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Studying capability of soil cadmium phytoremediation by sorghum in the presence of mycorrhiza fungi

Mehdi Moradi[1], Mojtaba Yousefi Rad[2*], Adeleh Darbani[3]

[1]*Department of Agronomy, Saveh Branch, Islamic Azad University, Saveh, Iran*

[3]*Department of Agronomy, Shahre Ray Branch, Islamic Azad University, Tehran, Iran*

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Abstract

This study was designed to investigate the capability of Sorghum plant to purify the cadmium-contaminated soil in the presence of mycorrhiza fungi. This study was performed in Farm of Agriculture Research of Saveh Islamic Azad University as a factorial experiment based on randomized complete blocks design with three replications. Seeds were cultivated in certain pots. In this study, two factors were considered including: the concentrations of cadmium nitrate at four levels (0, 100, 200 and 300 ppm) which was considered as the first factor, the contamination as well as the lack of contamination with mycorrhiza fungi (*Glomus Intraradices*) which was considered as the second factor. The results showed that the presence of mycorrhiza fungi has increased the concentration of cadmium in all plant organs. It was also observed that increasing the concentration of cadmium in the soil has increased the cadmium concentrations in various organs of the sorghum plant. The mycorrhiza inoculation at all levels of the cadmium intake has significantly increased the cadmium concentration in different organs of the sorghum plant. According to this study, the presence of mycorrhiza fungi has increased the absorption of cadmium toxic elements. It also resulted in purification of the soil by sorghum.

* **Corresponding Author:** Mojtaba Yousefi Rad ✉ m.yousefirad@iau-saveh.ac.ir

Introduction

Heavy metal contaminants are a serious problem worldwide to human health, animals and some plants(Revathi and Subhashree Venugopal, 2013). Heavy metal contamination is an increasing problem associated with agricultural areas because the soil is treated with contaminated wastes in these areas. Moreover, the phosphate fertilizers which contain cadmium (Cd) are indiscriminately overused in these areas. Therefore, heavy metal contamination should be managed as soon as possible in these areas. These kinds of metals are biologically durable and remain in the soil for a long time. This issue results in accumulation of these metals in the food chain which has potential adverse effects on human health. Heavy metals accessibility levels depends on following factors: the plant type, the amount required for the plant as a micronutrient, the capability of the plant to effectively regulate the metabolism through secretion of organic acids or protons to the root area (Boumman *et al.*, 2001). Cadmium is a bluish-white soft metallic element. It is obtained as a byproduct of in the process of zinc refining. Most properties attributed to the cadmium are similar to the ones attributed to the zinc. Cadmium and its compounds are highly toxic. Annually 25,000 tons of cadmium enters the environment naturally. About half of this amount enters the rivers through weathering of the rocks. The main sources of cadmium release include: the forest fires and volcanoes, the human activities such as industrial waste leachate, the production of synthetic phosphate fertilizers (Boominathan and Doran, 2003). This element primarily enters the human body through various food sources including: liver, mushrooms, river oysters, etc. which all contain high concentration of the cadmium element. This element eventually accumulates in the kidneys of the human body. The presence of this element in the body causes several undesirable effects including: diarrhea, severe abdominal pain and vomiting, bone fractures, infertility, damage to the central nervous system, damage to the immune system, psychological disorders, probable damage to DNA and cancer disease (Boumman *et al.*, 2001, Casio *et al.*, 2004). Cadmium reduces the rate photosynthesis as well as transpiration. It also increases the rate of respiration. Moreover, it causes so many risks for the plants including: the branches' axes are shortened; the yellowing process of the old leaves is intensified. Cadmium is not only absorbed through the roots but also branches and the leaves. There is also the risk of prolonged duration of the fall period for deciduous plants which is caused by cadmium contamination (Davies *et al.*, 2001). Cadmium shows various toxic effects in the mM concentrations including: it prevents the synthesis of protein, DNA and RNA, it inhibits the enzymatic activity (Danielson,1985). The maximum allowable amount of cadmium in the drinking water is 2.5 liter for a man whose weight is 70 kg based on the average daily consumption of drinking water (0.005 mg/lit)(Danielson,1985, Vierheiling *et al.*,2003).

