EFFECT OF INCREASING OF ZINC CONCENTRATION ON THE CHANGING CHEMICAL FORMS OF CADMIUM AND IT'S UPTAKE (CASE STUDY IN SORGHUM CROP)

*Sepideh Bagheri[1] and Seyyed Ali Noorhosseini[2]
[1]*Young Researchers and Elite Club, Bandar Anzali Branch, Islamic Azad University, Bandar Anzali, Iran*
[2]*Department of Agronomy, Gorgan Branch, Islamic Azad University, Gorgan, Iran*
*Author for Correspondence

ABSTRACT

Two soil samples one from top layer of a non polluted area (soil1) and the other from the vicinity of industerial factory (soil2) were used in the experiment. In both soil samples zinc contents were raised with zinc sulfate to three levels (250, 375 and 500 mg Zn/kg soil) based on initial Zn-HNO_3 content. The role of activity of plant root on chemical forms of cadmium was determined in vicinity different areas of root by using extractants. Soils and levels of zinc have significant difference on forms of extractable cadmium (P <0.01). In first level of zinc, root effect of sorghum caused to accumulate HNO_3 extractable Cd concentration (43% compared to the bulk soil) in rhizosphere of soil1. In third level of zinc, root effect of sorghum caused to accumulate HNO_3 extractable Cd concentration (84% compared to the bulk soil) in rhizosphere of soil2. In these two cases, the maximum uptake was observed in the plant. With increasing zinc concentration, Cd uptake reduced in soil1 and increased in soil2. The results showed that TF of total plant was more than 1 in both soils and all levels of zinc. In according to this factor confirmed that sorghum can be accumulator in low concentration of cadmium. The most of TF was related to soil2 and second level zinc. The maximum of cadmium reduce amount was this treatment of soil (44% reduce compared to initial planting).

Keywords: Cadmium, Chemical Forms, Extractant

INTRODUCTION

Cadmium occurs naturally in soil , it's concentration will be increased through atmospheric deposition , industrial pollution and waste, irrigation water and soil enhancer such as organic and mineral fertilizers and improvement in soil (Sheppard *et al.,* 2009). In general, Cd contents in soils ranged from 0.05 to 0.8 mg kg^{-1} soil (Kabata-Pendias and Pendi as, 2000). Zinc status of soils and plants plays an important role in Cd accumulation by crop plants (Moustakas *et al.,* 2011). The correlation between the metals concentration and uptake in the plant root and above-ground biomass depends on many factors affecting tranformation including species of plant, environmental conditions and competing (ions) (McBride, 1997). Rhizosphere processes is used to able operation in selected genotypes that were efficient in nutrient removal by plants and following increasing production. In deed, the rhizosphere has been recognized as a distinct microenvironment in which the properties and the intensity of soil processes differ from those of the bulk soil (Rengel and Marschner, 2005). Sorghum is the fifth most important cereal in the world, used as a main staple food or as animal feed and the agronomic requirements for successful production are well established. It has very good agronomic characteristics, such as fast growth and it will produce remarkable production in short time approximately 50 days (depending on the weather conditions) and highly productive (Ejeta and Axtell, 1992; Rooney *et al.,* 2007). It has very developed and broadcast root system (Fageria and Moreia, 2011). Several studies were taken on sorghum plant cultivation in the soils with different industrial pollutants, heavy metals and the importance of root for removaling them from soil (Mc Catcheon and Schnoor, 2003; Zhuang *et al.,* 2009; Guiwei *et al.,* 2010). The cadmium bioavailability is also strongly related to the specific characteristics of the various soil conditions under cultivation (Kabata-Pendiasand Pendias, 1992). Many studies emphasize that the total concentration of heavy metals in soils provides a poor indication of phytoavailability. To determine the

Research Article

content, mobility and bioavailability of elements, there is the extractants for removing them from the soil (Rao *et al.,* 2008). Determination of cadmium with extractent HNO$_3$ 4N provides an indication of nearly of total (non-silicate, non-solid phase). In addition, one of the main methods of measuring heavy metals in soil is organic extractent such as DTPA (Lindsay, 1978). The exchangeable Cd is easily absorbable by plants. Plant can uptake and accumulate exchangeable cadmium like other nutrients. Savonia *et al.,* (2005) reported exchangeable forms were determined by extraction with calcium nitrate in soil.

Several studies have been done about cadmium speciation in the rhizosphere of different soil conditions by using rhizobox (Séguin *et al.,* 2004; Jingchun *et al.,* 2008; Dechun *et al.,* 2009). Jingchun *et al.,* (2008) reported that available cadmium increased in rhizosphere of *andelia cand el* (L), Although the other forms were decreased (non-available cadmium). The lack of specific results from the redistribution of metals between the root-soil and dispersion of information about chemical forms of element recognition in rhizosphere than bulk soil cause further investigate of the role of plants in the bioavailability and concentration changes. Chemical changes of element in the rhizosphere of plants will help a lot to understand how uptake them by plant. In addation to, knowledge of chemical changes in this active area will result the building of extractants that simulated rhizosphere chemical area, may be more relevant uptake by plants. In this research such an approach has been used to study the changes in the cadmium content of two soils (polluted and non-polluted) under sorghum root effects at three levels of zinc concentration and investigating the concentration and cadmium uptake from soils in plant tissues of sorghum.

