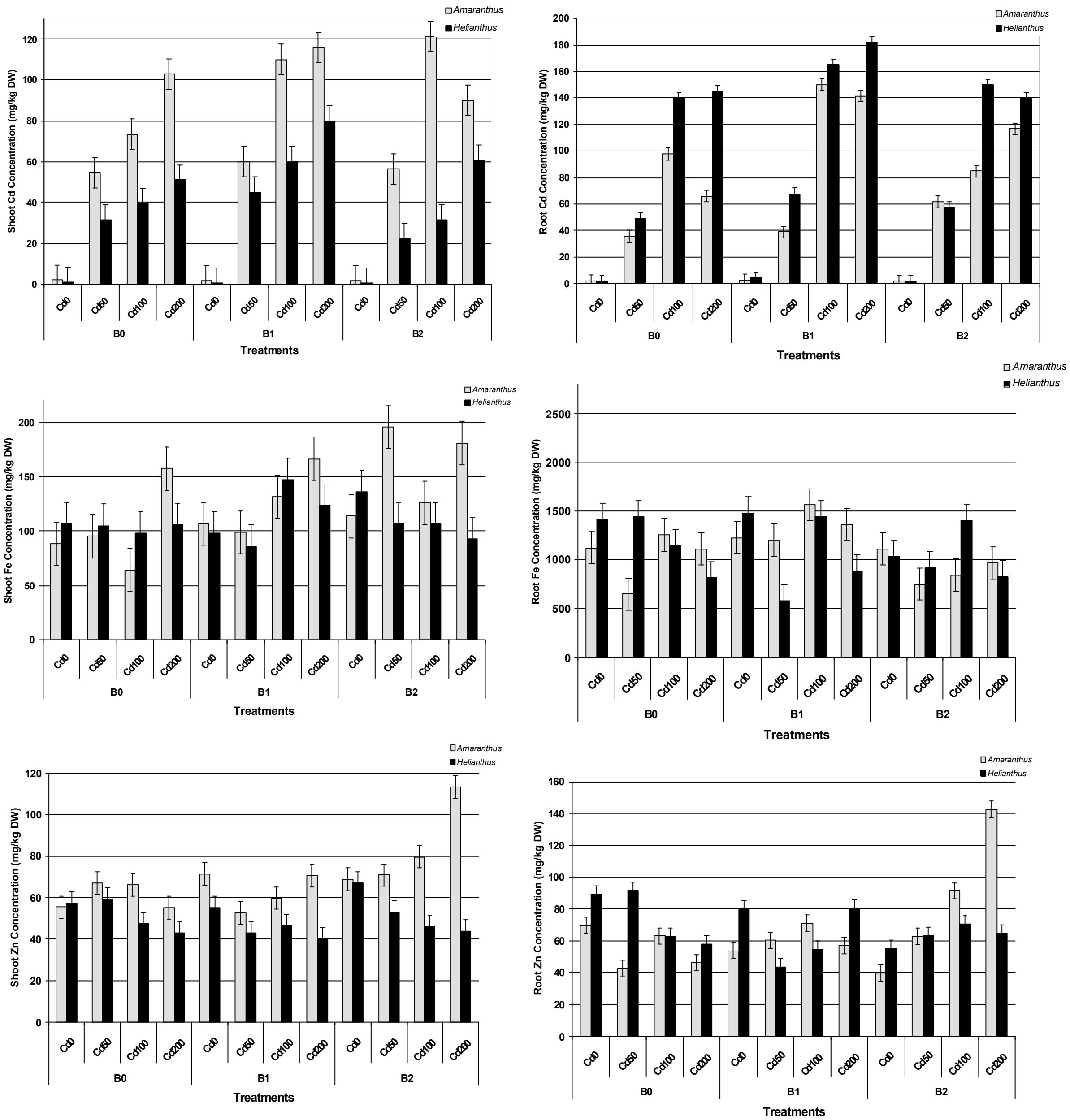

Fig. 1: The concentration of Cd, Fe and Zn in shoot and root of *A. retroflexus* and *H. annuus* grown in different cadmium and inoculant treatments. B0: Control, B1: *Bacillus mycoides* inoculant, B2:*Micrococcus roseus* inoculant. Significantly different at $P<0.01$

considered a suitable plant for phytoremediation through phytostabilization method. On the basis of research performed, plants confronting with tension of heavy metals use two ways [52,53]: 1- prevention from entrance of metal to shoot and association in root

2- Metal detoxication. On this basis, the plants which prevent from entrance and transfer of metals have low potential for phytoextraction, but, one can use them for stabilizing metal in soil and prevention from expansion of contamination as result of water erosion and wear. With regard to results of the present research and findings of other researchers in the use of plants with high biomass and ability to absorb metal in root in phytostablization method, use of plants like sunflower can be suitable option for phytoremediation [11,31].

