

Article

## Effects of biological fertilizers, EDTA chelate, urban compost and biochar on corn root remediation

Sadegh Bahamin<sup>1</sup>, Abbas Maleki<sup>2</sup>, Javad Dawoudian<sup>3</sup>, Behrouz Khalil Tahmasebi<sup>4</sup>, Shohreh Azizi<sup>5,6</sup>, Shamsolah Asgari<sup>7</sup>

<sup>1</sup>Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, Ilam Branch, Islamic Azad University, Ilam, Iran

<sup>3</sup>College of Natural Resources and Environmental Planning, Birjand University, Birjand, Iran

<sup>4</sup>Plant Protection Department, South Kerman Agricultural and Natural Resources Research and Education Center (AREEO), Jiroft, Iran

<sup>5</sup>UNESCO-UNISA Africa Chair in Nanoscience and Nanotechnology, College of Graduate Studies, University of South Africa, Muckleneuk Ridge, Pretoria, South Africa

<sup>6</sup>Nanosciences African Network (NANOAFNET), iThemba LABS-National Research Foundation, 1 Old Faure Road, Somerset West, 7129, PO Box 722, Somerset West, Western Cape, South Africa

<sup>7</sup>Soil Conservation and Watershed Management Research Department, Ilam Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

E-mail: shohrehazizi1379@gmail.com

Received 8 January 2022; Accepted 15 February 2022; Published 1 June 2022



### Abstract

This study was carried out based on a randomized complete block design with three replications in Ilam (Iran) under greenhouse conditions in 2019. This design includes biological fertilizer factor at two levels of inoculation and non-inoculation, mycorrhiza fungus at two levels of consumption and non-consumption, and fertilizer at four levels that include control treatment, EDTA chelate, urban compost and biochar application on the absorption of heavy metals, phytoremediation power and corn yield. In this study, the concentration of zinc, cadmium, Cadmium, nickel and chromium in the root as well as grain yield, TF indicator (transfer factor), BCF (biological aggregation factor), and BAC (biological aggregation coefficient) were measured. The data were analyzed with SAS software using Duncan's multiple range test. The highest transfer factor and BAC value were 1.82 and 1.59 respectively in the biochar treatment, mycorrhiza treatment, and bacterial inoculation, but there was no significant difference with the use of EDTA chelating agent or urban compost consumption. Furthermore, the lowest value of transfer factor and BAC were 0.59 and 0.76 respectively in the treatment of non-consumption of fertilizer levels. At different levels of control treatment (including bacterial inoculation or non-bacterial inoculation and consumption or non-consumption of mycorrhizae), BAC in the root was always lower than different levels of biochar consumption, EDTA chelating agent, and urban compost. The maximum BCF was obtained in the control treatment, compost consumption and non-bacterial inoculation at 1.2. In general, non-consuming any type of fertilizer increased this index. Moreover, the highest colonization was obtained in the biochar treatment, consumption of mycorrhiza, and bacterial inoculation at 39.7%. In this study, it was found that separate use of urban compost, especially biochar and EDTA chelating agent, as well as combination of these fertilizers with mycorrhizae and bacteria have increased the absorption of heavy elements in the roots and improved the potential of corn for phytoremediation.

**Keywords** mycorrhiza; urban compost; biochar; phytoremediation; biological accumulation factor.

Proceedings of the International Academy of Ecology and Environmental Sciences  
ISSN 2220-8860  
URL: <http://www.iaees.org/publications/journals/piaees/online-version.asp>  
RSS: <http://www.iaees.org/publications/journals/piaees/rss.xml>  
E-mail: [piaees@iaees.org](mailto:piaees@iaees.org)  
Editor-in-Chief: Wenjun Zhang  
Publisher: International Academy of Ecology and Environmental Sciences

## 1 Introduction

Phytoremediation is one of the most important biological methods that is applied to refine heavy metals in contaminated soils. This technology requires the use of metal-accumulating plants to remove, move, or stabilize metal contaminants in the soil. However, this technique is time-consuming and is considered as one of the main drawbacks (Wenzel et al., 1999). Accordingly, the efficiency of the phytoremediation technique is affected by the activity of rhizosphere microbes, metal species, and the concentration of metal elements in the soil (Khan, 2005). The heavy metals contaminated soils not only affect the health of communities but also high cost is required to remove and refine pollutants. In addition to the common strategies for dealing with heavy metal stress, biological methods should be considered to increase the ability of plants to absorb pollutants and reduce their harmful effects (Dawoudian et al., 2021). These methods include the application of plant growth-promoting bacteria, mycorrhizal fungi, and hyper accumulator plants. This solution is one of the most effective and safe methods compared to other cleaning technologies in terms of environmental compatibility, productivity and cost (Geoffrey and Gadd, 2004). Plant growth promoting rhizobacteria through various mechanisms such as improving plant growth and increasing the biodegradability of metals are able to increase plant efficiency of refining metal pollutants. For example, the use of *Pseudomonas* and *Asyntobacter rhizobacteria*, which have the ability to promote plant growth (Bahamin et al., 2013; Shamsibeiranvand et al., 2017), improved the plant's ability (Rezaei et al., 2015; Foladvand et al., 2017) to purify the non-biodegradable species of corn by increasing its growth and biomass (Lippmann et al., 1995; Bahamin et al., 2014; Zabet et al., 2015).

Microorganisms can adopt special mechanisms to tolerate the adsorption of metal ions (Fathi and Bahamin, 2018; Kardoni et al., 2019) and to reduce the intensity of heavy metals stress. These mechanisms include (1) pumping metal ions into the extracellular space; (2) accumulation of metal ions inside the cell; (3) conversion of toxic metals into forms with less toxicity, and (4) absorption or desorption of heavy metals (Wani et al., 2008; Maleki et al., 2020). Due to these properties, when plant growth-promoting rhizobacteria, inoculated with seeds or applied to the soil, significantly reduce metal toxicity while improving the overall growth and yield of crops such as chickpeas (*Cicer arietinum*), mung bean (*Vigna radiata* and *Pisum sativum*) (Zaidi and Khan, 2006; Khoshkhabar et al., 2015). Additionally, rhizobacteria can improve soil fertility and increase crop yields (Fathi et al., 2017) by providing the essential nutrients. Mycorrhizal fungi, after establishing a symbiotic relationship, change the root secretions of the host plant quantitatively and qualitatively, so they can play a considerable role to clean up the environment. Furthermore, mycorrhizal fungi can improve plant nutrition, plant water relationships, and increase plant tolerance to pollutants. (Belimov et al., 2005; Bahamin et al., 2019).

