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Phytoremediation of soils polluted by heavy metals using *Vetiver grass* and *Tall Fescue*

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Abstract

Phytoremediation is a biological method to improve soils contaminated with heavy metals. The objective of the present research was to study the capability of *Vetiver grass* and *Tall Fescue* in refining and reducing pollution of Cd, Cu and Zn from contaminated soils. The research was implemented in greenhouse during two separate tests (*Vetiver grass* and *Tall Fescue*) in a completely randomized design including seven levels of pollution (0, 50, 100, 200, 400, 600, 800 mg kg^{-1} soil) from three heavy metal types (Cd, Cu and Zn) in three replications. The effects of different levels of heavy metals on the growth characteristics (fresh and dry weights) of *Vetiver* were not significant. Besides, the effect of cadmium levels on shoot dry weight, the effect of Zn levels on shoot fresh and dry weights and the effect of Cu levels on shoot and root fresh and dry weights in *Fescue* were not significant compared to the control treatment. The maximum Cd, Cu and Zn concentrations in *Vetiver* related to 800 mg kg^{-1} treatment were found as 591, 298 and 356 mg kg^{-1}. The maximum content of Cd (96 mg kg^{-1}), Cu (27 mg kg^{-1}) and Zn (37 mg kg^{-1}) in *Fescue* was also measured at soils polluted with 800 mg kg^{-1}. Among different pollution treatments, Cd had the highest uptake and accumulation rate in shoot and root of plants. The results showed the higher capability of *Vetiver* compared to *Fescue* for remediation of environments contaminated with heavy metals especially Cd in Iran.

***Keywords**:* Cadmium; Growth characteristics; Pollution; Remediator plants

1. Introduction

As a part of biosphere, soil plays an important role in food production and environmental sustainability (Ghazban, 2011). Soil heavy metal pollution is among the most important environmental challenges through the world. Heavy metals are not biodegradable and tend to be accumulated in living organisms. Due to high mobility and plant availability, cadmium (Cd) has negative effects on root growth, root water hydraulic conductivity, uptake and translocation of nutrient elements, enzyme activity, and chlorophyll content (Alloway, 1990). Zinc (Zn) and copper (Cu) are among essential micronutrients for plants. However, high Cu and Zn concentrations are reported to have toxic effects on living organisms similar to Cd and Pb (Rout and Das, 2009).

Soil reclamation seems necessary according to human induced soil pollution (Mleczek *et al*., 2009). Several in-situ and ex-situ physical, chemical, and biological methods have been used for soil pollution remediation (Ghosh and Singh, 2005). Non-mobilization and extraction methods by physiochemical techniques are expensive and only useful for small size areas. Besides, soil leaching causes several negative effects on soil properties. That is why, phytoremediation which removes pollutants from soil and environment and conserves biological and physical nature of the soil with reasonable cost, is of great concern (Pulford and Watson, 2003; Banerjee *et al*., 2016).

Phytoremediation is a technology that transfers pollutants from soils and sediments to the plant tissues without soil structure degradation and soil productivity decrease (Lombi *et al*., 2001). Heavy metal uptake by plants is dependent to soil metal concentration and is also affected by plant physiology (Chen

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et al., 2004). There are three basic strategies for plant growth in polluted soils. Some plant species prohibit heavy metals to be transferred to their shoot. Some other species accumulate metals in the shoot and return them to the soil. The third group accumulates metals in both root and shoot (Neisi *et al*., 2014).

As a perennial rapidly growing intense plant with massive root system, *Vetiver* is among the plants that have been used for phytoremediation (Maffei, 2002). *Vetiver* has a very high tolerance for toxic elements, various soil pHs, pesticides, herbicides and climatic fluctuations (Percy and Truong, 2003). Tolerance of *Vetiver* for Cd, Pb, Cu, Zn, As and Hg is reported by several researchers (Truong *et al*., 2010; Vargas *et al*., 2016). *Vetiver* is reported as the best choice for phytoremediation due to uptake of Pb (98 %), Cu (54 %), Zn (41 %), and Cd (88 %) from soils in a greenhouse study (Chen *et al*., 2004). Comparing eight different plants in Thailand, Klomjek and Nitisoravut (2005) concluded that *Vetiver* showed the optimum capability. Besides, *Vetiver* has been used for remediation of soils polluted by phenol (Singh *et al*., 2008), and nuclear wastes and protozoa (Singh *et al*., 2008). Andra *et al*. (2010) investigated high ability of *Vetiver* to uptake and accumulate Pb in root and shoot of plants grown on a Pb polluted environment. High tolerance of *Vetiver* for As concentrations up to 225 mg kg^{-1} were reported by Datta *et al*. (2011). Doing a research on soils contained volcanic ash (0-25, 50 and 100 %), Ghosh *et al*. (2015) reported high capability of *Vetiver* to absorb and transfer As and Cd from soil after 18 months.

Tall Fescue is a perennial foliage grass with a thick and deep rooting system and a good adaptability with an extensive range of soil properties. Hutchinson *et al*. (2001) compared *Couch grass*, *Bermuda grass*, and *Fescue* in reducing aged petroleum sludge pollution as 68, 68 and 62 %, respectively. Reasonable growth of *Fescue* on soils polluted by Polychlorinated biphenyls was reported by Tu *et al*. (2011). *Tall Fescue* and *Kentaki water grass* were compared by Xu and Wang (2013). Results of the study showed that 200-500 mg kg^{-1} Cd stress in soil with 2559 and 4275 mg Cd kg^{-1} plant dry weight were concentrated in shoot by *Fescue* and *Kentaki blue grass*, respectively.

Limited data are available about soil remediation especially using *Vetiver* and *Fescue* in Iran. Besides, both of the plants seem to be tolerant to a wide range of soil properties and climatic conditions. The objectives of the present research were to study the capability of *Vetiver* and *Fescue* for remediation of soils polluted by different concentrations of Cd, Zn and Cu.