MATERIALS AND METHODS
Study Site and Plant
Two sample soils (chemical and physical properties were approximately similar) with for the experiment were collected from surface layer of soil (0-20cm) of a wheat field located near the city of Zanjan (northwest) and Taleghan (north), Iran. First sample (soil1) was without industrial pollution and second sample (soil2) was with heavy metals pollution that was from of industrial factory. Geographic position include Soil1: Longitude 36˚5.3′ 21″ N and Latitude 50˚28.9′ 12″ E, Soil2: Longitude 36˚40′ 18.8″ N and Latitude 48˚21′ 3″ E. Primary properties of soils were measured such as texture (Bouyoucos., 1962), pH (Saturation extract) (Thomas., 1996), EC (Saturation extract) (Rhodas., 1996) and %OC (Nelson and Sommers, 1982) %Өm and some heavy metals (Chang *et al.,* (1984) (Table 1). Sorghum seeds include Kimia, Sepideh and Payam cultivars was produced from Seed and Plant Improvement Institute Karaj, Iran. Pre-experiment, seeds were sown. In according to the higher germination percentage, seeds of Kimia cultivare was chosen for greenhouse cultivation.

Levels of Zinc and Incubation Period
In according to initial zinc concentration (HNO$_3$ extractable) and with using ZnSO4.H2O, zinc concentration in soils recieved three levels zinc (250, 375 and 500 mg kg^{-1}). After adding salt (zinc sulfate), planting boxes were maintained for three months in 25˚C temperature and moisture (70% MWHC) at 60% air humidity in incubator, Drying - wetting periods was carried out.

Rhizobox Design and Experimental Conditions
Sorghum was cultivated inside plastic boxes (with dimensions 18×15×20 cm) in a randomized complete nested-factorial design. For each soil and levels zinc, two boxes with plant (sorghum) and two boxes without plant (control) were used (The control box was not affected by the soil root). In each boxes with using nylon mesh (40µm); to prevent the direct influence of roots, inside each boxes three zones were designated as S$_1$ (called the rhizosphere: 4.5 Cm), S$_2$ (are affected by the rhizosphere: 2.5 Cm) and S$_3$ (distant from rhizosphere: 4 Cm) from which, subsamples were collected at two time periods of three and seven weeks after planting to measure the chemical properties Wang *et al.,* (2002). Rhizobox was taken at 25 °C, 70% air humidity and 14000 LUX light intensity in the growth chamber during 7 weeks.

Bioavailability of Cadmium
To measure the amount of cadmium available for plants, 10 gram soils with 20 ml DTPA+TEA 0.005M was shaked two hours and the solution was filtered with whatman 42 filter paper, Lindsay and Norvel

Research Article

(1978). For calcium nitrate extractable Cd, 10 gram soil with 50 mL 0.2 M calcium nitrate was shacked for two hours and the solution was filtered, Mench *et al.,* (1994). Water soluble of cadmium a solution was collected in soil:extractant of 1:1.5 ratio using 20 gram soil with 30 mL distilled water shacked for two hours and the solution was filtered, Abreu *et al.,* (2006). To measure the amount of cadmium extractable with nitric acid, 2 gram soils with 15 mL 4N HNO3, was digested for 12 hours in water bath and then filtered and collected in 50 mL volumetric flask, Chang *et al.,* (1984). Assessment the amount of cadmium in plant samples was taken (dry burning) digestion method combined by HCl 1N Waling *et al.,* (1984). Extracted cadmium contents were determined using atomic absorption spectrometery (Schimadzo model AA-670) and ICP (Perkin elemer Optima 2100) and were reported as mg Cd kg^{-1} in dry soil and plant. Cd uptake (μg Cd/pot) = yield (kg/pot) × Cd concentration in plant biomass (μg Cd/kg soil).

Statistical Analysis

Statistical data analysis was carried out with SAS 9.2 software and mean comparison was performed with LSD multiple ranges test at 5% level. Charting program also was performed EXCEL 2010. Variance analysis of plant's parameters and change cadmium forms was conducted factorial and nested – factorial design, respectively.

RESULTS AND DISCUSSION

Physical and Chemical Properties of Soils

Although most physical and chemical properties of soils which used in the experiment, before treatment for both soils were similar, but the concentrations of heavy metals except iron and copper of them (due to soil samples location) were different (Table 1).