Sorghum (*Sorghum bicolor*) is the most important crop in the semi-arid tropical areas because it produces large amounts of crops under extreme drought or heat conditions (Dogget, 1985, Fischer, 1975). The capability of sorghum to produce crops in the areas which are not suitable for other grains, except millet, may be considerably important in the future. This is probably because the environmental conditions will be highly unsuitable for production of various crops in the future. Therefore, sorghum, as a plant which can produce large amounts of crops in unsuitable conditions, may be considered as a target crop in the future. Sorghum is one of the most common forage crops of Poaceae family (Zand, 1997, Alipur, 1990). The sorghums that were cultivated from the past to the present, especially in different parts of Africa and India, are derived from the original sorghums (Eusorghum) (Alipur, 1990). Four main features can be attributed to the sorghum including: four-carbon photosynthetic pathway, tolerance to the heat, the capability to produce large amount of crops at drought conditions and high biomass. Sorghum as a crop with low water needs has a promising future (Zand, 1997).

Phytoremediation refers to a group of technologies in which the plants are used for the purpose of reduction, extraction, analysis, or immobilization of environmental toxins. Therefore, the contaminated areas are revitalized for general and specific applications in this method. The application of phytoremediation method has focused on accelerating the decomposition of organic pollutants. It has also emphasized on the extraction of hazardous heavy metals from either the soil or the water. This process is associated with the microorganisms living in the root area of the plant (Auge *et al.*, 1986, Halim *et al.*, 2003). Phytoremediation to clean up metal- and metalloid-contaminated soil or sediments has gained increasing attention as environmental friendly and cost effective (kotraba *et al.*, 2009). This method was at first used for wet areas as well as waterlogging plant system in order to treat contaminated water remains. However, nowadays phytoremediation is used to clean up both air and the soil. It was also used for the conservation of biodiversity in both soil and the plants. Identification and application of symbiotic organisms is important in phytoremediation process of the contaminated soils. Mycorrhiza fungi transfer some certain substances from soil to both roots and internal organs of the plants by increasing the number of Hyphae (Davies *et al.*, 1992). Mycorrhiza fungi reduce the toxicity of heavy metals in the host plants. The role of mycorrhiza fungi in reducing the stress caused by toxic metals in the host plants depends on the following factors: plant species, plant ecotypes, fungi type, fungal ecotype, type of the metal and its availability, specific conditions of the soil such as the fertilizer content and plant growth conditions such as light intensity and root density (Yang *et al.*, 2004). This study aimed to assess the capability of sorghum to purify the cadmium-contaminated soil in the presence of mycorrhiza fungi. This study also aimed to identify whether the lead (Pb) is present in the sorghum or not and how this element is transferred to different organs of this plant (roots and shoots).

Materials and methods

Location and experimental design

This experiment was performed in a greenhouse at Research Farm of Saveh Islamic Azad University. This experiment was conducted as factorial model in a randomized complete block design with three replications.

Experimental treatments

Two factors were studied in this experiment. The first factor was cadmium nitrate concentrations at four levels (0, 100, 200 and 300 ppm) and the second factor the contamination and lack of contamination with mycorrhiza fungi (*Glomus intraradices*). In early stages, lead nitrate was added to each pot in certain amounts: 0.8 gr, 1.6 gr, 2.4 gr to the amount of 8 kg soil (In each pot). In other words, certain amount of lead nitrate was dissolved in water. Then, this solution was added to the soil, so that the level of soil in the pot would be the same as field capacity, i.e. 8 kg. Then, this prepared soil was placed in nylon for three weeks, so that the metal would be stabilized in the soil. Then, the stabilized soil was transferred to the pots whose capacity was 8 kg. In order to contaminate the pots' soil with mycorrhiza fungi, following steps were done: 1. the surface soil was removed from the pot; 2. Fifty grams of the inoculum was added to each pot; 3. the pots were filled with soil. Then the sorghum seeds, which were pre-germinated, were treated with the inoculum. Then, the pots were planted in a proper depth from the ground.

Traits

After the growing procedure, the harvesting procedure was done 90 days after the planting. Then, various sorghum organs, including roots and the shoots, were separately maintained in the oven for 48 hours at 72 ° C. when the organs were dried, samples were transported to the laboratory for dry digestion procedure. At this stage, the samples were crushed with the Crushing Machine and 1 gr of the crushed material were weighted and was placed in the furnace for 8 h at 600 ° C. Then, the digestion procedure was done with 2 M hydrochloric acid at 80 ° C. After filtering the extract with Whatman 40 filter paper, the heavy metal concentrations were measured by the atomic absorption machine.

Statistical analyses

Analysis of variance was performed by SAS software. The charts were drawn by Excel software. Comparison of the means was done by Duncan test (P<0.05).