2. Materials and Methods

A non-polluted surface soil (0-30 cm) was collected from Shahid Bahonar University of Kerman research farm. Routine Physicochemical soil properties including pH in saturated paste (Thomas, 1996), Electrical conductivity of saturated extract (Rhoades, 1996), organic carbon (Walkley and Black, 1934), soil texture (Gee and Bauder, 1986), equivalent calcium carbonate (Allison *et al*., 1965), field capacity (Hillel, 1982), and available Cd, Zn and Cu using DTPA-TEA (Page *et al*., 1982) with an ASS Vario 6 atomic absorption spectroscopy were determined. The studied soil was calcareous with a sandy loam textural class and low organic carbon (Table 1).

Table 1. Selected properties of studied soil

Cd mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	$CaCO_3$ %	Clay %	Silt %	Sand %	FC %	OC %	pH	EC dS m^{-1}
0.08	0.21	0.41	20	15	21	64	19.72	0.87	7.70	2.64

The study performed as two separate experiments including *Vetiver* grass and *Tall Fescue* in a completely randomized design with three replications. The treatments were 7 concentration levels (0, 50, 100, 200, 400, 600 and 800 mg kg^{-1} soil) of Cu, Zn and Cd which separately added to soils from $CuSO_4$, $ZnSO_4$ and $CdSO_4$ sources, respectively. *Vetiver* seedlings and *Fescue* seeds were prepared from Tak Jem, Iran and Isfahan Pakan seed companies, respectively.

About three kg of air dried and sieved (2mm) soil samples were transferred into the pots. *Vetiver* seedlings were transferred to the pots and incubated in non-polluted soil for 60 days to be adopted before pollutant treatments. Ten *Fescue* seeds were planted in each pot for the second experiment. After the incubation period, Zn, Cu and Cd treatments were added gradually to soils. Essential Nutrient Elements (NPK) were also added according to soil testing results to avoid nutritional stress during the experiment. Based on the evapotranspiration, the pots were irrigated every 3 days up to 70 % of soil field capacity to avoid drainage water. The pots were then kept under greenhouse condition for 80 days.

The root and shoot for each pot were harvested separately after 80 days and their fresh weights were investigated. Root and shoot dry weights were determined after washing and drying (70 °C during 72 hours) using a digital scale with 0.0001 gr precision.

Dry digestion and solution of ash in 2 N HCl was used for Zn, Cu and Cd determination in plant samples (Rothery, 1988). Besides, soil available Cd, Cu and Zn were extracted from polluted soils using DTPA-TEA (Page *et al*., 1982) and heavy metal concentrations in the supernatant were measured by an atomic absorption spectrometer (ASS Vario 6). The data were analyzed using SAS software and mean comparison performed using Duncan test ($P< 0.01$).

3. Results and Discussion

3.1. Growth characteristics

3.1.1. Cadmium

The results showed that *Vetiver* root fresh weight was the only growth parameter that was significantly ($P< 0.01$) affected by Cd levels in soil (Table 2). Soil addition of Cadmium higher than 100 mg kg^{-1} caused significant decrease of *Vetiver* root fresh weight compared to the control (Table 3). On the other hand, no significant relationship was found between Cd levels and shoot fresh and dry weights compared to control treatment as was also supported by Aibibu *et al*. (2010). Potters *et al*. (2007) also reported that heavy metals decreased plant growth due to their effect on plant hormones such as Auxin.

Moreover, the results showed that the maximum root fresh weight was found in control and a significant decreasing trend for root fresh weight with pollutant levels ($p< 0.01$) observed with no significant differences among the treatments (Table 3). The minimum shoot fresh weight (18.40 gr pot^{-1}) was found in 800 mg kg^{-1} Cd treatment for *Fescue* (Table 4) compared to control plant. Xu and Wang (2013) reported that *Blue kentaki* and *Tall Fescue* are capable of accumulating Cd in their shoot without biomass decrease when 5-200 mg kg^{-1} of Cd was applied in soil. *Fescue* shoot fresh weight was only significantly affected by Cd levels compared to other pollutants (Zn, Cu).

3.1.2. Zinc

The maximum root dry weight of 4.33gr pot^{-1} was found in 50 mg kg^{-1} Zn concentration. Although the difference with control plant was not significant, but it seems that Zn concentrations up to 50 mg kg^{-1} could enhance *Vetiver* shoot growth. *Vetiver* root dry weights were not significantly affected by Zn levels, either (Table 3). Ghaemi and Majdeddin (2016) concluded that *Vetiver* is a zinc tolerant plant and increasing Zn concentration in plant doesn't cause any problem compared to other heavy metals. Mean data comparison showed that the maximum *Fescue* dry and fresh weights were determined for 200 and zero mg kg^{-1} Zn levels, respectively ($p< 0.01$).

3.1.3. Copper

Effect of different Cu levels on root fresh weight of *Vetiver* was significant ($p< 0.01$) and it decreased with increasing Cu levels in soil. No significant effect of Cu levels on fresh and dry shoot weights was found for *Vetiver grass* (Table 2). The same results were also reported by Truong (1999) and Roongtanakiat and Chairoj (2001). Meanwhile, root dry and fresh weights of *Fescue* were not significantly affected by different levels of Cu (Table 2). El-Tayeb *et al*. (2006) also concluded that Cu levels decrease both fresh and dry root weights in *Sun Flower*.