Table 1: Some physicochemical Properties of soils (before planting)

Soil	pH	EC (dsm^{-1})	Texture -	OC (%)	Өm (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})
1	8.4	1.1	Sandy Clay Loam	0.85	34.7	242.5	38.26	18.8	25.8	15.5
2	8.2	1.3	Sandy Clay Loam	1.47	35.4	220.7	223.7	21.9	327.5	227

Table 2: Initial extractable Cd (mg/kg dw) before treatment by ZnSO4.H$_2$O

	HNO$_3$	DTPA+TEA	Ca(NO$_3$)$_2$	H$_2$O
Soil1	0.3	0.1	0.01	0.005
Soil2	0.7	0.4	0.08	0.02

Table 3: Changes of extractable Cd concentrations (means ± SD) in soil (mg/kg dw) of bulk soil used in the experiment

		Soil1				Soil2			
Time	Levels of Zinc	HNO$_3$	DTPA+TEA	Ca(NO$_3$)$_2$	H$_2$O	HNO$_3$	DTPA+TEA	Ca(NO$_3$)$_2$	H$_2$O
Primary of planting	1	0.55±0.05	0.107±0.007	0.012±0.007	0.0025±0	0.7±0.06	0.35±0.02	0.017±0.007	0.0025±0.002
	2	0.7±0.025	0.192±0.02	0.065±0.02	0.004±0	0.98±0.1	0.33±0.007	0.017±0.0007	0.0025±0.002
	3	0.8±0.025	0.09±0.006	0.055±0.012	0.001±0	1.11±0.03	0.36±0.01	0.0115±0.0025	0.006±0.002

Research Article

Chemical Forms of Cadmium

Type soils and levels of zinc have significant differences on extractable cadmium by four extractants. These results confirmed with Chen *et al.*, (2000). They reported that the different conditions of soils influence chemical forms of cadmium. Amounts of HNO_3 extractable cadmium showed a significant difference in cultivation sorghum ($p<0.05$) (Table 4).

Table 4: Results of analysis variance of chemical forms of cadmium

Source of Variation	df	Mean of Square			
		H_2O(d.w)	$Ca(NO_3)_2$	DTPA	HNO_3
Section	2	0.000094^{ns}	0.0012^{*}	0.00009^{ns}	0.001^{ns}
Section (plant)	3	0.000067^{ns}	0.00083^{ns}	0.0011^{ns}	0.03^{**}
Time	1	0.0025^{**}	0.0061^{**}	0.0008^{ns}	0.69^{**}
Time×Section	2	0.000055^{ns}	0.00095^{ns}	0.00061^{ns}	0.012^{**}
Time×Section (plant)	3	0.000037^{ns}	0.00075^{ns}	0.0005^{ns}	0.024^{**}
Error a	12	0.00014	0.00021	0.0011	0.026
Zinc	2	0.0003^{ns}	0.0022^{**}	0.012^{**}	7.05^{**}
Soil	1	0.0056^{**}	0.053^{**}	0.92^{**}	0.018^{**}
Error b	60	0.000106	0.0003	0.00129	0.0022

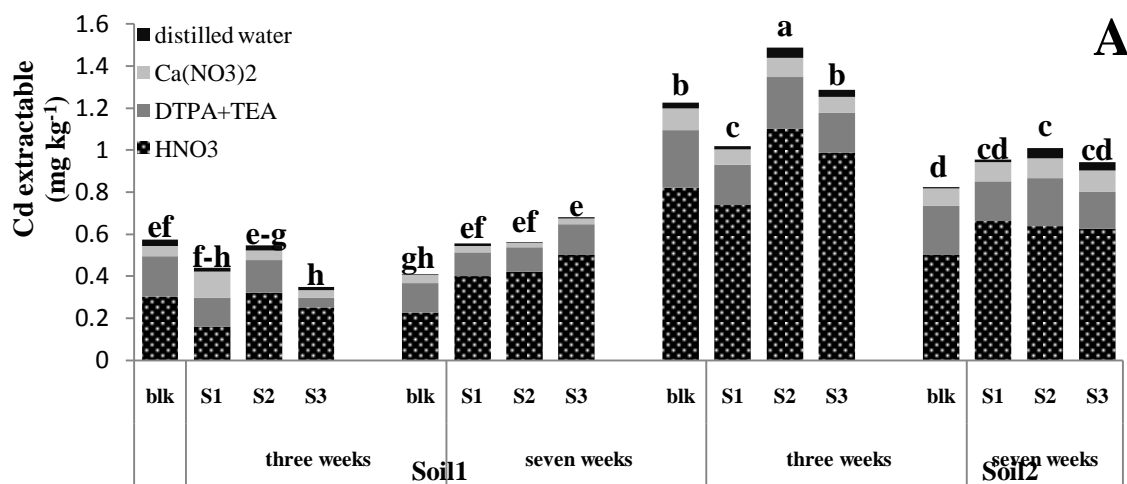*** significant at ($p<0.01$), * significant at ($p<0.05$), ns non-significant.*