Roots are the first plant organs to expose to heavy metals in contaminated soil, and metal ions are transferred to various organs of plant after adsorption by roots. Plants in heavy metals contaminated soils can be damaged by inactivating photosynthesis, protein and DNA synthesis, stomatal activity, and the production

of free radicals. However, plants can maintain their survival in metal-contaminated soils by absorbing and accumulating heavy metals or by synthesizing metal-binding complexes and chelates (Grill et al., 1985). Reactions between plants and beneficial microspheres of rhizosphere can increase biomass production and plant tolerance to heavy metals, so it can be an important component of refining plant technology (Bernard and Glick, 2003). Rhizobacteria stimulate plant growth through various mechanisms can improve soil contaminated with metal, thus increasing the efficiency of the crop process. These mechanisms are atmospheric nitrogen stabilization, phosphate dissolution, secretion of iron-containing chelating agents, and production of growth-promoting hormones such as auxin and gibberellin, and inhibition of overgrowth of mitochondrial growth by increasing ethylene.

The aim of this study was to investigate the effect of inoculation of plant growth-promoting bacteria and arbuscular mycorrhiza on the growth and absorption of heavy metals by corn plants in soils treated with different levels of heavy metals.

## 2 Study Area and Methodology

### 2.1 Study site

In this research, a factorial pot experiment with three factors was performed in a completely randomized design with three replications. This study was carried out based on a randomized complete block design with three replications that was conducted in Ilam city (Ilam province, Iran) in 2019.

### 2.2 Data collection

This design includes biological fertilizer factor at two levels of inoculation and non-inoculation; mycorrhiza fungi at two levels of consumption and non-consumption and fertilizer at four levels of control, EDTA chelate (ethylene diamine tetraacetic acid), urban compost and biochar on the absorption of heavy elements, phytoremediation power and corn. Consumption of EDTA chelate (ethylene diamine tetraacetic acid), municipal compost and biochar was 1% of soil weight in pots (Jafari et al., 2017).

**Table 1** Physical and chemical decomposition results of some soil properties used.

Soil texture	pH	EC (ds.m <sup>-1</sup> )	CaCO <sub>3</sub> (%)	Organic matter (%)	P mg kg <sup>-1</sup>	DTPA-Zn mg kg <sup>-1</sup>
Clay loam	7.21	1.03	26.7	1.01	17.4	0.45

**Table 2** Some chemical decomposition results of biochar and urban waste compost

	pH	EC(ds.m <sup>-1</sup> )	C (%)	N (%)	Moisture (%)	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )
Biochar	8.9	6.5	11.8	0.78	-	-	-	-
Urban compost	7.1	3.5	16.7	1.09	8.8	18.1	180	2.5

After analyzing and measuring the concentration of some nutrients in several soil samples, suitable soil in terms of nutrient proportions was selected for corn cultivation and experimentation. Then, sufficient amounts of soil were prepared. The used soil (Table 1) was a non-gypsum and non-saline arable soil with loamy clay texture that its classification was Typic Haploceracid.

The microbial strains used in this study were *P. fluorescens* and *Glomus mosseae* microbial fungi, which were provided from the Microbial Bank of the Soil Biology Department of the Soil Research Institute of Iran. To carry out microbial treatments, 100 g of fungal inoculation with a potential of about 250 prologues per cm<sup>3</sup> was added as a layer approximately 2 cm thick in pots for fungal treatment before planting and under seeds.

For bacterial treatment, 50 ml of liquid culture medium containing *Pseudomonas* bacteria, which grew at a temperature of 28.5°C in incubators in the Nutrient Brass culture medium was given to each of the pots and the population were about 10<sup>8</sup> bacteria per ml (He et al., 2009).

For each pot, Chelate EDTA was applied at a rate of 10 mg kg<sup>-1</sup> and compost at 30 tons per hectare. Before distributing the soil in pots, first 100 mg kg<sup>-1</sup> of heavy metal (zinc (ZnSO<sub>4</sub>), cadmium, nickel, Pb and chromium) were calculated for each treatment to contaminate the soil (Table 2). Then, certain values from the prepared mother solution were taken for each level and added to a small part of the desired soil and mixed with all the soil of the desired treatment to create a uniform distribution of zinc on the soil. In the next step, the soils were soaked in plastic pots for about 5 months in consecutive dry-wet periods at room temperature. During each dry-wet period, the soil in the pots was saturated and then the dry air was applied so that the soil moisture reached a relatively constant level. Drainage pots were used to prevent leaching. Each dry-wet period lasted 40 days, and after each period the soil in the pots was thoroughly mixed to create uniformity in concentration. At the end of each period, the total amount of zinc and solution was measured. After the reactions, the contaminated soils were disinfected twice (within a week) with an autoclave at a temperature of 121°C and pressure of 1.5 atmospheres for 2 hours in canned bags. After performing the treatments, eight seedlings of single Cross 703 were sown, therefore five plants were kept in each pot. During the growing season, the pots were irrigated to the capacity of the crop by weight method.

Three months after planting, the plants were removed from the crown, washed, and dried at 70°C for 48 hours. After determining the dry weight of the plants, the concentration of heavy elements in the aerial parts were measured by the digestion method (use of sulfosalicylic acid) using the atomic absorption device of Perkin Almer model AA200 (Khattak and Jabeen, 2012). DTPA (Tetrix) solution was used to extract heavy metals (Boguta and Sokolowska, 2012). Finally, the concentrations of cadmium, Pb, zinc, chromium and nickel metals were measured.

To assess plant potential for phytoremediation, after determining the amount of heavy metals, TF indicators (transfer factor; ratio of metal concentration in aerial parts of the plant to the concentration of metal in the roots), BCF (biological accumulation factor; ratio of metal concentration in plant roots to metal concentration in soil), and BAC (biological accumulation coefficient; ratio of metal concentration in plant shoots to metal concentration in soil) were measured. Based on these indicators, ability of the plant should be evaluated for soil contamination.