Table 2. ANOVA results of treatments affecting some growth characteristics of *Vetiver* and *Fescue*

Sources of change	df	Mean of square			
		Shoot dry weight	Shoot fresh weight	Root dry weight	Root fresh weight
Vetiver					
Cadmium treatment	6	4.68^{ns}	20.35^{ns}	1.01^{ns}	42.06^{**}
Zinc treatment	6	7.46^{ns}	25.90^{ns}	0.98^{ns}	42.43^{**}
Copper treatment	6	5.23^{ns}	56.16^{ns}	1.53^{ns}	30.79^{**}
Fescue					
Cadmium treatment	6	0.66^{ns}	27.84^{**}	7.67^{**}	18.02^{**}
Zinc treatment	6	1.20^{ns}	16.31^{ns}	4.08^{**}	8.08^{**}
Copper treatment	6	1.64^{ns}	4.44^{ns}	5.00^{ns}	36.31^{ns}

^{ns}: non-significant, **: significant (0.01 probability level)

3.2. Heavy metal contents

3.2.1. Cadmium

Results of the study showed that Cd concentration levels significantly affected root and shoot Cd concentrations in both *Vetiver* and *Fescue* plants (Table 5). The maximum *Vetiver* root (215 mg kg^{-1}) and shoot (375 mg kg^{-1}) Cd concentration levels were observed in 800 mg kg^{-1} Cd concentration which proves the capability of *Vetiver* to transfer Cd from root to the shoot (Fig. 1a). The same results were also reported by Wei *et al*. (2008, 2009) for *Taraxacum mongolicum* and *Conyza Candensis* plants, respectively. The higher concentration of Cd in shoot compared to root was attributed to the characteristic of accumulator plants. The maximum threshold level for *Taraxacum mongolicum* (Cd accumulator) and *Solamum nigrum* (Cd hyper accumulator) was reported as 25 mg kg^{-1} (Wei *et al*., 2008). Bidenstripartite as a Cd accumulator plant was only tolerant to 10 mg kg^{-1} Cd (Wei *et al*., 2009). *Vetiver* which is not considered as a hyper accumulator plant was capable of accumulating high levels of Cd in this study.

On the other hand, no significant difference among 50, 100 and 200 Cd treatments was found in *Fescue*. The maximum Cd concentrations in 800 mg kg^{-1} treatment were 63.5 and 32.9 mg kg^{-1} for *Fescue* root and shoot, respectively (Fig. 1b). Cadmium accumulation in root and little transfer to the shoot were also reported for most plant species by Kadukova and Kalogerakis (2007). The difference between root and shoot Cd concentrations was also determined in *Fescue* shows the rapid uptake of the element by root and the limited internal translocation of the element from root toward the shoot (Monterio *et al*., 2009). This could be due to non-mobility and the trap of metal ions by cell wall ligands and out cellulose carbohydrate together with rapid absorption and translocation of heavy metal in *Fescue* root compared to shoot (Monterio *et al*., 2009). Root could be accounted for Cd accumulation and precipitation in this case (Mohammadian *et al*., 2016).

Table 3. Effect of different levels of Cd, Cu and Zn on dry and fresh weights of *Vetiver* root and shoot

Elements	Concentration levels (mg kg^{-1})	Shoot fresh weight (gr pot^{-1})		Shoot dry weight (gr pot^{-1})		Root fresh weight (gr pot^{-1})		Root dry weight (gr pot^{-1})	
	0	a	16.95	ab	5.81	a	13.73	a	2.94
	50	a	9.63	ab	5.38	ab	10.96	a	3.57
Cadmium	100	a	14.30	ab	6.22	abc	10.35	a	2.87
	200	a	11.55	a	3.73	bcd	7.61	a	1.86
	400	a	12.07	a	4.3	bcd	8.21	a	3.02
	600	a	16.09	a	7.48	cd	5.45	a	3.52
	800	a	14.28	ab	4.97	d	3.84	a	2.62
	0	a	16.95	ab	5.81	a	13.73	a	2.94
	50	a	17.81	ab	5.64	b	10.13	a	2.79
Copper	100	a	16.47	a	7.02	bc	7.79	a	4.20
	200	a	15.83	b	4.11	c	7.15	a	2.10
	400	a	14.92	a	7.19	c	6.58	a	3.55
	600	a	15.51	ab	5.22	cd	5.79	a	2.44
	800	a	11.67	b	3.72	d	3.92	a	2.59
	0	a	16.95	a	5.81	ab	13.73	a	2.94
	50	a	25.80	a	10.31	a	17.68	a	4.33
Zinc	100	a	18.26	a	7.27	bc	12.95	a	2.71
	200	a	19.61	a	7.02	c	10.82	a	2.97
	400	a	18.57	a	6.85	c	9.27	a	3.26
	600	a	18.10	a	5.73	bc	8.38	a	3.68
	800	a	18.32	a	8.25	bc	8.01	a	3.77

Different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test.

3.2.2. Zinc

Effect of Zn on root and shoot metal concentration of both *Vetiver* and *Fescue* was significant (Table 5). The maximum Zn accumulations of 166 and 188 mg kg^{-1} for *Vetiver* and 19.6 and 17.2 mg kg^{-1} for *Fescue* in soils treated with 800 mg kg^{-1} Zn were found in root and shoot, respectively (Fig. 2). Root Zn concentrations for both plants were higher for almost all concentration levels compared to the shoot.

3.2.3. Copper

ANOVA showed that Cu accumulation as affected by Cu concentration in *Vetiver* root was significant (Table 5). The highest 47 and 250 mg kg^{-1} Cu in *Vetiver* root and shoot were found in 800 mg kg^{-1} Cu treatment (Fig. 3a). On the other hand, the maximum Cu concentrations for

Fescue were 20.4 and 7 mg kg^{-1} in root and shoot, respectively (Fig. 3b). Metal absorption in low concentration levels of treatments was not significant for all three metals under study due to selective cell membrane permeability of root, but the absorption difference in treatments of more than 400 mg kg^{-1} was considerable. Cell membrane conservation is a mechanism conducted by plants to resist heavy metals. Cell membrane is the first structured part of plant that could be affected by toxic levels of heavy metals. It seems that the selective permeability of the membrane for the element would be decreased due to excess concentration of the metal around root, which in turn increased metal absorption (Hall, 2002). Results of the study showed that the highest concentrations for all three metals were investigated in *Vetiver* shoot. Besides, Cd absorption by both *Vetiver* and *Fescue* was higher than Zn and Cu.