Iron Concentration in Shoots and Roots: Results of iron analysis in shoot of two plants showed that with increase in concentration of cadmium, concentration of iron in shoot of both plants increased. Although, increase in concentration of iron in both plants decreased in Cd200 treatments in comparison to Cd100, this decrease was due to toxic concentration of cadmium and statistically, it was not significant. In amaranthus, the highest iron concentration in shoot was observed in B2Cd50, B2Cd200 and B1Cd200 treatments respectively and in sunflower; it was observed in B1Cd100, B2Cd0 and B1Cd200.

The highest iron concentration in root of amaranthus was observed in B1Cd100, B1Cd200 and B0Cd100 respectively and in sunflower; it was found in B1Cd0, B1Cd100 and B0Cd50 respectively. B1Cd100 treatment in the amaranthus had the highest concentration. In terms of effect of inoculant,, similar to results of shoot, Bacillus inoculant had obtained better results.

Zinc Concentration in Shoots and Roots: The results showed that zinc concentration in shoot of amaranthus was generally higher than that in sunflower. The highest concentration of zinc in amaranthus was observed in B2Cd200, B2Cd100 and B1Cd0 respectively and in sunflower; it was found in B2Cd0, B0Cd50 and B0Cd0 respectively. In sunflower, increase in concentration of cadmium led to decrease in zinc concentration in the shoot in such a way that there was significant difference between concentration of zinc in Cd200 and Control (Cd0) ($P<0.01$). Results obtained from application of bacterium inoculant showed that there was not a significant difference between two inoculants in sunflower, but in amaranthus, there was significant difference among some of the treatments Figure 1.

Zinc concentration in different treatments of amaranthus had significant difference in terms of concentration of cadmium and consumption of inoculant. The highest concentration of zinc in amaranthus was observed in B2Cd200, B2Cd100 and B1Cd100 respectively and in sunflower; it was found in B0Cd50, B0Cd0 and B1Cd0 treatments respectively and seems that decrease in concentration of zinc in Cd200 was due to toxicity of cadmium in this treatment Figure 1.

Study on Characteristics of Bacteria: In table 2, some of the specifications of bacteria were identified and two strains were used in greenhouse test. Two strains of bacteria (B1 and B2) had some characteristics of Plant Growth Promoting Rhizobacteria (PGPR). Application of inoculant of these bacteria in greenhouse test showed better results in comparison to Control treatment (B0). These bacteria were resistant to high concentrations of heavy metals and were able to produce siderophores. Results obtained from determination of concentration of iron in different organs of the tested plants showed that concentration of iron in root of sunflower was higher than that in shoot. In fact, in some part of the plant which there is the maximum association of cadmium (root), concentration of iron was also high. Observations of this result and findings of other researchers indicated that the plant confronting with stress of heavy metals increases extent of iron absorption. This increase is a reply to metal stress and also is as result of effect of plant growth promoting rhizobacteria. Ability to produce microbial siderophores can be one of the important factors of iron concentration increase in shoot and root of the plant. Synergistic effects of bacterium and plant in these conditions are so close that this synergism in decrease of metal stress on the one hand and increase of iron concentration in root and shoot of the plant and facilitation in absorption of iron and zinc on the other hand leads to improvement of phytoremediation conditions. Ability to reduce ACC Deaminase Enzyme which prevent from synthesis of ethylene in the plant, reduces ethylene tension in the plant and is one of the ways of plant's confrontation with undesirable conditions. Similar research with isolation of resistant bacteria from contaminated soils and application of

Table 2: Some specification of growth promoting rhizobacteria – identification of bacteria on the basis of [45]