Results and Dscussion

Cadmium concentration of different organs

According to the result of variance analysis in Table 1, the effect of cadmium concentration in the soil on cadmium concentration of the root was statistically significant (P<0.01). Based on comparison of the means in Table 2, the highest cadmium concentration was seen in Cd4 treatment while the lowest one was seen in Cd1 treatment. In other words, increasing the concentration of cadmium in the soil may increase the cadmium absorption and cadmium concentration in the root part. According to analysis of variance in Table 1, the effect of mycorrhiza inoculation on cadmium concentration in the root is significant (P<0.05). Based on Table 2, the presence of mycorrhiza increased cadmium concentrations in the roots of inoculated plants; however it did not increase cadmium concentrations in the roots of non-inoculated plants. According to the results of analysis of variance in Table 1, the interactive effect of mycorrhiza fungi and soil cadmium concentration on the root cadmium concentration is statistically significant (P<0.01). Comparison of treatment means in Table 3 showed that the highest cadmium concentration is seen in Cd*fl treatment while the lowest one is seen in Cd1*fl and Cd1*f2 treatments. It was reported that the concentration of heavy elements in the root and shoots parts of the inoculated plants was not higher than the one attributed to non-inoculated plants in the soil contaminated with heavy metals. The results of this study are in line with the results of other researchers who conducted the same study as this study on the wheat (Boumman and Doran, 2003).

Table 1. The results of variance analysis of studied factors.

The source of variation	df	Root cadmium concentration (ppm)	Shoot cadmium concentration (ppm)	Root dry weight (gr)	Shoot dry weight (gr)
Block	2	0.645	0.004 n.s	12.84**	2.89**
Fungi	1	0.173**	0.006 n.s	871*	1.64*
Cd	3	1.36*	0.298**	0.851*	3.67**
F*cd	3	0.023**	0.009*	0.179*	0.092 n.s
Error	62	0.091	0.010	0.347	183

n.s: not significant, *: significant at 5% level of significance, **: significant at 1% level of significance.

According to the results of analysis of variance in Table 1, the effect of soil cadmium concentration on cadmium concentration in the shoot organs is statistically significant (P<0.01). Based on Table of comparison of the means 2, the highest cadmium concentration of the shoot organs is seen in Cd4 treatment while the lowest one is seen in Cd1 treatment. According to Table of variance analysis1, it can be seen that the effect of mycorrhiza inoculation on cadmium concentration in the shoot organs is not statistically significant. Based on means comparison Table 2, it is seen that the mycorrhiza inoculation has not a significant effect on cadmium concentration in the shoot organs. The variance analysis Table 1 shows that the interactive effect of mycorrhiza fungi and cadmium concentration of soil on cadmium concentration in the shoot is statistically significant (P<0.05). Comparison of the treatments in Table (3) showed that the highest concentration of cadmium in the shoot organs is seen in Cd4*f2 treatment while the lowest one is seen in Cd1*f2 and Cd1*f1 treatments. The results of this study are in line with the results of researches including Yang *et al.*, (2002 and 2004) and Chaney *et al.*, (2005).

Dry weight of root and shoot

According to the result of variance analysis (Table 1), the effect of cadmium concentration in the soil on dry weight of the roots is statistically significant (P<0.05). Comparison of the means in Table 2 showed that the lowest root dry weight in seen in Cd4 treatment while the highest one is seen in Cd1 and Cd2 treatments. According to analysis of variance in Table 1, it is seen that the effect of inoculation with mycorrhiza fungi on the root dry weight is statistically significant (P<0.05). Based on comparison of the means in Table 2, the highest dry weight is seen in the roots of inoculated plants while the lowest one is seen in the roots of non-inoculated plants.

According to result of variance analysis in Table 1, it is seen that the interactive effect of mycorrhiza fungi and soil cadmium concentration on the root dry weight is statistically significant (P<0.05). Based on comparison of the means in Table 3, the highest root dry weight is seen in Cd2*f1 and Cd1*f1 treatments while the lowest one is seen in Cd4*f2 treatment. Although by increasing absorption of various substances from the soil, the root dry weight increases. This is caused by the interaction between the plants and the fungi. However, sometimes the presence of heavy metals such as lead inverse this relation. This is because heavy metals are toxic. Then, the plant prevents root development to nullify the toxic effects of heavy metals. The absorption of toxins is reduced since the plants' roots are not grown. Our results prove this issue. There are also some other reports on this issue (Fuentes *et al.*, 2004, Cunningham and Ow 1996). Heavy metal contamination increased the toxicity, as a result, the growth of sorghum roots were prevented. This phenomenon was seen in our study.