Table 4. Effect of different levels of Cd, Cu and Zn on dry and fresh weights of *Fescue* root and shoot

Elements	Concentration levels(mg kg^{-1})	Shoot fresh weight (gr pot^{-1})		Shoot dry weight (gr pot^{-1})		Root fresh weight (gr pot^{-1})		Root dry weight (gr pot^{-1})	
Cadmium	0	a	28.20	a	6.36	a	12.77	a	5.88
	50	ab	26.41	ab	6.15	b	9.78	b	1.88
	100	ab	24.74	ab	5.72	bc	7.97	b	1.60
	200	ab	24.97	ab	5.42	c	6.60	b	1.38
	400	ab	25.47	ab	5.74	c	6.17	b	1.39
	600	b	24.00	ab	5.73	c	6.82	b	1.93
	800	c	18.42	b	4.92	c	6.15	b	2.15
Copper	0	a	28.20	ab	6.36	ab	12.77	a	5.91
	50	a	27.50	b	5.37	a	18.67	a	5.00
	100	a	27.64	ab	6.95	ab	17.93	a	4.77
	200	a	29.80	a	7.84	ab	12.05	a	3.68
	400	a	26.38	ab	6.36	ab	10.92	a	3.05
	600	a	29.75	ab	6.59	ab	11.19	a	2.75
	800	a	28.35	ab	6.55	b	10.07	a	2.43
Zinc	0	a	28.20	a	6.36	ab	12.77	a	5.88
	50	a	27.50	a	6.20	bc	11.63	bc	3.87
	100	a	29.20	a	6.30	abc	12.29	bc	4.03
	200	a	29.00	a	7.20	a	13.75	a	6.30
	400	a	31.00	a	7.90	dc	11.01	ab	5.27
	600	a	31.70	a	6.90	de	9.90	bc	4.08
	800	a	34.00	a	7.25	e	9.04	c	3.16

Different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test.

Table 5. ANOVA results of treatments effect on heavy metal uptake and accumulation in *Vetiver* and *Fescue*

Sources of change	df	Amount of element in shoot	Amount of element in root
Vetiver			
Cadmium treatment	6	144640.37^{**}	65304.99^{**}
Zinc treatment	6	19032.65^{**}	439.85^{**}
Copper treatment	6	11965.68^{**}	7452.43^{**}
Fescue			
Cadmium treatment	6	566.17^{**}	1885.43^{**}
Zinc treatment	6	19.15^{**}	186.80^{**}
Copper treatment	6	151.25^{**}	185.24^{**}

**: significant (0.01 probability level)

3.3. Translocation and bioaccumulation factors

Equation 1 (Raskin and Ensley, 2000) was used to calculate the translocation factor from root to the shoot. The maximum translocation factors (TF) were found for Cu (5.33), Cd (1.74), and Zn (1.12) in *Vetiver* grass (Fig. 4). As figure 4 shows, the TF contents for *Vetiver* were more than 1 compared to the amounts observed for *Fescue* (TF <1). According to TF values, *Vetiver* is highly capable to translocate heavy metals from root to shoot. Considering the TF values calculated for *Fescue*, it seems that *Fescue* roots play a role in phytoremediation of heavy metals under study.

Translocation factor from root to shoot = metal concentration in shoot/metal concentration in root Equation (1).

Bioaccumulation (BF) factor as affected by soil properties, plant species, and pollutant element is a common investigation tool for phytoremediation and environmental pollution studies (Sipter *et al*., 2008). BF was calculated using the equation 2 (USEPA, 2004). Figure 5 shows the BF factors for *Vetiver* and *Fescue* at 800 ppm level. The maximum BF for Cd (2.2) and Cu (1.27) were calculated for *Vetiver*. The low BF contents for *Fescue* show the lower ability of this plant compared to *Vetiver* to uptake and accumulate metals from the soil. The BF factors prove that *Vetiver* could be accounted as an accumulator plant for phytoremediation practices.

At the same time, Roongtanakiat and Sanoh (2011) reported that *Vetiver* is not a hyper accumulator plant, but due to high biomass content, it shows a high capability for phytoremediation. In another study, Chen *et al*. (2000) concluded that *Vetiver* with high biomass content had more efficiency in Cd absorption compared to *Thlaspi caerulescens* as a hyper accumulator plant. Moreover, deep roots of *Vetiver* caused remediation of different soil layers. These strong points of *Vetiver* compared to slow growth, and low biomass content of other hyper accumulator plants proved *Vetiver* as an optimum choice for phytoremediation (Chen *et al*., 2000).

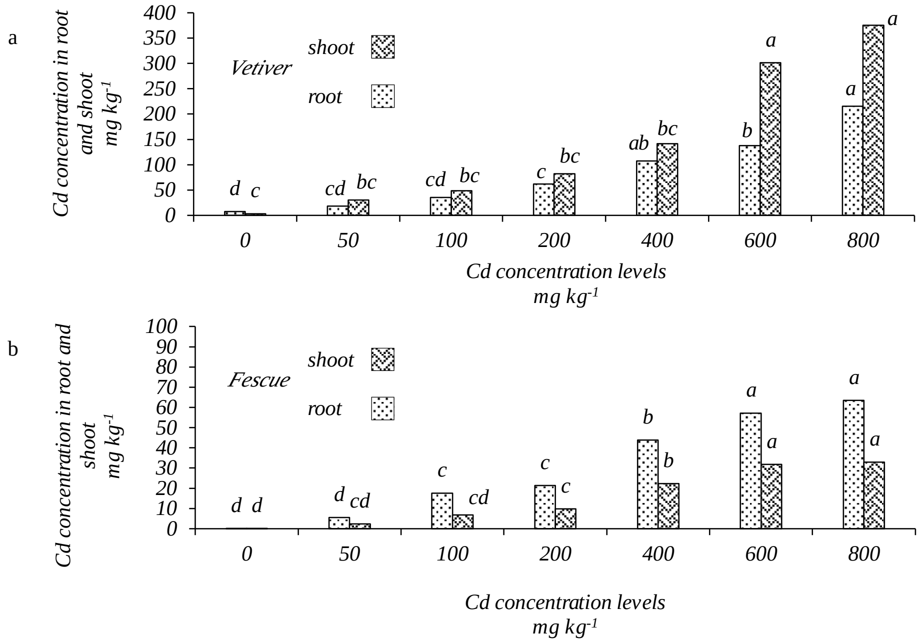

Fig. 1. Effect of different concentration levels on Cd concentration in *Vetiver* and *Fescue* root and shoot (different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test)

