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Assessment Effect of Organic Matter and Arsenic on Transfer Coefficient, Tolerance Index and Phytoremediation in Cress (*Lepidium sativum* L.)

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ABSTRACT

Soil contamination with heavy metals such as arsenic has harmful effects on human health and agricultural products. Arsenic (AS) is one of the heavy metals which are highly toxic and carcinogenic. This research was conducted to study the effect of organic manure on increasing the Arsenic absorption ability by Cress plant in the greenhouse of the Agriculture and Natural Resources Research Center, Khuzestan, Iran, according factorial experiment based randomized completely design with three replications. Main factor included two levels of organic substance (0 and 10 tons per hectar) and three levels of arsenic concentration (0, 20 and 40 ppm) belonged to sub factor. Mean comparison result revealed increasing arsenic concentration led to increase arsenic accumulation in shoot plant (14.1 ppm), also this trend seems in transfer coefficient trait so cress can be used as a purifier plant to reduce arsenic contamination of the soil. It need to mention by increase arsenic concentration, shoot dry weight decreased (0 ppm arsenic concentration had 20.1 gr per flowerpot but 40 ppm treatments had 0.7 gr). Finally according to contamination symptoms (Necrosis and chlorosis) on cress plant at 40 ppm concentrations and reduced plant shoot dry weight at this concentration, it is recommended to use the cress plant for purification of soil contaminated less than 40 ppm arsenic concentrations. It is noteworthy advised according plant's ability to absorb arsenic and other heavy elements, sowing of this plant as food should be avoided in the contaminated fields.

Keywords*:* *Contamination, Heavy Metal, Purifier plant.*

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INTRODUCTION

Any change in the characteristics of soil constituents that limits the soil use is called soil contamination. Heavy metals due to non-biodegradability and harmful biological effects on the organisms at low concentrations have a significant importance in environmental contamination area (Alloway, 1990). These metals find their ways from various sources to the environment, plant body and eventually to the food chain of humans and animals and cause serious damages. For example, in humans, anemia, hypertension, mental retardation and a variety of cancers are of consequences due to consumption water or foods contaminated with heavy metals. Arsenic is one of the most toxic elements that are used in various industries such as glassworks, metallurgy, electronics, and ball making, steel, paint industries and enter into the components of environment such as soil and water through many ways. There are some methods to reduce soil and water contamination that are so expensive. Washing soils contaminated with heavy metals by acid, treatment of industrial wastewaters in refineries and excavation and soil burial in a safe place can be mentioned as examples. Another technology that is a very low-cost and simple method comparing with other refining approaches includes using plants to remove soil contamination, which is called green refinement. Since the modifying material such as organic modifiers can increase the plant ability to absorb the contaminants, a variety of animal manures can be used for this purpose (Gholami *et al*., 2012). Arsenic (As) is found in sedimentary rocks and in groundwater in many countries. This element is also found in soils, mainly due to irrigation, mining, industrial, and other anthropogenic inputs. Arsenic concentrations range from 0.01 $mg.L^{-1}$ to 2100 $mg.L^{-1}$ in water and from 0.1 to 90 $mg.kg^{-1}$ in soils (Mandal and Zuzuki, 2002; Smedley and Kinniburgh, 2002). Soils with high concentration of As negatively affect crop production and food safety, a phenomenon that has been documented in several countries (Brammer and Ravenscroft, 2008; Dahal *et al*., 2008). Plants exposed to high As concentrations show reduced germination, decreased chlorophyll content and photosynthesis rate, reduced height, tillering and/or ramification, and decreased root and aerial biomass growth and yield; As also negatively affects the *Bradyrhizobium*-legume symbiosis, and may even cause death (Talano *et al*., 2013). Arsenate is toxic to plants because it acts like phosphate and is transported through the plasmatic membrane by the phosphate carriers (Smith *et al*., 2010). The As concentration in plants followed the order roots > leaves > shoots > pods > grains (Lee and Yu, 2012). Dong *et al*. (2008) studied the effect of Arbuscular -Mycorrhizal fungus *Glomus mosseue* in a culture including *Trifolium repens* L. and *Lolium perenne* L. on the tolerance of these plants in arsenic contamination conditions. The plants inoculated with Mycorrhiza caused an increase in phosphorous absorption versus decrease in arsenic transport from roots to shoots, which suggested an increase in plants resistance to arsenic. Moreno-Jimenez *et al*. (2008) by studied *Myrtus communis*, *Arbutus unedo* and *Retama sphaerocarpa* against the presence of arsenic contamination reported species of *Arbutus unedo* show the greatest sensitivity against arsenate. In general, the arsenic accumulated mostly in the roots of these plants. Although *Myrtus communis* has had the highest absorption compared to two other species, but *Retama sphaerocarpa* showed the highest amount of arsenic transportation from the plant root to its shoots. Finally, *Myrtus communis* and *Retama sphaerocarpa* were determined as appropriate species for re-developing vegetation cover in soils contaminated with average concentrations of arsenic. Ladan (2011) examined the potential of green refining of arsenic contaminated soils by two plants of green onion and ornamental cabbage. The results showed that, given the low absorption of arsenic by green onion, this plant cannot be a good option for removing arsenic contamination from the contaminated soils. The results showed that by increasing the arsenic concentration in the soil, its absorption by ornamental cabbage plant increased and this trend continued up to the concentration of 200 $mg.kg^{-1}$; in concentrations higher than 200 $mg.kg^{-1}$, due to the occurrence of arsenic toxicity (even with apparent symptoms as necrosis), the absorption of this element reduced. The objective of the present study was to investigate effect of organic manure on increasing the arsenic absorption ability by Cress plant in the greenhouse laboratory situation.