In this regard, if the TF is greater than 1, the plant is suitable to extract contaminants. Also plants with the TF and BAC index values greater than 1 are appropriate phyto stabilization. Additionally, plants with the TF value less than one and the BCF value more than 1 are appropriate for the phyto stabilization (Yoon et al., 2006). In order to determine the percentage of mycorrhizal symbiosis, the roots were stained with trypan blue (Norris et al, 1992) and the percentage of root length coexistence was determined by McGonigle et al (1990) method.

### 2.3 Analysis data

The data obtained were analyzed with SAS software using LSD (least significant difference) test.

## 3 Results and Discussion

### 3.1 Zinc concentration in the root

The results represented that the main effect of fertilizer, mycorrhiza, and bacteria on zinc concentration in the root were significant (Table 3). Moreover, the highest zinc content was obtained in the treatment of urban compost consumption (448.8) but there was no significant difference with the use of EDTA chelator. In the case of mycorrhiza application or bacterial inoculation, the Zn concentration in the root was higher than non-

mycorrhiza or non-bacterial inoculation (Table 4). Zinc is one of the essential micronutrients and when its concentration in the leaf is more than 400 mg/kg of dry weight, toxicity symptoms appear as necrotic spots on the leaf and high concentrations of zinc causes plant death. The roots of plants are associated with a large number of different living things. The reaction of the two with each other and with soil conditions determines the growth and reproduction of plants (Varvara et al, 2000).

**3.2 Cadmium concentration in the root**

The results illustrated that the main effect of fertilizer, mycorrhiza, and bacteria on cadmium concentration in the root were significant (Table 3). Furthermore, the highest root cadmium was recorded in the biochar treatment at 329.8, but there was no significant difference with EDTA or urban compost. In the case of mycorrhiza application or bacterial inoculation, the concentration of cadmium in the root was higher than in non- mycorrhiza or non-bacterial inoculation (Table 4).

**3.3 Nickel concentration in the root**

The results showed that the main effect of fertilizer, mycorrhiza, bacteria, and the triple interaction of fertilizer, bacteria and mycorrhiza on nickel concentration in the root were significant (Table 3). The highest nickel concentration in the root was recorded in the biochar treatment, mycorrhiza and bacterial inoculation at 22.4 mg kg<sup>-1</sup> (a), but there was no significant difference with the use of EDTA chelating agent. The lowest value was 4.7 mg kg<sup>-1</sup> (h) in the non-consumption of any type of fertilizer. At different levels of control treatment (including bacterial inoculation or non-inoculation, use or not of mycorrhiza), nickel concentration in the root was always lower than different levels of biochar, EDTA chelator and urban compost (Fig. 1).

**Table 3** ANOVA results of heavy metal concentration, yield and transfer factor in shoot organs.

	Zinc concentration in root	Cadmium concentration in root	Nickel concentration in root	Pb concentration in root
Replication	7127.7*	573.4ns	0.92ns	263.7ns
Fertilizer	35587**	23420**	37.42**	915.2**
Error a	748.1	398.7	0.41	68.8
mycorrhiza	16752**	19409**	91.6**	2441**
Mych.fert	2171.0ns	1001ns	0.43ns	52.5ns
Error b	739.0	442.0	1.59	27.4
Bacteria	72824**	40111**	181**	3134**
Bac.fert	1543ns	711.1ns	1.28ns	110.8ns
Bac.mych	4940ns	1422.6ns	0.09ns	505.7*
Bac.mych.fert	1160ns	200.3ns	7.48**	205.1ns
Residual	1527.9	989.9	0.59	92.7
CV	9.9	11.2	5.2	12.1

\*: Significant at 5%; \*\*: Significant at 1%; ns: Non-significant

**Table 3 (continued)** ANOVA results of heavy metal concentration, yield and transfer factor in shoot organs.

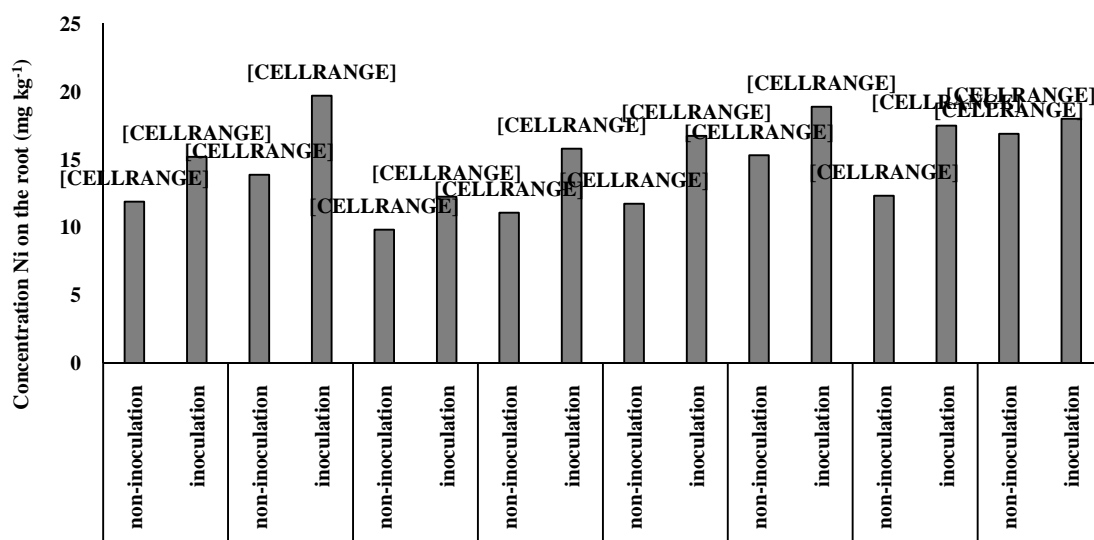
	Chromium concentration in root	Transfer factor	Biological aggregation coefficient	Biological accumulation factor	Colonization
Replication	1464ns	0.031ns	0.010ns	0.018ns	46.1*
Fertilizer	899ns	1.158**	0.209*	0.13*	245.6**
Error a	313.4	0.014	0.022	0.014	8.1
mycorrhiza	1304*	0.856**	0.706**	0.038ns	316.0**
Mych.fert	16.3ns	0.039ns	0.009ns	0.008ns	5.5ns
Error b	230.7	0.014	0.007	0.007	14.5
Bacteria	1171ns	2.871**	1.278**	0.153**	457**
Bac.fert	1.9ns	0.082**	0.016ns	0.012ns	36.4**
Bac.mych	9.3ns	0.068*	0.015ns	0.106**	0.1ns
Bac.mych.fert	41.9ns	0.087**	0.032*	0.028*	24.4**
Residual	272	0.013	0.009	0.008	4.5
CV	12.8	8.9	8.5	9.2	6.8