Strain of bacteria	Resistance to Pb (1000 mg/l)	Resistance to Zn (1000 mg/l)	Resistance to Ni (1000 mg/l)	Resistance to Cd (1000 mg/l)	Posetive or Negative Gram	ACC deaminase	Salinity (60 ds/m)	Siderophore
Bacillus mycoides M1	+	+	+	+	+	+	+	-
Micrococcus roseus M2	-	-	+	-	+	+	+	+
Bacillus circulans M3	+	-	-	-	-	-	+	+
Bacillus pumilis M4	+	-	-	-	+	+	+	-
Bacillus coagularis M5	-	+	-	-	-	+	+	+
Bacillus cereus M6	+	-	-	-	+	+	+	+
Micrococcus luteus M7	+	-	-	-	+	-	+	+

+ = proper reply to the intended characteristic - = lack of the intended characteristic

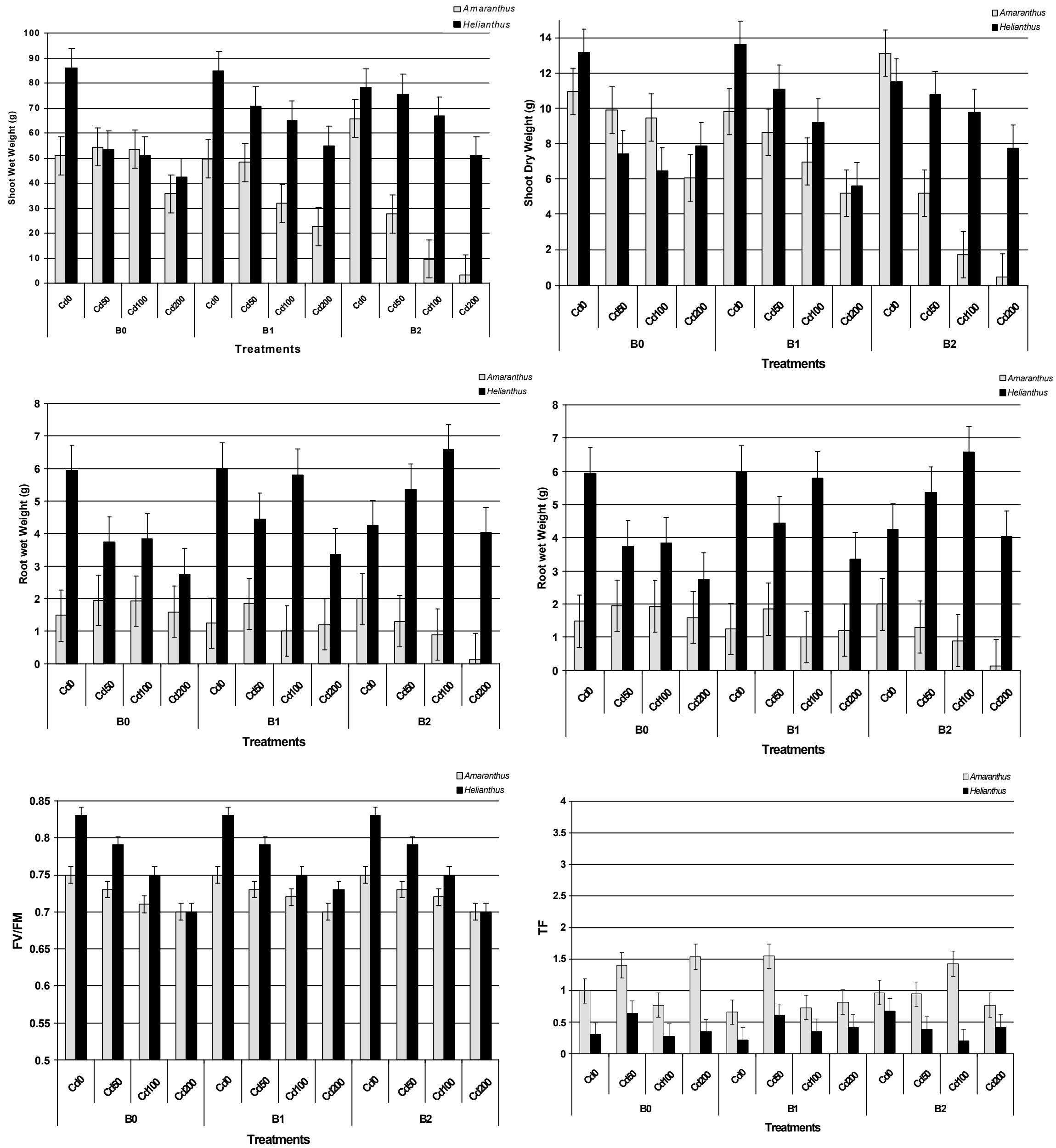

Fig. 2: Shoot and root wet, dry weight, Fv/Fm and TF of *A. retroflexus* and *H. annuus* grown in different Cadmium and inoculant treatments. B0: Control, B1: *Bacillus mycoides* M1 inoculant, B2: *Micrococcus roseus* M2 inoculant. Significantly different at P<0.01