MATERIALS AND METHODS

Field and Treatments Information

This study was carried out to evaluate the effect of organic material to increasing the absorption ability of arsenic element by Cress plant. Thus, the research was performed in the flowerpot format in greenhouse of agricultural and natural resources research center, Khuzestan province (At southwest of Iran), according factorial experiment based randomized completely design with three replications at 2011. Main factor included two levels of organic matter (OM_1: 0 and OM_2: 10 $t.ha^{-1}$) and three levels of arsenic concentration (AC_1:0, AC_2: 20 and AC_3: 40 ppm) belonged to sub factor. The soil texture used in this study was clay loam and was prepared from the field of agricultural research center of Safiabaf, Dezful (Fig.1).

Traits Measure

The studied soil was air dried for 24 hours and then was sieved through a 2 mm sieve; then it was contaminated with the desired concentrations. To contaminate the soil, a certain amount of sodium hydrogen arsenate, 7 hydrated ($Na_2HAsO.7H_2O$) was dissolved in water based on experiment treatments and was sprayed as a solution on the soil surface and fully mixed with the soil. Then, the organic matter (rotten and powdered sheep manure) up to the amount required was weighted based on 10 tons per hectare and was added to the pots having treated with organic material. Then, considering the specific gravity of soil (1.35 $g.cm^{-3}$) and based on the size of the pots (Average diameter of 18 cm and height equal to 12 cm), the amount of soil required was calculated and potting was performed. Finally, the pots were left for 10 days for pollutants and soil interactions and creating conditions close to nature in the greenhouse environment. Then, the seeds of cress (broad-leaved cress) with 90% viability were planted in pots and were watered lightly to prevent drought stress. At the end of growth period, the plant was completely removed from the pot; then it was well washed with distilled water and dried; after drying in oven at 75 degrees Celsius temperature for 24 hours, the plant was ground and then was extracted with hydrochloric acid, and finally arsenic concentration in cress was read by atomic absorption device. The soil samples were also extracted with nitric acid and hydrochloric acid and arsenic concentration in soil were read by atomic absorption device (Hudson-Edwards *et al.*, 2004).

Fig.1. Cultivation Cress in the laboratory

Soil texture

Fifty grams of dry and sieved soil and was placed in a container and 50 ml of Calgon solution and 300 ml of distilled water were added to it and were mixed with electric mixer for 5 minutes. The resulting mixture was transferred to a one-liter graduated cylinder and was brought to 1 liter of distilled water. Then it was mixed by manual mixer and after 40 seconds and in different times (5 hours) the particles were read by hydrometer. Then, by doing the necessary corrections the percentage of sand, silt, and clay was calculated (Gee and Bauder, 1986). Soil properties of studied soil was mentioned in table 1.