Figure 1A

Research Article
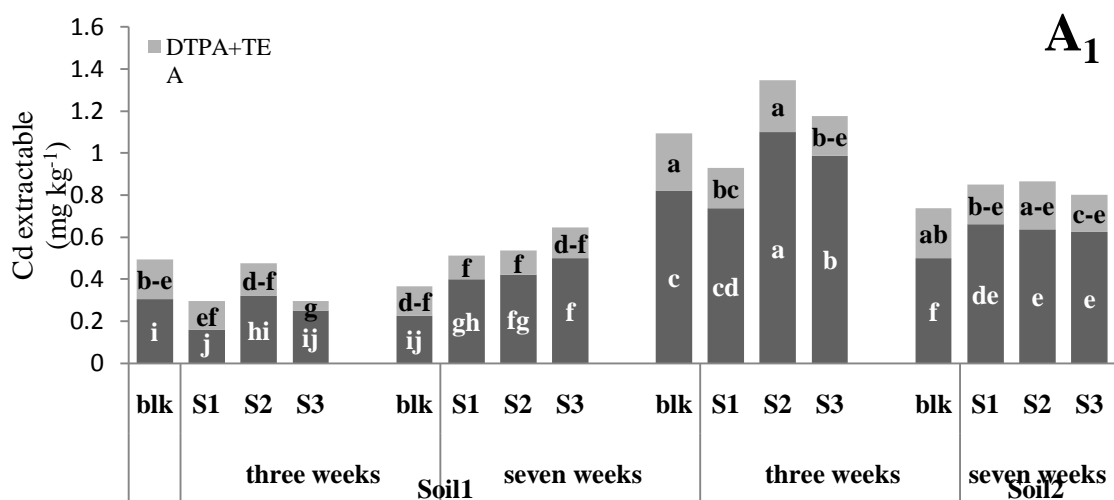
Figure 1A₁
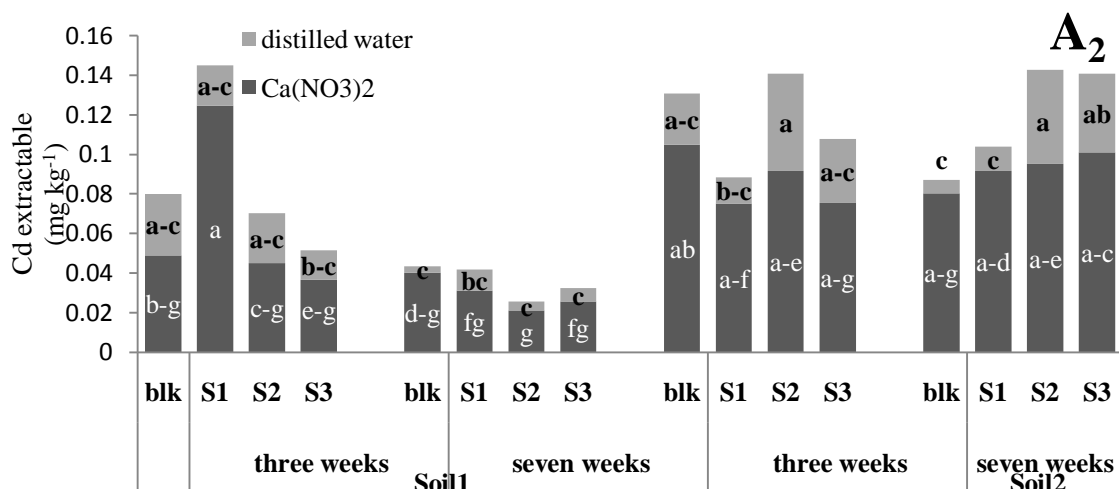
Figure 1A₂