\*: Significant at 5%; \*\*: Significant at 1%; ns: Non-significant

**Table 4** Mean comparison of main effect of mycorrhiza, bacterium and fertilizer on Pb, Zn, Cd and Cr.

	Pb concentration in root (mg kg <sup>-1</sup> )	Zinc concentration in root (mg kg <sup>-1</sup> )	Cadmium concentration in root (mg kg <sup>-1</sup> )	Chromium concentration in root (mg kg <sup>-1</sup> )
<b>Fertilizer</b>				
Biochar	77.5 b	388.5 b	329.8 a	132.1
Control	66.6 c	320.1 c	222.5 c	115.6
EDTA chelating agent	81.3 ab	414.2 ab	278.1 b	132.3
Urban compost	87.4 a	448.8 a	288.1 ab	134.1
<b>Mycorrhiza</b>				
Non-application	71.1 b	374.2 b	259.5 b	123.3 b
Application	85.3 a	411.6 a	299.7 a	133.7 a
<b>Bacterium</b>				
Non inoculation	70.1 b	354.0 b	250.7 b	123.6
Inoculation	86.3 a	431.9 a	308.5 a	133.5

Means with similar letters in each column, show non- significant difference according to LSD tests at 5% level.



**Fig. 1** Triple interaction effect on nickel concentration in plant root. Means with similar letters in each column, show non-significant difference according to LSD tests at 5% level.

### 3.4 Cadmium concentration in the root

The results demonstrated that the main effect of fertilizer, mycorrhiza, bacteria, and the interaction of bacteria and mycorrhiza on the lead concentration in the root were significant (Table 3). The highest concentrations of lead in the root were obtained at 96.4 mg kg<sup>-1</sup> (a) in the treatment of mycorrhiza consumption and bacterial inoculation. The lowest value was 43.7 mg kg<sup>-1</sup> (c) in the non-consumption of each type of fertilizer (Fig. 2).

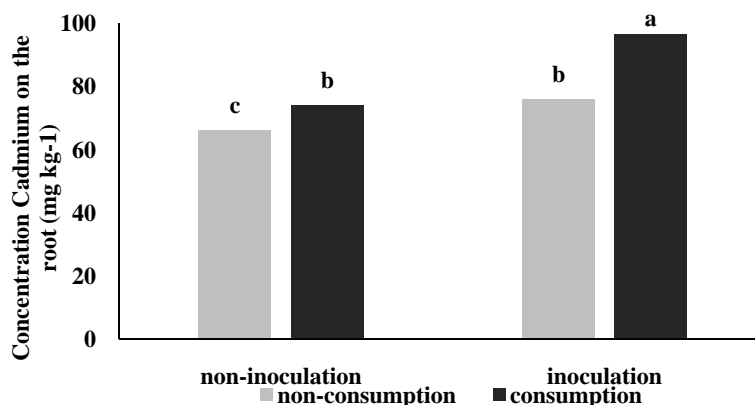


Fig. 2 Double interaction effect of mycorrhiza and rhizobium inoculation on root Cadmium concentration. Means with similar letters in each column, show non- significant difference according to LSD tests at 5% level.

### 3.5 Chromium concentration in the root

The results showed that the main effect of mycorrhiza on chromium concentration in the root was significant (Table 3). In the case of consumption of mycorrhiza or bacterial inoculation, chromium concentration in the root was higher than non- consumption of mycorrhiza or non- bacterial inoculation (Table 4).

### 3.6 Transfer factor (TF)

The results represented that the main effect of fertilizer, mycorrhiza, bacteria, and the triple interaction of fertilizer, bacteria and mycorrhiza on the transfer factor were significant (Table 3). The maximum transfer factor was found in the biochar treatment, mycorrhiza consumption and bacterial inoculation at 1.82 (a), but there was no significant difference with EDTA or consumer compost. The lowest value was 0.59 (i) in the treatment of non-use of any type of fertilizer. At different levels of control treatment (including bacterial inoculation or non-inoculation, or use or not of mycorrhiza), the transmission factor was always lower than different levels of biochar, EDTA chelator and municipal compost (Fig. 3).