Table 1. Properties of studied soil in lab

AS (ppm)	K (ppm)	P (ppm)	O.C (%)	Soil lime (%)	EC ($ds.m^{-1}$)	pH	Soil texture
1.75	270	16.3	0.77	47.2	4.2	7.4	Clay loam

Transfer Coefficient (TC)

The index is the ratio of the concentration of the metallic element in shoot to the total concentration of the same element in soil or growing medium (Sun *et al*., 2008) that is known as one of the indicators in assessing the efficiency of plant purification. Due to the potential toxicity of arsenic to humans, livestock and poultry, it is very crucial to purge the environment of this element. In order to purge the polluted sites usually excavation, leaching and mechanical separation are used and or to assess the plant's ability to transfer metal from root to shoot translocation factor is calculated according to the following formula (Ross, 1994):

Equ.1. T.C= Arsenic concentration in stem (mg/kg)/ Arsenic concentration in root (mg/kg)

Tolerance index (TI)

This index is the plant dry weight in contaminated soil (Arsenic) to the Plant dry weight in non-contaminated soil. Index of less than one indicates a significant reduction in plant dry weight and happen stress, equal one means make no difference in dry weight and index more than one indicated increasing of plant dry weight and reduction of pollution. Mention index is calculated according to the following formula (Peer *et al*., 2005):

Equ.2. T.I= Plant dry weight in contaminated soil/ Plant dry weight in non-contaminated soil

Absorb index (UI)

This index indicated shoot dry weight multiple to arsenic concentration in shoot dry weight. Mention index used for compare phytoremediation ability of different plants (Singh *et al*., 2007; Marchiol *et al*., 2004).

Statistical Analysis

The data obtained from lab reading were ultimately analyzed by MSTAT-C software, and means comparison were done by Duncan multiple range test at 5% probability levels, also diagrams were drawn with Excel software.

RESULTS

Arsenic concentrations in shoot

According result of analysis of variance effect of different level of organic matter and arsenic concentration on arsenic concentrations in shoot of plant was significant at 1% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of organic matter indicated that minimum plant height (6.43 ppm) was noted for non-organic matter application and maximum of that (9.29 ppm) belonged to 10 $t.ha^{-1}$ organic matter treatments (Fig.1). Maximum arsenic concentrations in shoot of plant (14.1 ppm) was obtained for 40 ppm arsenic concentrations and minimum of that (0.2 ppm) was for non arsenic concentrations application (Fig.2). By increasing organic matter and arsenic application accumulation of arsenic concentration in the plant shoot significantly increased, so that matter indicating the cress plant has been able to absorb more arsenic and can be considered as a purifier plant for the mentioned arsenic levels. Mention result confirmed by Spagnoletti and Lavado (2015).

Arsenic concentrations in soil

Analysis of variance result revealed effect of different level of organic matter and arsenic concentration on arsenic concentrations in soil was significant at 5% and 1% probability level, respectively but interaction effect of treatments was not significant (Table 2).

Table 2. The result of analysis of variance of studied characteristics

S.O.V	df	Arsenic concentrations in shoot	Arsenic concentrations in soil	Shoot dry weight	Transfer coefficient	Tolerance index	Absorb index
Replication	2	35.87^{*}	122.6^{ns}	0.153^{ns}	0.103*	0.096^{*}	30.9^{*}
Organic matter (OM)	1	30.3^{**}	564.9^{*}	0.271^{ns}	0.001^{ns}	0.009^{ns}	16.1^{ns}
Arsenic Concentrations (AC)	2	284.59^{**}	4561^{**}	1.74^{*}	0.165^{*}	1.093^{*}	60.25^{*}
OM×AC	2	12.87^{ns}	530.3^{ns}	0.259^{ns}	0.066^{ns}	0.092^{ns}	13.22^{ns}
Error	12	10.52	11.61	0.159	0.021	0.039	12.54
CV (%)	-	3.55	2.98	4.66	3.88	2.55	1.87

ns, * and ** : non-significant, significant at the 5% and 1% probability level, respectively.

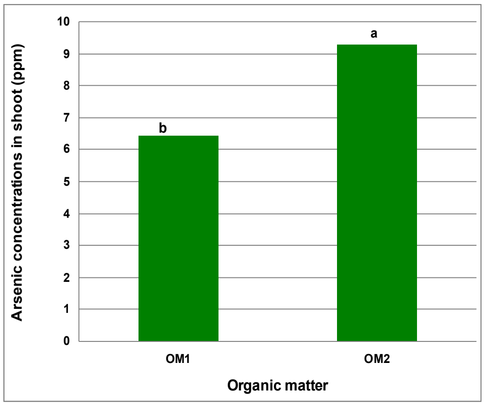

Fig.1. Mean comparison effect of different rate of organic matter on arsenic concentrations in shoot via Duncan test at 5% probability level.