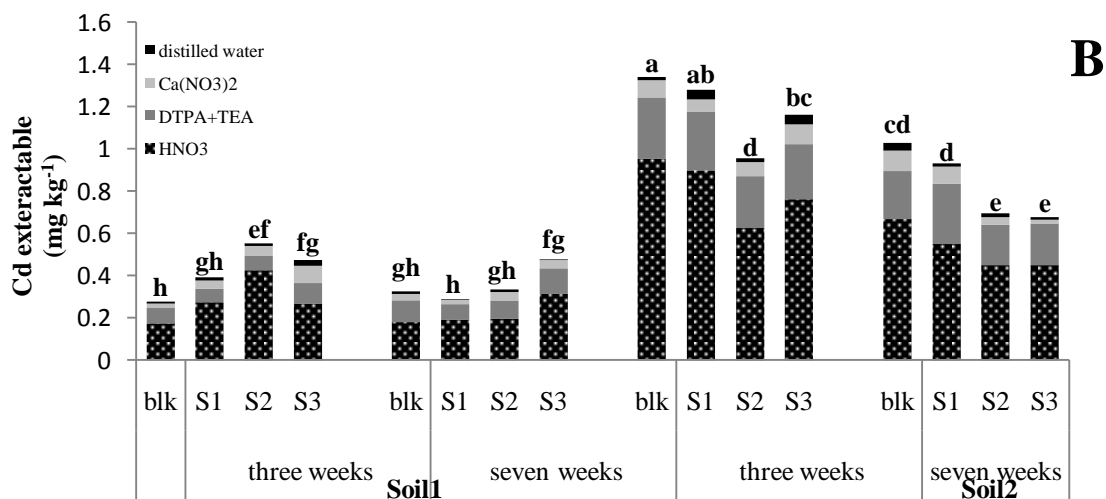
Figure 1B
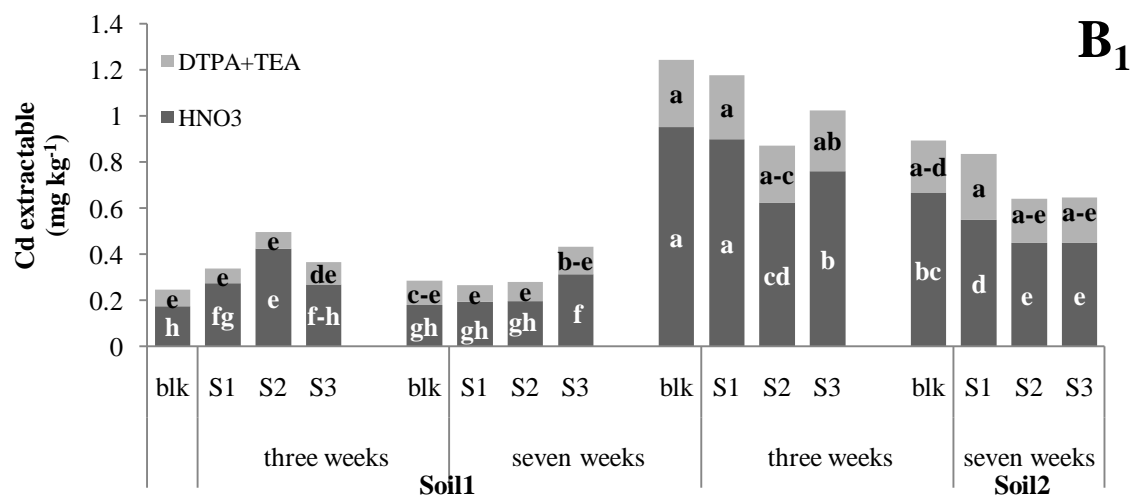
Research Article
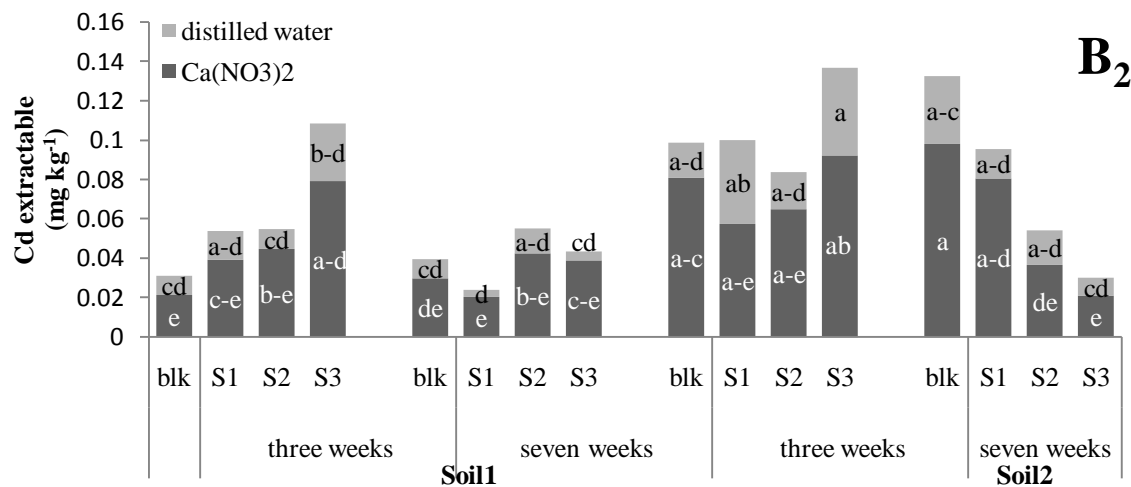