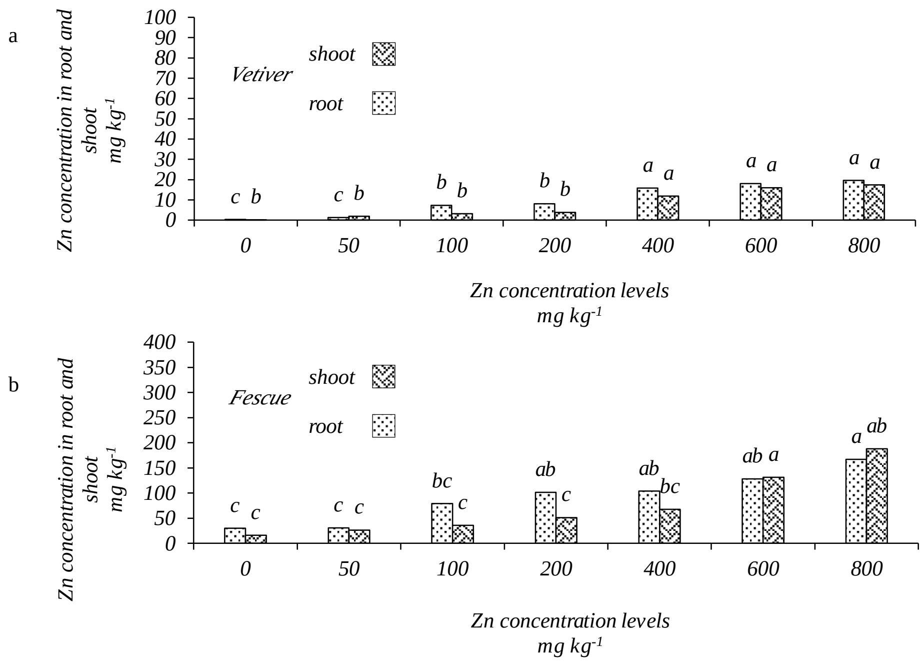

Fig. 2. Effect of different concentration levels on Zn concentration in *Vetiver* and *Fescue* root and shoot (different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test)

Equation 2 shows Bioaccumulation factor from soil to the plant as fo llows:

Bioaccumulation factor from soil to the plant= metal concentration in plant/ metal concentration in soil (Equation 2).

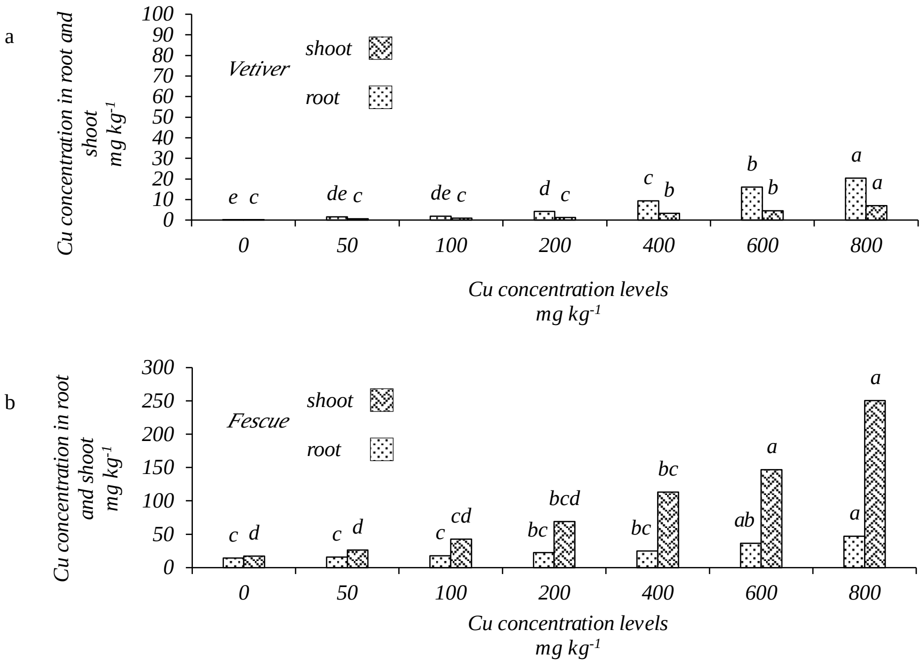

Fig. 3. Effect of different concentration levels on Cu concentration in *Vetiver* and *Fescue* root and shoot (different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test)

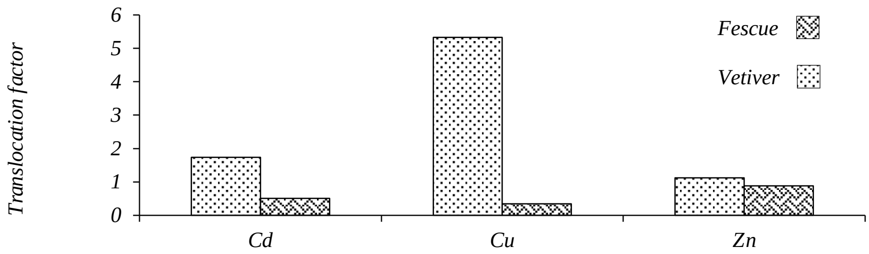

Fig. 4. Translocation factor (TF) from root to shoot in *Vetiver* and *Fescue*

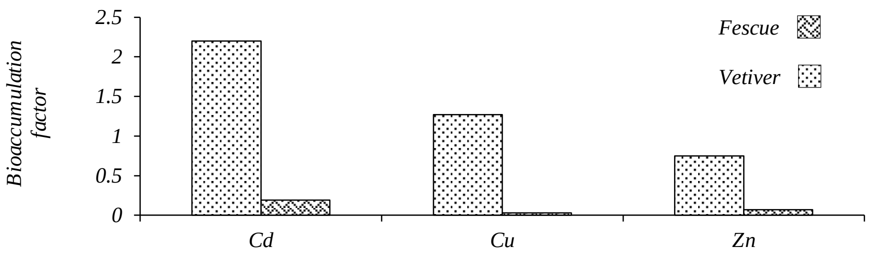

Fig. 5. Bioaccumulation factor (BF) from soil to the plant for *Vetiver* and *Fescue*