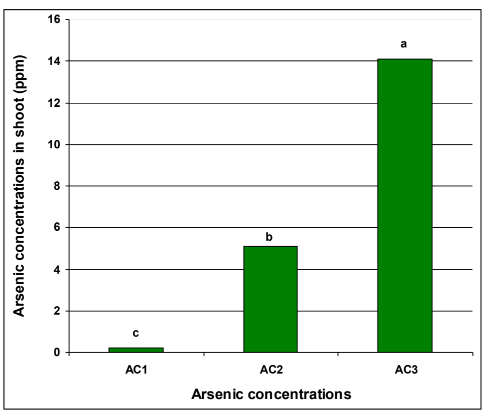

Fig.2. Mean comparison effect of different rate of arsenic on arsenic concentrations in shoot via Duncan test at 5% probability level.

Mean comparison result showed the maximum arsenic concentrations in soil (32.3 ppm) was observed for 10 $t.ha^{-1}$ organic matter treatment and minimum of that (19.1 ppm) was for non-organic matter application (Fig.3). Arsenic concentrations in soil increased by arsenic application, so the maximum arsenic concentrations in soil (26.3 ppm) was obtained for 40 ppm arsenic application and minimum of that (2.1 ppm) was for non arsenic application (Fig.4). Another researchers reported same result (Robinson *et al*., 2003).

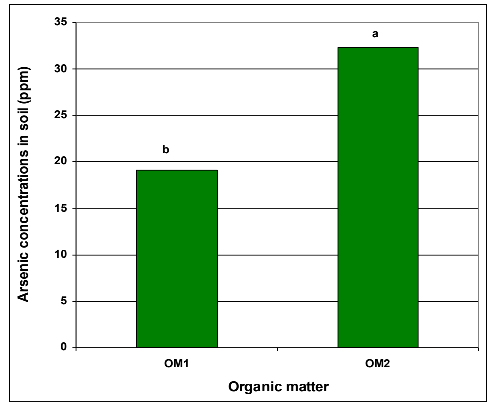

Fig.3. Mean comparison effect of different rate of organic matter on arsenic concentrations in soil via Duncan test at 5% probability level.

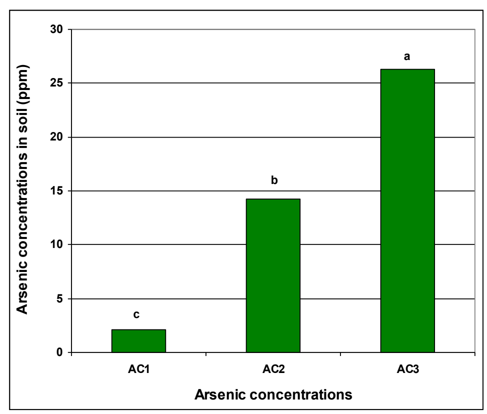

Fig.4. Mean comparison effect of different rate of arsenic on arsenic concentrations in soil via Duncan test at 5% probability level.

Shoot dry weight

According result of analysis of variance effect of different level of arsenic concentration on shoot dry weight was significant at 5% probability level, but effect of different amount of organic matter and interaction effect of treatments was not significant (Table 2). Mean comparison result revealed the maximum and the minimum amount of shoot dry weight belonged to non arsenic application (2.1 gr) and 40 ppm arsenic application (0.7 gr) (Fig.5). Another researchers such as Pickering *et al*. (2000) reported same result.

Transfer Coefficient

Result of analysis of variance revealed effect of different level of arsenic concentration on transfer coefficient was significant at 5% probability level, but effect of different amount of organic matter and interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of arsenic concentration indicated that minimum transfer coefficient (0.1) was noted for non arsenic application and maximum of that (0.45) belonged to 20 ppm arsenic application (Fig.6). Mention result confirmed by Audet and Charest (2007).

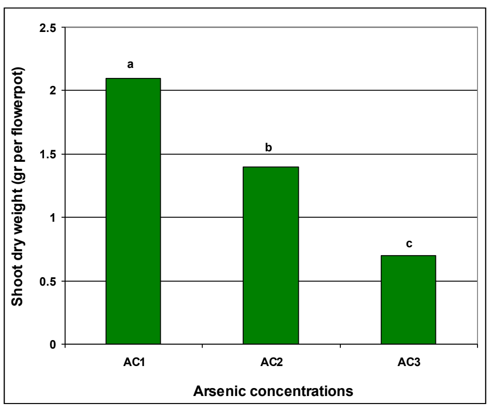

Fig.5. Mean comparison effect of different rate of arsenic on shoot dry weight via Duncan test at 5% probability level.