Figure 1B$_1$
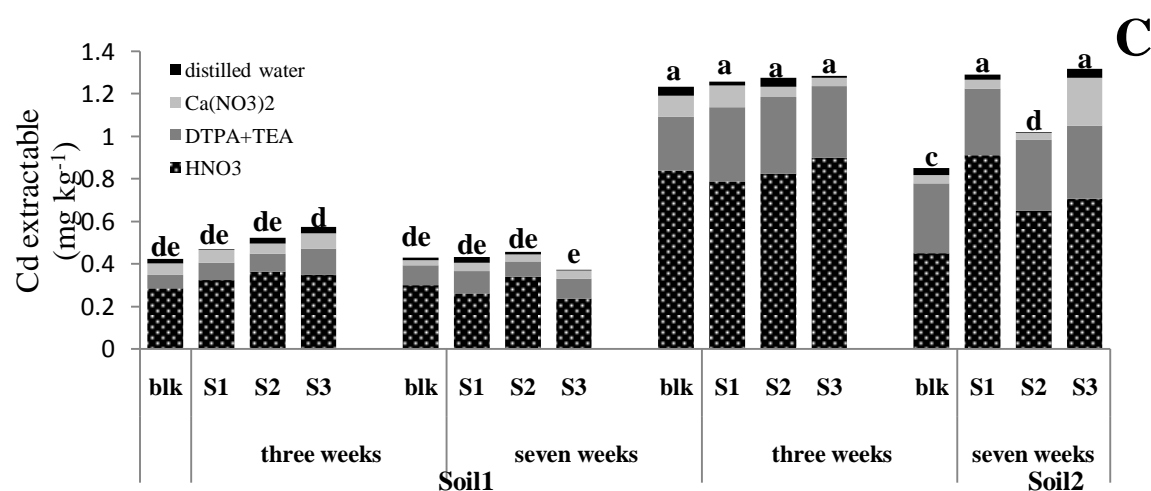

Figure 1B$_2$

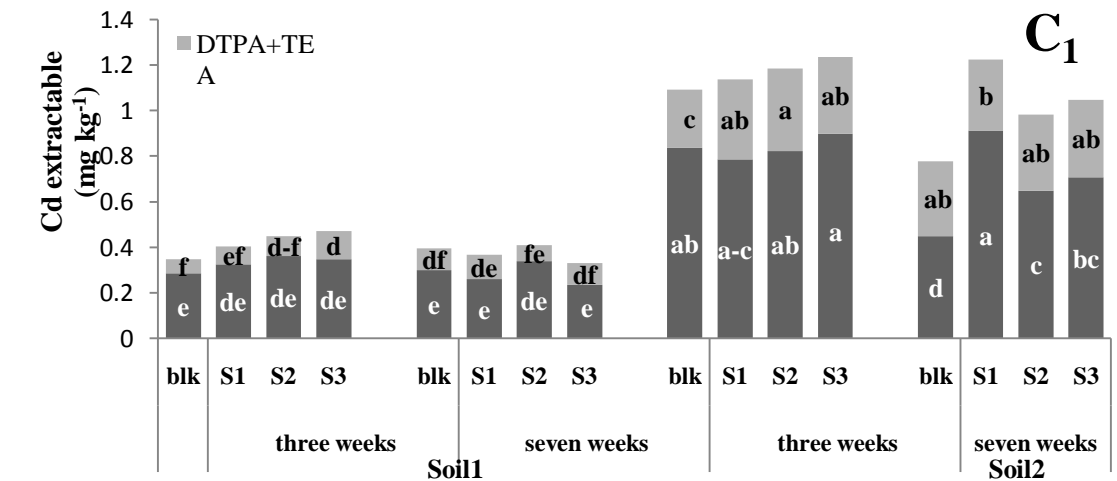**Figure 1C**

Research Article
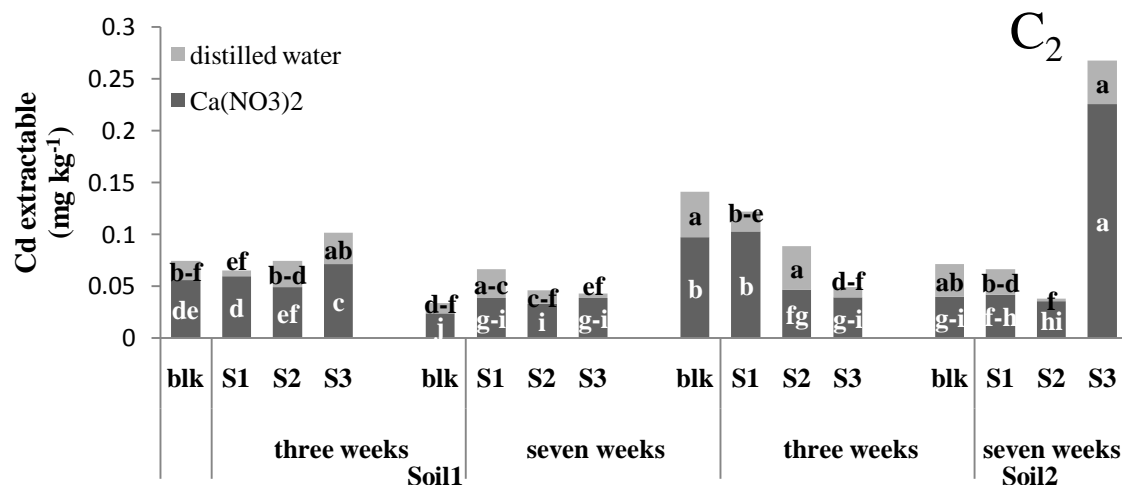

Figure 1C₁

Figure 1C₂

Figure 1: Comparison between cadmium concentrations (mg.kg^{-1}) extractable with distilled water, Ca(NO$_3$)$_2$ in soils of different zones (S1, S2 and S3) in the medium sorghum and bulk soil (blk) at times three(3)and seven (7)weeks after planting. A (First level of zinc (250 mg kg^{-1}) B (Second level of zinc (375 mg kg^{-1}) C (Third level of zinc (500 mg kg^{-1})

In according to (Figure 1-A1, A2), HNO$_3$ extractable Cd decreases (47%) and Ca(NO$_3$)$_2$ extractable cd increases (1.5 time) relative to bulk soil1 at three weeks. Mehmood *et al.,* (2009) showed that acid and sulfate compounds were secrete from sorghum root. In addation to, root exudates were considered as source of carbon and energy for soil microorganisms. So, continued increasing organic matter to the soil caused increasing microbial activity in the rhizosphere. This could change non-available compounds to more available. It was accumulated exchangable cadmium in plant (Vivas *et al.,* 2006). In soil1, total cadmium decrease 40% significantly relative to bulk soil in S$_3$ zone at three weeks, significant increase of 70% total cadmium relative to bulk soil was taken at seven weeks (Figure 1-A).

According to (Figure 1-B1, B2), in soil1 HNO$_3$ extractable cadmium content in S$_1$ and S$_2$ increase (56% and 1.4 time relative to bulk soil, respectively) at three weeks. In Soil2, it was decreased in S$_2$ and S$_3$ (25 and 52% relative to bulk soil, respectively) at three weeks. In soil1, Ca(NO$_3$)$_2$ extractable cadmium

Research Article

content is reduced in S_1 zone (50% relative to S_3) at three weeks soil. In soil2, $Ca(NO_3)_2$ extractable cadmium content was reduced the distance from the root. In soil1, total extractable cadmium significantly increased S2 and S3 (2 times and 70% relative to bulk soil, respectively) at three weeks. In soil2, total extractable cadmium significantly