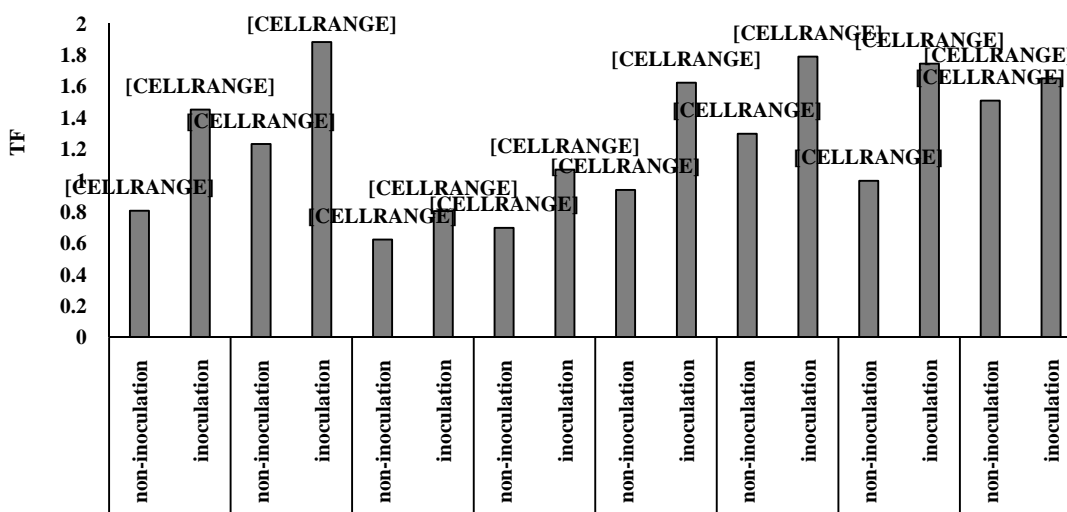
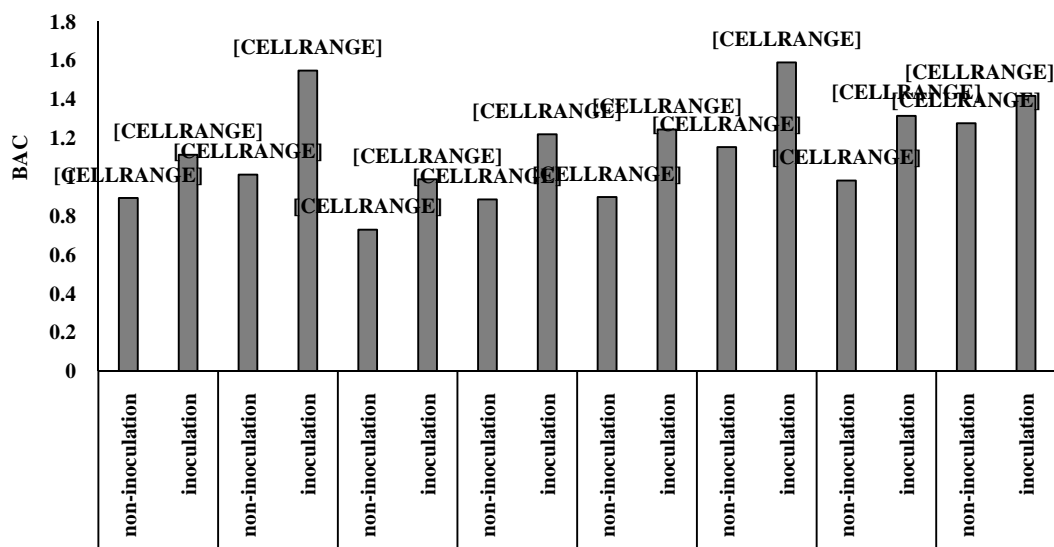


Fig. 3 Triple interaction effect on transfer factor. Means with similar letters in each column, show non- significant difference according to LSD tests at 5% level.

### 3.7 Biological aggregation coefficient (BAC)

The results demonstrated that the main effect of fertilizer, mycorrhiza bacteria, and the interaction of triple fertilizer, bacterium and mycorrhiza on BAC were significant (Table 3). The highest BAC was obtained in the biochar treatment, consumption of mycorrhiza, and bacterial inoculation at 1.59 (a), but there was no significant difference with EDTA or compost. The lowest value was recorded in the treatment of non-consumption of any type of fertilizer at 0.76 (g). At different levels of control treatment (including bacterial inoculation or non-inoculation, or consumption or non-consumption of mycorrhizae), BAC in root was always lower than at different levels of biochar consumption, EDTA chelating agent and urban compost (Fig. 4).



**Fig. 4** Triple interaction effect on BAC. Means with similar letters in each column, show non-significant difference according to LSD tests at 5% level.

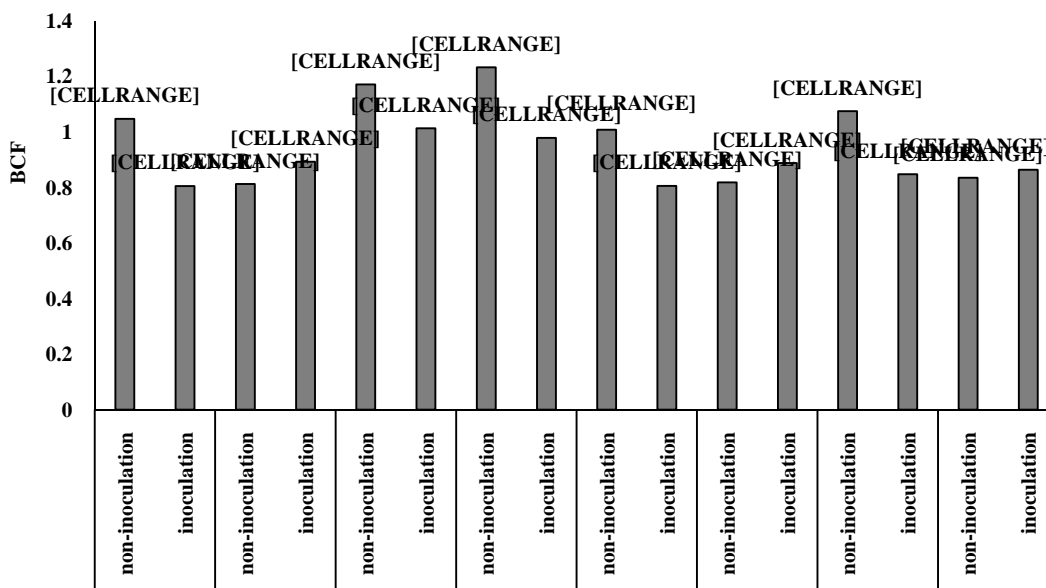
### 3.8 Biological accumulation factor (BCF)

The results illustrated that the main effect of fertilizer, bacteria and the triple interaction of fertilizer, bacteria and mycorrhiza on BCF were significant (Table 3). The maximum BCF was obtained in the control treatment, compost consumption, and non-bacterial inoculation at 1.2 (a). In general, non-use of any type of fertilizer increased this index (Fig. 5).