3.4. Availability of Cd, Zn and Cu in soil

ANOVA showed that DTPA extractable Cd, Cu and Zn contents were significantly affected by different levels of pollutants ($p< 0.01$) (Table 6). Mean data comparison indicated that available content of all three metals in soil were increased with increasing of heavy metal levels. The maximum values related to 800 mg kg^{-1} soil treatment for Cd, Cu and Zn were 268, 232, 470 and 503, 766, 516 mg kg^{-1} for soils under *Vetiver* and *Fescue*, respectively (Fig. 6).

Fixed, exchangeable and soluble are among different forms of elements in the soil. Soil pH, antagonistic effects of elements in soil environment, clay content, and moisture content highly affect the uptake of elements by plants (Brooks, 1998). Results of heavy metal investigation extracted by DTPA showed that available Cu, Cd and Zn concentrations in soils under *Fescue* were more than soils cultivated by *Vetiver*. This clearly shows the higher capability of *Vetiver* to remove Zn, Cu and Cd metals from polluted soils, compared to *Fescue*.

Table 6. ANOVA results of available amounts of Cd, Cu and Zn in different soil treatments

Sources of change	df	CV	Mean square
Vetiver			
Cd	6	11.03	471625.61**
Zn	6	21.49	108947.83**
Cu	6	15.20	26090.53**
Fescue			
Cd	6	10.73	1023120.23**
Zn	6	19.10	113767.60**
Cu	6	13.13	253722.27**

**: significant (0.01 probability level)

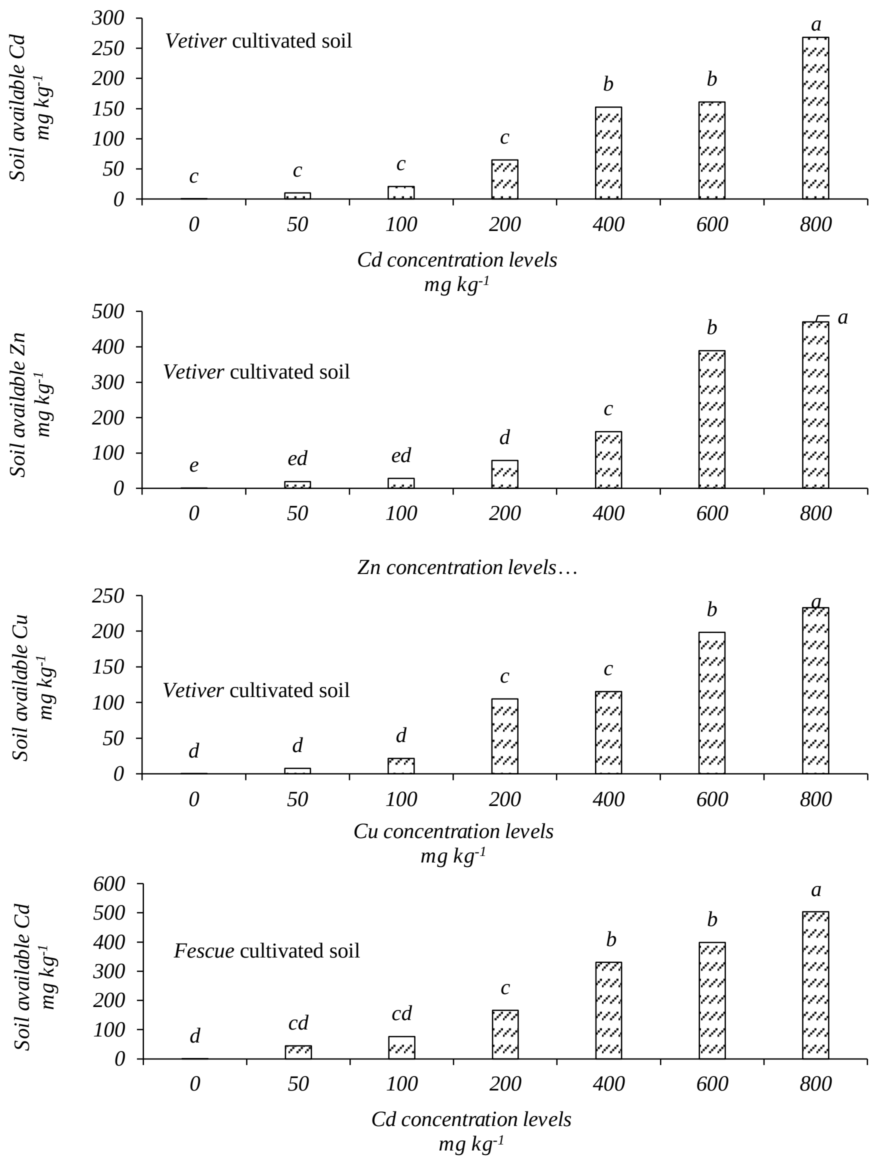

Fig. 6. Effect of different concentration levels on available concentration of Cd, Zn and Cu in *Vetiver* and *Fescue* cultivated soil (different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test)