decreased S_2 and S_3 (50% relative to bulk soil) at three weeks. Root exudates such as organic and amino acids can change solubility, adsorption, desorption, fractionation and transport of heavy metals in soil with through chelated, solubility, redox changes (Kuang *et al.,* 2003). Amount and type of root exudates are differences to age of plant, environmental condition, physical, chemical and biological stress (Mimmo *et al.,* 2011). Thus significant difference between the cadmium extraction with different extractent among the levels of zinc is justify.

The $Ca(NO_3)_2$ and distilled water extractable increased in S_3 zone (Figure 1-C). In according to, S_3 is the distance from the root, so it could be resulted that increasing in S_1 and S_2 was taken in between three and seven weeks but decrease due to more uptake and unsoluble complex transformation. Comparison Figure 1-C and Table 7 showed that increasing this form caused more Cd uptake by sorghum. Cadmium uptake takes place with cadmium inorganic complexes such as $CaCl^+$, $CdCl_2$ and $CdSO_4$ etc. or organic complexation (McLaughlin, 1996). The main mechanism due to microbial activity of root and the release of CO_2 to the soil solution from root growth (Dechun *et al.,* 2009). This caused to increase available cadmium concentration in the rhizosphere of Canola. In according Table7, maximum uptake was taken in first level zinc concentrations in soil1 and third level zinc concentrations in soil2. In this condition, DTPA extractable Cd decreased and proportion DTPA and HNO_3 extractable Cd percentage in the rhizosphere and bulk soil is similar. The most variable has been taken in these levels at seven weeks. The bioavailability of heavy metals in soils depend on numerous soil characteristics, influenced by the activity of plant roots and associated microorganisms (Wenzel 2009). Wei-Hong *et al.,* (2007) observed root exudates change under effect of levels of zinc concentrations. This caused to changing uptake and concentration.
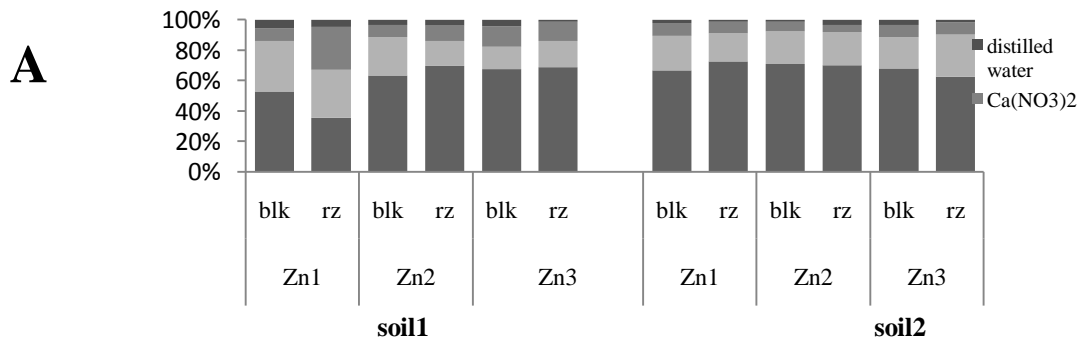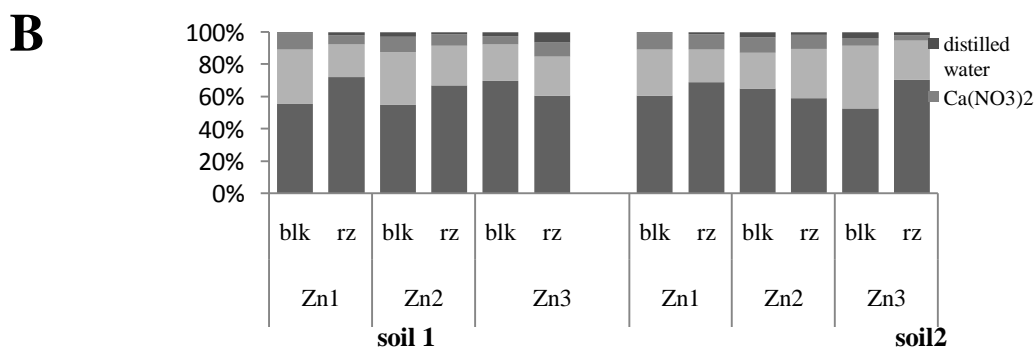