inoculant showed that in zinc and cadmium contamination conditions, motion of these metals in Rhizosphere increased and concentration of iron increased in the plant. Bacteria had abilities of siderophores production, ACC Deaminase Enzyme and IAA (Oxine) and their consumption increased phytoremediation in the contaminated soils [1,21]. Bacterial complex of Iron-siderophore can be used by the plant and be a suitable iron resource for the plant. This case is the best reason for prevention from formation of chlorosis and yellowness of leaves in confrontation with high densities of heavy metals. Results showed that the role of effective bacterium was to facilitate provision of iron necessary for the plant for growth in case of presence of heavy metal. On the other hand, bacteria applying mechanisms such as decrease in ethylene stress, decrease in pH of

Rhizosphere and development of roots growth provide better opportunities for settlement and promotion of plant growth [2,21].

Shoots Wet Weight: Since presence of heavy metals leads to decrease in growth and function of the products [2,4,5], in this expriment, similar results were also obtained. In different treatments with increase in concentration of cadmium in the media, shoot wet weight and the highest extent of this decrease was found in Cd200 treatment for both plants Figure 2. The highest shoot wet weight of shoot of the plant in sunflower was found in B0Cd0, B1Cd0 and B2Cd0 and the lowest was found in B0Cd200, B0Cd100 and B2Cd200 treatments. which were not different statistically ($P<0.01$). In addition amount of biomass of sunflower was more than that of amaranthus. In amaranthus, the highest shoot wet weight was found in B2Cd0, B0Cd50 and B0Cd100 and the lowest shoot wet weight was found in B2Cd200, B2Cd100 and B1Cd200 treatments. In terms of effect of inoculant in shoot wet weight, there was statistically significant difference between two plants ($P<0.01$) and in amaranthus, Bacillus incoulant showed better results except in B2Cd0. in sunflower, Bacillus and Micrococcus inoculants showed better results than control in wet weight increase Figure 2.

Shoots Dry Weight: With increase in concentration of metal in media, shoot dry weight decreased in both plants and the lowest amount was observed in Cd200. in sunflower which had higher biomass than amaranthus, the highest shoot dry weight was observed in B1Cd0, B0Cd0 and B2Cd0 treatments and the lowest shoot dry weight was observed in B1Cd200, B0Cd100 and B0Cd50 treatments. In amaranthus, the highest dry weight of shoot was observed in B2Cd0, B0Cd0 and B0Cd50 treatments and the lowest shoot dry weight was observed in B2Cd200, B2Cd100 and B1Cd200 treatments. Observations showed that there was difference between sunflower and amaranthus in terms of effects of inoculant and Bacillus inoculant in amaranthus. Micrococcus inoculant in sunflower showed better results in dry weight increase Figure 2.

Roots Wet Weight: Root wet weight of sunflower in all treatments was higher than that of amaranthus. In most treatments, increase in concentration of metal led to decrease in wet weight especially in concentration of Cd200. Effect of inoculant can be discussed in this regard. The highest root wet weight in sunflower was observed in B2Cd100, B1Cd0 and B0Cd0 treatments and the lowest wet weight was observed in B0Cd200, B1Cd200 and B0Cd50 treatments. In amaranthus, the highest wet weight of root was observed in B2Cd0, B0Cd50 and B0Cd100 treatments and the lowest wet weight was observed in B2Cd200, B2Cd100 and B1Cd100 treatments. The obtained results showed that application of inoclant in sunflower was effective to concentration at Cd100 and in higher concentration, it had no considerable effect. There was not significant difference between two inoculants ($P<0.01$). In amaranthus, similar results were obtained Figure 2.