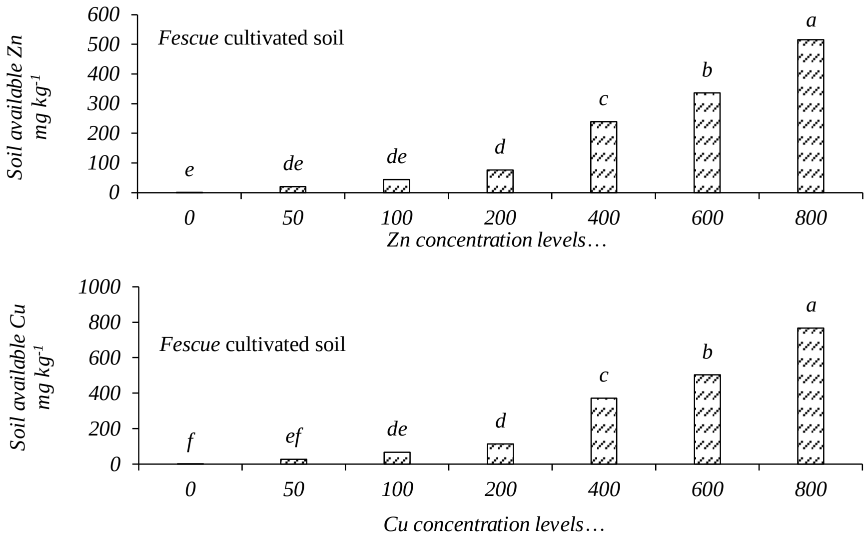

Fig. 6. Continued. Effect of different concentration levels on available concentration of Cd, Zn and Cu in *Vetiver* and *Fescue* cultivated soil (different letters show significant difference among means of each characteristic, probability level 0.01, Duncan test)

4. Conclusion

Results of the study showed that *Vetiver* was tolerated to Cu, Zn and Cd treatments and no significant effect was found among pollutant levels and shoot fresh and dry weights and root dry weights. ANOVA showed that Cd had the most accumulation in root and shoot parts. Uptake and accumulation of heavy metals were significantly increased with increasing pollutant levels without any toxic symptoms in *Vetiver*. It also revealed that the maximum capability of *Vetiver* to translocate Cu from root to shoot, also the maximum ability for Cd uptake from soil. That is why *Vetiver* could be accounted as an accumulator plant. *Vetiver* shoots and *Fescue* roots accumulated the maximum heavy metal contents. Considering three basic strategies for plants to grow in polluted soils, *Vetiver* concentrated pollutants especially Cd in root and shoot parts.

References

Aibibu, N., Y. Liu, G. Zeng, X. Wang, B. Chen, H. Song, L. Xu, 2010. Cadmium accumulation in Vetiveria zizanioides and its effects on growth, physiological and biochemical characters. Bioresource Technology, 101; 6297-6303.

Allison, L.E., W.B. Bollen, C.D. Moodie, 1965. Total carbon. In: Black CA, editor. Methods of soil analysis. Madison, Wisconsin, USA: American Society of Agronomy Inc; p. 1379-1396.

Alloway, B.J., 1990. Soil processes and the behavior of metals. In: Alloway BJ, editor. Heavy metals in soils. Glasgow: Blackie Academic and Professional; p. 7-28.

Andra, S.S., R. Datta, D. Sarkar, K.C. Makris, C.P. Mullens, S.V. Sahi, S.B.H. Bach, 2010. Synthesis of phytochelatins in Vetiver grass upon lead exposure in the presence of phosphorus. Plant and Soil, 326; 171-185.

Banerjee, R., P. Goswami, K. Pathak, A. Mukherjee, 2016. Vetiver grass: an environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecological Engineering, 90; 25-34.

Brooks, R.R., 1998. Plants those hyperaccumulate heavy metals. CAB. Pub., UK.

Chen, H.M., C.R. Zheng, C. Tu, Z.G. Shen, 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. Chemosphere, 41; 229-234.

Chen, Y., Z. Shen, X. Li, 2004. The use of Vetiver grass (Vetiveria zizanioides) in the phytoremediation of soils contaminated with heavy metals. Applied Geochemistry, 19; 1553-1565.

Datta, R., M.A. Quispe, D. Sarkar, 2011. Greenhouse study on the phytoremediation potential of Vetiver grass, Chrysopogon zizanioides L., in arsenic-contaminated soils. Bulletin of Environmental Contamination and Toxicology, 86; 124-128.

El-Tayeb, M.A., A.E. EL-Enany, N.L. Ahmad, 2006. Salicylic acid-induced adaptive response to copper stress in sunflower (Heliantus annus L.). Plant Growth Regulation, 50; 191-199.

Gee, G.W., J.W. Bauder, 1986. Particle size analysis. In: Klute A, editor. Methods of soil analysis. Madison, Wisconsin, USA: American Society of Agronomy Inc; p. 388-409.

Ghaemi, A.A., F. Majdeddin, 2016. Investigation of the phytoremediation of Vetiver and Eucalyptus by absorption of heavy metals from sewage in a contaminated soil with landfill leachate. Water Engineering, 9; 95-106.

Ghazban, F., 2011. Environmental geology. University of Tehran Press, Tehran.

Ghosh, M., S.P. Singh, 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. Asian Journal on Energy and Environment, 6; 214-231.