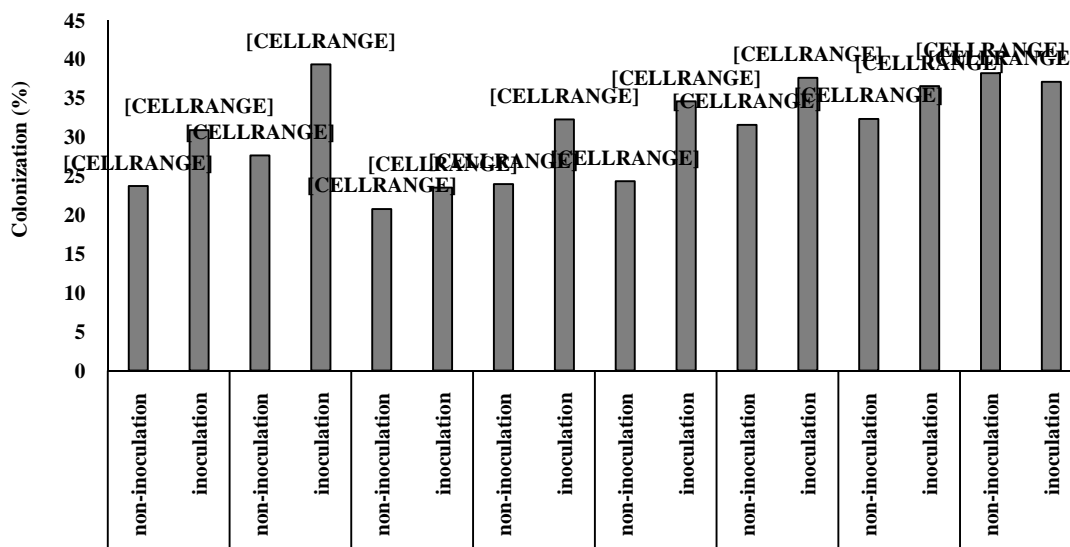
### 3.9 Colonization

The results demonstrated that the main effect of fertilizer, mycorrhiza, bacteria, the interaction of fertilizer and bacteria, and the effect of triple interaction on colonization were significant (Table 3). The highest colonization was found in the biochar treatment, consumption of mycorrhiza, and bacterial inoculation at 39.7 (a), but there was no significant difference with the use of EDTA or compost. The lowest value was 21.2 (f) in the treatment of non-use of any fertilizer. At different levels of control treatment (including bacterial inoculation or non-inoculation, or mycorrhiza use or non-use), root colonization was always lower than different levels of biochar, EDTA chelator and municipal compost (Fig. 6).





**Fig. 5** Triple interaction effect on BCF. Means with similar letters in each column, show non- significant difference according to LSD tests at 5% level.



**Fig. 6** Triple interaction effect on colonization percentage. Means with similar letters in each column, show non- significant difference according to LSD tests at 5% level.

#### 4 Conclusions

Based on the results of the transfer factor (TF) and the biological aggregation coefficient (BAC), the use of compost, chelating agent or biochar can increase this index individually or in combination with bacteria and mycorrhiza. In this study, using the mentioned treatments, the transfer factor and biological accumulation

coefficient were higher than 1, which indicates the power of phytoremediation and transfer of heavy elements in these treatments. Additionally, in this research colonization was enhanced with the use of mycorrhizae and bacteria, as well as used fertilizers.

Plant growth-promoting rhizobacteria are able to reduce the effects of stress imposed by high levels of endogenous ethylene on the plant by stimulating the synthesis of enzyme 1-aminocyclopropane 2-carboxylase deaminase (ACC). These beneficial microbes, due to their multiple properties such as resistance to metals and their ability to convert into less toxic forms, as well as the ability to promote plant growth through various mechanisms, are one of the most suitable options to use in researches related to bioremediation of contaminated soils. Due to the high nutrient content of roots, rhizosphere soil absorbs more bacterial species than non-rhizosphere soil (Penrose and Glick, 2001). These bacteria, including growth-promoting rhizobacteria, facilitate plant growth and, on the other hand, have been shown to be more effective in minimizing bioavailability and biological toxicity of heavy metals (Wani et al., 2008). Although plant growth-promoting rhizobacteria has been used primarily to facilitate plant growth in agricultural operations, significant emphasis is still being placed in order to exploit their bioremediation potential (Khan, 2004).

Due to the loss of beneficial microorganisms in metal-contaminated soils, these soils often have little fertility or even in some cases lack the essential nutrients for the proper growth of plants. However, these soils can be enriched in terms of nutrients through the use of microbial-induced inoculation, especially plant growth-promoting rhizobacteria. On the other hand, these rhizobacteria not only provide essential nutrients for plants in contaminated soils, but also play an important role in detoxifying metal ions, enabling plants to refine heavy metals (Mayak et al., 2004). For example, when rhizobacteria were used to stimulate the growth of *Kluyvera ascorbata* SUD165 in soils treated with heavy metals such as nickel, zinc, Cadmium and chromium, not only it improved the growth of rapeseed (*Brassica napus*) but also protected the plant against nickel toxicity (Burd et al., 1998). Similarly, the use of nickel-resistant *Kluyvera ascorbata* in soil treated with nickel, Pb and zinc protected *Lycopersicon esculentum* L., indian mustard (*Brassica juncea*) and rapeseed against the toxicity of these metals (Burdica et al., 1998).

Mycorrhizal fungus are involved in the process of immobilizing (organizing) heavy metals in contaminated soils by secreting certain enzymes and reducing their accumulation in plants (Joner and Leyval, 2001). Audet and Charest (2006) showed that by increasing zinc levels the concentration and uptake of this element in the shoots of mycorrhizal plants was higher than non-mycorrhizal plants and most of the zinc uptake accumulated in the shoots. Moreover, it has been reported that the total adsorbed zinc was significantly lower in mycorrhizal plants grown in zinc contaminated soils compared to non-mycorrhizal plants. The results of many studies illustrate that the presence of growth-promoting bacteria in the rhizosphere of plants grown in heavy metals contaminated soils has led to an increase in the concentration of some metals such as zinc, copper, lead and chromium in plant organs (Abou-Shanab et al., 2007). Li et al (2007) stated that inoculation of growth-promoting bacteria in soils treated with different zinc levels significantly enhanced the dry weight of aerial organs and zinc uptake in plants compared to non-inoculated plants.