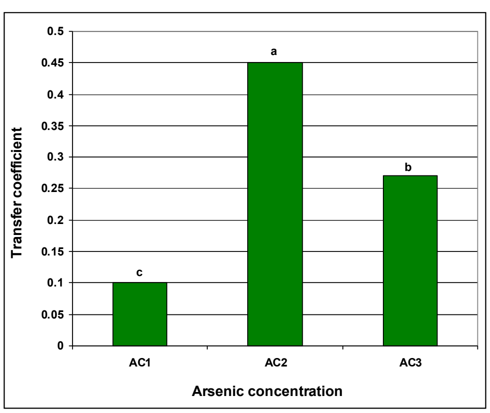

Fig.6. Mean comparison effect of different rate of arsenic on transfer coefficient via Duncan test at 5% probability level.

Tolerance index

According result of analysis of variance effect of different level of arsenic concentration on tolerance index was significant at 5% probability level, but effect of different amount of organic matter and interaction effect of treatments was not significant (Table 2). According result of mean comparison maximum tolerance index (1.09) was observed for non arsenic application and minimum of that (0.23) was for 40 ppm arsenic application (Fig.7). Another researchers reported same result (Meharg and Hartley-Whitaker, 2002; Pickering *et al*., 2000).

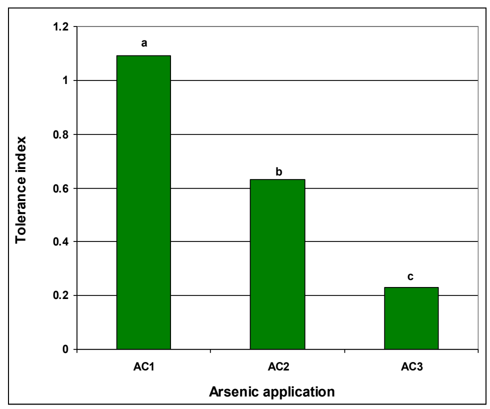

Fig.7. Mean comparison effect of different rate of arsenic on tolerance index via Duncan test at 5% probability level.

Absorb index

Analysis of variance result revealed effect of different level of arsenic concentrations on absorb index was significant at 5% probability level, but effect of different amount of organic matter and interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of arsenic concentrations indicated that minimum absorb index (0.33) was noted for non arsenic application and maximum of that (6.77) belonged to 20 ppm arsenic application (Fig.8).

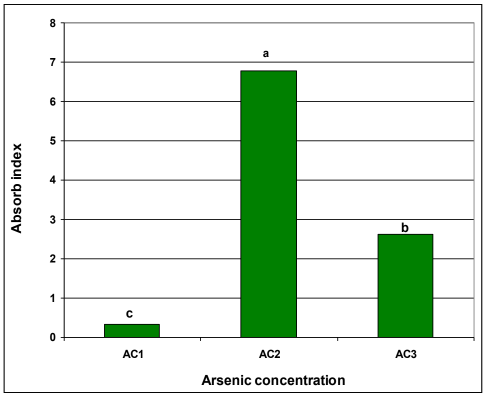

Fig.8. Mean comparison effect of different rate of arsenic on absorb index via Duncan test at 5% probability level.

Another researchers such as Heikens *et al*. (2007) reported same result.

DISCUSSION

By increasing absorption of arsenic in the soil, its absorption by plants increased and this trend continued up to the concentration 40 ppm, even with appearing the signs of contamination (Apparent symptoms as necrosis and chlorosis). Therefore, considering the cress plant's ability to absorb the arsenic element, this plant can be used as a purifier plant to reduce arsenic contamination of the soil. Examining the different level of arsenic showed that with increasing As concentration the plant transfer coefficient has increased but tolerance index is decreased. Since the cress plant is consumed by human and livestock, after planting it in the arsenic-contaminated areas, the plants should be transfer to a secure area and dispose to prevent possible their usage. Given the ability of plant to absorb arsenic and other heavy elements, it is recommended to avoid planting of this plant for growing food vegetables in the lands suspected to be contaminated with such elements. Also, according to contamination symptoms on cress plant (at 40 ppm concentrations) and reduced plant shoot dry weight at this concentration, it is recommended to use the cress plant for purification of soil contaminated with arsenic at concentrations of 0 ppm to less than 40 ppm.

CONCLUSION

It is suggested with considering the ability of the cress plant to absorb arsenic element, this plant use as a purifier plant to purify the contaminated soils with arsenic. It is noteworthy advised according plant's ability to absorb arsenic and other heavy elements, sowing of this plant as food should be avoided in the contaminated fields.

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