Ghosh, M., J. Paul, A. Jana, A. De, A. Mukherjee, 2015. Use of the grass, Vetiveria zizanioides (L.) Nash for detoxification and phytoremediation of soils

contaminated with fly ash from thermal power plants. Ecological Engineering, 74; 258-265.
Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany, 53; 1-11.
Hillel, D., 1982. Introduction to soils physics. Academic Press Inc., San Diego, California.
Hutchinson, S.L., M.K. Banks, A.P. Schwab, 2001. Phytoremediation of aged petroleum sludge: effect of inorganic fertilizer. Journal of Environmental Quality, 30; 395-403.
Kadukova, J., N. Kalogerakis, 2007. Lead accumulation from non-saline and saline environment by Tamarix smyrnesis Bunge. European Journal of Soil Biology, 43; 216-223.
Klomjek, P., S. Nitisoravut, 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere, 58; 585-593.
Lombi, E., F.J. Zhao, S.J. Dunham, S.P. McGrath, 2001. Phytoremediation of heavy metal-contaminated soils. Journal of Environmental Quality, 30; 1919-1926.
Maffei, M., 2002. Vetiveria: the genus Vetiveria. Taylor & Francis Inc., Uk.
Mleczek, M., I. Rissmann, P. Rutkowski, Z. Kaczmarek, P. Golinski, 2009. Accumulation of selected heavy metals by different genotypes of salix. Environmental and Experimental Botany, 66; 289-296.
Mohammadian, Z., A. Gholamalizadeh Ahangar, M. Ghorbani, Z. Mohkami, 2016. The effect of potassium fertilizers on lead and cadmium phytoremediation by Lavender (Lavendula officnalis) in a polluted soil. Soil and Water Conservation Research, 23; 273-287.
Monterio, M.S., C. Santos, A.M.V.M. Soares, R.M. Mann, 2009. Assessment of biomarkers of cadmium stress in lettuce. Ecotoxicology and Environmental Safety, 72; 811-818.
Neisi, A., M. Vosoughi, M.J. Mohammadi, B. ohammadi, A. Naeimabadi, 2014. Phytoremediation of by Helianthus plant. Journal of Torbat Heydariyeh University of Medical Sciences (Journal of Health Chimes), 2; 55-65.
Page, A.L., R.H. Miller, D.R. Keeney, 1982. Methods of soil analysis: chemical and microbiological properties. American Society of Agronomy Inc., Madison, Wisconsin, USA.
Percy, I., P. Truong, 2003. Landfill leachate disposal with irrigated Vetiver grass. In: Proceedings of Third International Conference on Vetiver, Guangzhou, China.
Potters, G., T.P. Pasternak, Y. Guisez, K.J. Palme, M.A.K. Jansen, 2007. Stress-induced morphogenic responses: growing out of trouble? Trends in Plant Science, 12; 98-105.
Pulford, I.D., C. Watson, 2003. Phytoremediation of heavy metal contaminated land by trees- a review. Environment International, 29; 529-540.
Raskin, I., B.D. Ensley, 2000. Phytoremediation of toxic metals: using plants to clean up the environment. John Wiley & Sons Inc., New York.
Rhoades, J.D., 1996. Salinity: electrical conductivity and total dissolved solids. In: Sparks DL, editor. Methods of soil analysis. Madison, Wisconsin, USA: American Society of Agronomy Inc; p. 417-436.
Roongtanakiat, N., P. Chairoj, 2001. Uptake potential of some heavy metals by Vetiver grass. Kasetsart Journal (Natural Science), 35; 46-50.
Roongtanakiat, N., S. Sanoh, 2011. Phytoextraction of zinc, cadmium and lead from contaminated soil by Vetiver grass. Kasetsart Journal (Natural Science), 45; 603-612.
Rothery, E., 1988. Analitical methods for graphite tube atomizers. Varian Techtron Pty. L td, 193, Victoria, Australia.
Rout, G.R., P. Das, 2009. Effect of metal toxicity on plant growth and metabolism: I. zinc. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C, editors. Sustainable agriculture. Dordrecht: Springer; p. 873-884.
Singh, S., J.S. Melo, S. Eapen, S.F. D`Souza, 2008. Potential of Vetiver (Vetiveria zizaniodes L. Nash) for phytoremediation of phenol. Ecotoxicology and Environmental Safety, 71; 671-676.
Sipter, E., E. Rózsa, K. Gruiz, E. Tátrai, V. Morvai, 2008. Site-specific risk assessment in contaminated vegetable gardens. Chemosphere, 71; 1301-1307.
Thomas, G.W., 1996. Soil pH and soil activity. In: Sparks DL, editor. Methods of soil analysis. Madison, Wisconsin, USA: American Society of Agronomy Inc; p. 475- 490.
Truong, P., 1999. Vetiver grass technology for mine rehabilitation. PRVN Tech. Bull. No. 1999/2. ORDPB, Bangok, Thailand.
Truong, P.N.V., Y.K. Foong, M. Guthrie, Y.T. Hung, 2010. Phytoremediation of heavy metal contaminated soils and water using Vetiver grass. In: Wang L, Tay JH, Tay S, Hung YT, editors. Handbook of environmental engineering, environmental bioengineering. Totowa, New Jersey: Humana Press; p. 233-275.
Tu, C., Y. Teng, Y. Luo, X. Sun, S. Deng, Z. Li, W. Liu, Z. Xu, 2011. PCB removal, soil enzyme activities, and microbial community structures during the phytoremediation by alfalfa in field soils. Journal of Soils and Sediments, 11; 649-656.
USEPA. 2004. Office of solid waste and emergency response, hazardous waste land treatment. SW-874.
Vargas, C., J. Pérez-Esteban, C. Escolástico, A. Masaguer, A. Moliner, 2016. Phytoremediation of Cu and Zn by Vetiver grass in mine soils amended with humic acids. Environmental Science and Pollution Research, 23; 13521-13530.
Walkley, A., I.A. Black, 1934. An examination of the degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37; 29-38.
Wei, S., Q. Zhou, U.K. Saha, H. Xiao, Y. Hu, L. Ren, G. Ping, 2009. Identification of a Cd accumulator Conyza canadensis. Journal of Hazardous Materials, 163; 32-35.
Wei, S.H., Q. Zhou, S. Mathews, 2008. A newly found cadmium accumulator Taraxacum mongolicum. Journal of Hazardous Materials, 159; 544-547.
Xu, P., Z. Wang, 2013. Physiological mechanism of hypertolerance of cadmium in Kentucky bluegrass and tall Fescue: chemical forms and tissue distribution. Environmental and Experimental Botany, 96; 35-42.