Salt represented that the use of 5 mmol kg<sup>-1</sup> of EDTA of soil was very effective to improve the phytoremediation of the superabsorbent Indian mustard plant. The roots of this plant have a high capacity to absorb cadmium in their cell walls, therefore the application of this substance to improve the movement of this element from the roots to the aerial parts was very useful. EDTA has a special ability to destroy root cells (Salt, 1997). Some other findings are based on the fact that the lead or cadmium complex is transferred by EDTA from the root to the shoots, when the level of these elements in the root are high enough until the symptoms of toxicity appear and the plant feels dangerous, so the root removes this extra substance through the vessels

(Vasil, 1998). Chen et al. reported that application of 10 mmol kg<sup>-1</sup> EDTA of soil caused toxic effects on Indian mustard leaves and reduced plant dry weight compared to control (Chen et al., 2004).

Pb uptake index compared to cadmium and nickel is composed of smaller numbers due to lower Cadmium concentration in the shoots of the plant. Increasing adsorption index in compost treatments can be related to the positive effect of organic matter on physical, chemical and biological properties of soil. Sometimes the concentration of the element in the shoot increases but the absorption index decreases. For example, decreasing absorption index in treatments with high levels of EDTA or compost can be related to the toxicity of plant.

In this study, application of biochar, compost, chelating agent or rhizosphere inoculation, lead to increase the transfer coefficient of heavy elements and their uptake into the roots, which is a desirable result. Plants with the TF value less than one and the BCF value greater than 1 are suitable for the phyto establishment process (Yoon et al., 2006). The minerals and organic part of the compost may have been able to reduce the availability of heavy elements in the soil by increasing the absorption properties of the soil and consequently reduce the bioaccumulation factor of the plant roots. It is possible that the application of compost has been able to increase plant biomass in heavy metal contaminated soil by reducing plant stress to heavy metals. Due to the decomposition of organic fertilizers over time and on the other hand the redistribution of heavy metals in the soil, phytoremediation is necessary. In this regard, the physical and chemical conditions of the soil such as the percentage of organic matter in the soil can be an effective factor in to remove heavy metal from the ground and of course its uptake by plants (Mashayekhi et al., 2017).

Refining of heavy metals contaminated soils using biological systems (including microbes and plants) is one of the new research fields that has brought made remarkable achievements in recent years. At the same time, the need to empower emerging bioremediation technology is felt through the implementation of numerous field tests under different climatic conditions. On the other hand, understanding the mechanism of physical, chemical, and biological processes in the rhizosphere and the interactions between overgrown, non-overgrown and plant growth-promoting rhizobacteria can be important to better simulate the phytoremediation effects of heavy metal contaminated areas. In addition, the treatment of heavy metal-contaminated sites with plant-growth-promoting rhizobacteria is a cost-effective technique for treating metal contaminants, as these microorganisms can easily, without cost, produce large numbers of bacterial cells. However, in order to turn phytoremediation into optimal option for refining heavy metals contaminated soils, some of the issues related to this technology should be considered, such as mechanisms involved in the absorption and detoxification of metal ions, as well as factors affecting the mobility and transfer of heavy metals. In this study, it was found that separate consumption of municipal compost, especially biochar and EDTA chelator, as well as the combination of these fertilizers with mycorrhiza and bacteria has increased the absorption of heavy elements in the roots and improved the phytoremediation power of corn.

### Acknowledgement

The authors are gratefully acknowledging the Islamic Azad University of Ilam for supporting this research grant.

### References

- Abou-Shanab R, Angle JS, van Berkum P. 2007. Chromate tolerant bacteria for enhanced metal uptake by *Eichhornia crassipes* (Mart). International Journal of Phytoremediation, 9: 91-105
- Audet P, Charest C. 2006. Effect of AM colonization on wild tobacco plants grown in Zinc contaminated soil.

- Mycorrhiza, 16: 277-283
- Bahamin S, Koocheki A, Nassiri Mahallati M, Beheshti SA. 2021. Effect of nitrogen and phosphorus fertilizers on yield and nutrient efficiency indices in maize under drought stress. *Environmental Stresses in Crop Sciences*, 14(3): 675-690
- Bahamin S, Koocheki A, Nassiri Mahallati M, Beheshti S. 2019. Effect of biological and chemical fertilizers of nitrogen and phosphorus on quantitative and qualitative productivity of maize under drought stress conditions. *Environmental Stresses in Crop Sciences*, 12(1): 123-139
- Bahamin S, Parsa S, Ghoreishi S. 2013. The examination of effects of growth stimulating and salinity bacteria on the characteristics of *Mentha spicata* leaves. *International Journal of Agronomy and Plant Production*, 4(9): 2119-2125
- Bahamin S, Sohrab M, Mohammad AB, Behroz KT, Qorbanali A. 2014. Effect of bio-fertilizer, manure and chemical fertilizer on yield and reproductive characteristics of sunflower (*Helianthus annuus* L.). *International Journal of Agriculture and Environmental Research*, 3(1): 36-43
- Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR. 2005. Cadmium-tolerant plant growth promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biology and Biochemistry*, 37: 241-250
- Bernard R and Glick BR, 2003. Synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances*, 21: 383-393
- Boguta P, Sokolowska Z. 2012. Influence of phosphate ions on buffer capacity of soil humic acids. *International Agrophysics*, 26(1): 7-14
- Boroumand N. 2016. Effects of arbuscular mycorrhizal fungi on cadmium phytoremediation by marigold (*Tagetes erecta*). *Journal of Soil Management and Sustainable Production*, 6(1): 191-204
- Burd GI, Dixon DG, Glick BR. 1998. A plant growth promoting bacterium that decreases nickel toxicity in seedlings. *Applied and Environment Microbiology*, 64: 3663-3668
- Chen YH, Li XD, Shen ZG. 2004. Leaching and uptake of heavy metals by ten different species of plants during an EDTA-assisted phytoextraction process. *Chemosphere*, 57: 187-196
- Dawoudian J, Bahamin S, Tantoh HB. 2021. Environmental impact assessment of cement industries using mathematical matrix method: case of Ghayen cement, South Khorasan, Iran. *Environmental Science and Pollution Research*, 28(18): 22348-22358
- Fathi A, Bahamin S. 2018. The effect of irrigation levels and foliar application (zinc, humic acid and salicylic acid) on growth characteristics, yield and yield components of roselle (*Hibiscus sabdariffa* L.). *Environmental Stresses in Crop Sciences*, 11(3): 661-674
- Fathi A, Kardoni F, Bahamin S, Khalil Tahmasebi B, Naseri R. 2017. Investigation of management strategy of the consolidated system of organic and biological inputs on growth and yield characteristics in corn cultivation. *Applied Research of Plant Ecophysiology*, 4(1): 137-156
- Foladvand F, Khoshkhabar H, Naghdi N, Hosseinabadi M, Bahamin S, Fathi A. 2017. The effect of sowing date and nitrogen on yield, and essential oil of German chamomile. *Scientia*, 19(3): 85-92
- Geoffrey M and Gadd GM. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma*, 122: 109-119
- Grill E, Winnacker EU, Zenk MH. 1985. Phytochelatin: the principal heavy-metal complexing peptides of higher plants. *Science*, 230: 674-676
- He LY, Chen ZJ, Ren GD, Zhang YF, Qian M, Sheng XF, 2009. Increased cadmium and lead uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. *Ecotoxicology and Environmental Safety*, 72: 1343-1348