Roots Dry Weight: Results of root dry weight in different treatments showed that although increase in concentration of cadmium led to decrease in root dry weight, this decrease was more intense in control treatment (without inoculant). Between two plants, root dry weight of sunflower was more than that of amaranthus and the highest dry weight in sunflower was observed in B0Cd0, B1Cd0 and B2Cd100 treatments and the lowest dry weight was observed in B0Cd200, B0Cd100 and B1Cd200 treatments. In amaranthus, the highest dry weight of root was observed in B0Cd100, B0Cd0 and B0Cd50 treatments and the lowest dry weight was observed in B2Cd200, B2Cd100 and B1Cd100 treatments. There was significant difference among different treatments in terms of application of inoculant in both plants, in similar levels of cadmium, there was not significant difference between two inoculants statistically and application of inoculant showed better results than that of control treatment Figure 2. Study on data obtained from wet weight and dry weight of shoot and root showed that heavy metal stress led to decrease yield of wet and dry material. This stress was highest in concentrations of 200 mg/kg. Results of research showed that increase in concentration of heavy metals in soil decreased biomass of the root and shoot in *Zea mays*, sorghum, *Helianthus annuus*, *Cynodon dactylon* and *Conyza discordies*[54]. In another research, amount of biomass in shoot and root of sunflower in contaminated soil was less than that in uncontaminated soil [31]. With regard to effects of growth promoting rhizobacteria, yield in inoculant treatments (B1, B2) showed lower decrease in comparison to control and intensity of cadmium stress decreased Figure 1.

Photosynthetic Photochemical Efficiency: Green plants have two types of chlorophyll a and b. These two materials are green colored under white light, because they absorb radiation located in the limit of blue and red spectrum and reflect radiation located in green spectrum. Energy obtained from absorption is spent for performance of photosynthesized chemical reactions and its remaining is lost in the form of heat. Researchers measuring parameters of F0 (low level of photosynthesis), Fm(maximum level of photosynthesis) and their difference(Fv) obtained new ratio called Fv/Fm(photochemical quenching) which shows very close correlation with optical function of pure photosynthesis of healty leaves[43]. Data obtained from measurement of photochemical quenching showed that there was statistically significant difference between two plants in all treatments. On the other hand, photosynthetic quenching in sunflower was higher than that in amaranthus. The highest amount in sunflower was observed in B1Cd0, B2Cd0 and B0Cd0 treatments and the lowest amount was observed in B0Cd200, B2Cd200 and B1Cd200 treatments. In amaranthus, the highest photosynthetic quenching was observed in control treatments (Cd0) and the lowest was observed in B1Cd200, B2Cd200 and B0Cd200 treatments Figure 2.

Translocation Factor: Comparison of translocation factor showed that this factor in amaranthus was much better than that in sunflower and amaranthus had more successful phytoremediation with “phytoextration” mechanism, though the amount of biomass obtained in amaranthus was less than that in sunflower. The highest amount of translocation factor in amaranthus was observed in B1Cd50, B0Cd200 and B2Cd100 treatments. In sunflower, translocation factor in all treatments was less then 1 and was not considerable Figure 2. In fact, one can mention that sunflower acted successfully for use in phytoremediation through “phytostabilization” mechanism and prevention from expansion of contamination by its stabilization in root and this case shows its capability to be used in phytostabilization with regard to weight of biomass of sunflower [11,55]. Purpose of this research in the first step was to study absorption and translocation of heavy metals in shoot and root of the plants and to determine translocation factor (TF) and to study possibility of using them in phytoremediation of soils contaminated with heavy metals. In principle, in order to have necessary efficiency for performance of phytoremediation, amount of plant biomass production should be high and its capacity metals absorption should be high and should be resistant to pollution of heavy metals [56]. In addition, its growth period should be short. Results obtained from this research showed that amaranthus had metal translation factor higher than 1 and naturally, one can continue studies in this field by aiming at its applied use. Since many native and nonnative plants don’t have major characteristics necessary for use in phytoremediation, effort to recognize and study this method and ways of phytoremediation efficiency increase is very important. As mentioned, phytoremediation of contaminated soils is a relatively new and developing technology with special benefits [12-14]. On the basis of results obtain from greenhouse and laboratory tests, it seems that by continuation of research in the fields of botany and study of characteristics of growth promoting rhizobacteria and synergism between plant and bacterium, one can be successful in development of application of phyotremediation in soils contaminated with heavy metals with economic and applied approach. In addition that some of the plants such as sunflower can be used for control and prevention from distribution of metals contamination (phytostabilization) and phytoexraction method can be used by identification of native and nonagricultural plants which are hyperaccumulator for phytoremediation of the contaminated regions. Potential of phytoremediation depends on interactions of soil, heavy metals, bacterium and plant. Recognition of these factors and mechanism of their effect can have important role in development of phytoremediation application.

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