According to the results of analysis of variance in Table 1, it is shown that the effect of cadmium concentration in the soil on the shoot dry weight is statistically significant (P<0.01). Comparison of the means in Table 2 showed that the highest shoot dry weight is seen in Cd1 treatment while the lowest one is seen in Cd4 treatment. Based on the results of Analysis of variance in Table 1, it is seen that the effect of mycorrhiza inoculation on the shoot dry weight is statistically significant at (P<0.05). Comparison of the means in Table 2 showed that inoculated plants have higher dry weight compared to non-inoculated plants.

Table 2. Comparison of the means of cadmium concentration and dry weight of sorghum's organs under studied factors.

Factor level	Root dry weight (gr)	Shoot dry weight (gr)	Root cadmium concentration (ppm)	Shoot cadmium concentration (ppm)
F1	1.01 a	0.89 a	0.35 a	0.15 a
F2	0.73 b	0.68 b	0.27 b	0.16 a
Cd1	1.05 a	1.34 a	0.00 c	0.00 c
Cd2	0.94 a	0.67 b	0.27 b	0.15 b
Cd3	0.87 ab	0.7 b	0.42 b	0.21 ab
Cd4	0.61 b	0.43 c	0.56 a	0.26 a

F1: using mycorrhiza fungi, F2: lack of using mycorrhiza fungi
Cd1: the first level of cadmium concentration-0ppm, Cd2: the second level of cadmium concentration-100ppm, Cd3: the third level of cadmium concentration-200 ppm, Cd4: the fourth level of cadmium concentration-300 ppm.

According to the results of analysis of variance in table 1, it is shown that the interactive effect of mycorrhiza fungi and the soil cadmium concentration on the shoot dry weight is statistically significant (P<0.05). According to Table 3, the highest dry weight in the shoot parts is seen in Cd1*f1 treatment while the lowest one is seen in Cd4*f2 treatment. Several studies showed that the plants' dry weight show positive reaction to the mycorrhiza inoculation. A significant increase is seen in the dry weight of inoculated plants compared to non-inoculated plants. Improved plant growth in the presence of mycorrhiza was not confirmed in lots of researches. The results of examining the interactive effect of mycorrhiza fungi and heavy metals concentrations (copper and cadmium) on the corn dry weight showed that the dry weight of non-inoculated roots is lower than the one attributed to inoculated roots (Halim *et al.*, 2003, Vierheiling *et al.*, 2003).

Table 3. Comparison of the means of the effect of Cd and fungi on cadmium concentration and the dry weight of sorghum's organs.

Factor level	Root dry weight (gr)	Shoot dry weight (gr)	Root cadmium concentration (ppm)	Shoot cadmium concentration (ppm)
Cd1*f1	0.98 abc	1.15 b	0.005 d	0.00 c
Cd1*f2	1.12 a	1.53 a	0.006 d	0.00 c
Cd2*f1	0.62 bc	0.6 cd	0.23 c	0.15 b
Cd2*f2	1.13 a	0.74 c	0.31 bc	0.16 b
Cd3*f1	0.77 abc	0.58 cd	0.35 bc	0.2 b
Cd3*f2	1.1 ab	0.82 bc	0.48 ab	0.21 b
Cd4*f1	0.53 c	0.38 d	0.49 ab	0.29 a
Cd4*f2	0.68 abc	0.48 cd	0.63 a	0.22 ab

F1: using mycorrhiza fungi, F2: lack of using mycorrhiza fungi
Cd1: the first level of cadmium concentration-0ppm, Cd2: the second level of cadmium concentration-100ppm, Cd3: the third level of cadmium concentration-200 ppm, Cd4: the fourth level of cadmium concentration-300 ppm.

The results of this study showed that the concentration of cadmium heavy metals in sorghum's organs have a direct relationship with the soil cadmium concentrations. By increasing the cadmium levels in the soil, its concentration will increase in roots and shoots parts of the sorghum. On the other hand, cadmium concentrations in the roots are higher than its concentration in the shoots. Therefore, it can be concluded that cadmium is less transferred to the shoot parts. Research findings indicated that sorghum has a good symbiotic relationship with the mycorrhiza fungi. The mycorrhiza fungi have increased the absorption and transportation rates of different substances from soil to the roots and shoots in sorghum. This is because an extensive network of hyphae is creating the plant; moreover, these hyphae easily penetrate the soil and absorb the required nutrients. As the results revealed, inoculated fungi have higher cadmium concentration compared to non-inoculated plants in their various organs. The research results showed that the mycorrhiza fungi have increased the capability of sorghum to purify and refine the cadmium-contaminated soil.

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