- Jafari M, Moameri M, Jahantab A, Zargham N. 2017. The effect of municipal waste compost and biochar on the ability of the plant to purify the species *Boiss tomentellus* in greenhouse conditions. *Rangeland Scientific Research Journal*, 11(2): 194-206
- Joner EJ, Leyval C. 2001. Time course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. *Biology and Fertility of Soils*, 33: 351-357
- Kardoni F, Bahamin S, Khalil Tahmasebi B, Ghavim-Sadati SH, Vahdani SE. 2019. Yield comparisons of mung-bean as affected by its different nutritions (chemical, biological and integration) under tillage systems. *Journal of Crop Ecophysiology*, 13(49(1)): 87-102
- Khan AG. 2004. Mycotrophy and its significance in wetland ecology and wetland management. In: *Developments in Ecosystems Vol. 1* (Wong MH, ed). 97-114, Elsevier, Northampton, UK
- Khan AG. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology*, 18: 355-360
- Khattak MI, Jabeen R. 2012. Detection of heavy metals in leaves of *Melia azedarach* and *Eucalyptus citriodora* as biomonitoring tools in the region of Quetta valley. *Pakistan Journal of Botany*, 44(2): 675-681
- Khoshkhabar H, Jafari M, Feilinezhad A, Bahamin S. 2015. Effect of sodium silicate on the yield and yield components of pea under salinity stress. *Biological Forum – An International Journal*, 7(1): 1045-1049
- Li WC, Ye ZH, Wong MH. 2007. Effects of bacteria on enhanced metal uptake of the Cd/Zn hyperaccumulating plant, *Sedum alfredii*. *Journal of Experimental Botany*, 58: 4173-4182
- Lippmann B, Leinhos V, Bergmann H. 1995. Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. 1. Changes in root morphology and nutrient accumulation in maize (*Zea mays* L.) caused by inoculation with indole-3-acetic acid (IAA) producing *Pseudomonas* and *Acinetobacter* strains or IAA applied exogenously. *Angewandte Botany*, 69: 31-36
- Maleki A, Fathi A, Bahamin S. 2020. The effect of gibberellin hormone on yield, growth indices, and biochemical traits of corn (*Zea Mays* L.) under drought stress. *Journal of Iranian Plant Ecophysiological Research*, 15(59): 1-16
- Mashayekhi HR, Baghaie1 AH, Gomarian M. 2017. Effect of EDTA chelate and cow manure on Cd uptake by pot marigold in a polluted soil. *Water and Soil Research*, 31(3): 405-417
- Mayak S, Tirosh S, Glick BR. 2004. Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Physiology*, 166: 525-530
- Mc Gonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytology*, 115: 495-501
- Norris JR, Read DJ, Varma AK. 1992. *Methods in Microbiology Vol. 24. Techniques for the Study of Mycorrhiza*. Academic Press, London, UK
- Penrose DM, Glick BR. 2001. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth promoting bacteria. *Canadian Journal of Microbiology*, 47: 368-372
- Rezaei A, Lotfi B, Jafari M, Bahamin S. 2015. Survey of effects of PGPR and salinity on the characteristics of *Nigella* leaves. *Biological Forum–An International Journal*, 7(1): 1045-1049
- Saghir Khan M, Zaidi A, Ahmad Wani P, Oves A. 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, 7: 1-19
- Salt A. 1997. Effect of EDTA on metal accumulation by aquacultured seedling of Indian mustard.

- Environmental Science and Technology, 316(1997): 1936-1644
- Shamsibeiranvand Z, Sadeghi Z, Khoshkhabar H, Hosseinabadi M, Bahamin S. 2017. Survey some physiological characteristics of medicinal plant *Scrophularia striata* Boiss in Ilam province. *Scientia Agriculturae*, 19(3): 62-68
- Varvara P, Grichko, Brendan F, Bernard, Glick R. 2000. Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. *Journal of Biotechnology*, 81: 45-53
- Vasil AD. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiology*, 447-453
- Wani PA, Khan MS, Zaidi A. 2008. Chromium reducing and plant growth promoting Mesorhizobium improves chickpea growth in chromium amended soil. *Biotechnology Letters*, 30: 159-163
- Wenzel WW, Adriano DC, Salt D, Smith R. 1999. Phytoremediation: a plant-microbe-based remediation system. In: *Bioremediation of Contaminated Soils* (Adriano DC, et al., eds). *Agronomy Monographs* 37. 457-508, ASA, CSSA and SSSA, Madison, USA
- Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, 368: 456-464
- Zabet M, Bahamin S, Ghoreishi S, Sadeghi H, Moosavi S. 2015. Effect of deficit irrigation and nitrogen fertilizer on quantitative yield of aboveground part of forage pear millet (*Pennisetum glaucum*) in Birjand. *Environmental Stresses in Crop Sciences*, 7(2): 187-194