Figure 2A

Figure 2B

Figure 2. Changes of percentage chemical forms in rhizosphere (rz) and bulk soil (blk) at three (A) and seven weeks (B)

Research Article

Accumulation of Cd in the Plant

Levels of zinc concentrations and two soil type have significant effects on TF of total plant. This is inherent character and is not influenced environmental condition, while these changes have significant effect on uptake and concentration.

Table 5: Results of analysis of variance of chemical forms of cadmium Cd uptake, concentration and Translocation factor

| Source of Variation | df | Mean square | | | | | | | | |
| | | Uptake | | | Concentration | | | Translocation Factor | | |
		Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
soil	1	0.011^{ns}	7.11^{**}	7.68^{*}	4.99^{**}	8.49^{**}	26.5^{**}	8.6^{*}	6.48^{**}	0.15^{ns}
zinc	2	9.32^{*}	0.028^{ns}	7.52^{*}	0.87^{*}	0.103^{*}	0.57^{*}	0.69^{ns}	2.7^{**}	4.23^{ns}
Soil × zinc	2	27.3^{**}	0.109^{*}	24.4^{**}	0.29^{ns}	0.11^{**}	0.617^{*}	0.38^{ns}	0.35^{ns}	0.62^{ns}
Error	6	0.806	0.01	0.75	0.093	0.01	0.41	0.89	0.108	1.16

**significant at (p<0.01), * significant at (p<0.05), ns non-significant

Results of work were shown that Cd concentrations in shoot had been decreased in soil1 (influenced only increasing Zn concentrations), while this is adverse in soil2 (with other heavy metals pollution). These results is similar some studies (Grant *et al.,* 1998; Khoshgoftarmanesh *et al.,* 2013). Results from some studied were shown that Cd concentration in the plant tissues of pot marigold were largely dependent on the Zn levels (Moustakas *et al.,* 2011). Khoshgoftarmanesh *et al.,* (2013) reported that increasing Zn concentration in the soil caused decreasing cadmium concentration in arial organ of some wheat cultivars. They expressed that cultivar plant is main factor of it's concentration change. This work was shown not only cd concentrations change in plant tissue depended on plant cultivars but also type soils was importance factor. In according to levels Zn, the Cd concentration changes in total organ plant were different altogether, dependent on the type soils.

Table 6: Cd concentration in plant tissue (mg kg^{-1})

| Treatment | Soil1 | | | Soil2 | | |
	Root	Shoot	Total	Root	Shoot	Total
Zn$_1$	0.261 ± 0.005^{b}	2.5 ± 0.3^{a}	2.76 ± 0.3^{a}	1.79 ± 0.01^{b}	3.18 ± 0.22^{a}	4.97 ± 0.23^{ab}
Zn$_2$	0.533 ± 0.014^{a}	1.16 ± 0.1^{b}	1.69 ± 0.1^{b}	1.97 ± 0.07^{ab}	2.65 ± 0.2^{a}	4.62 ± 0.125^{b}
Zn$_3$	0.297 ± 0.016^{b}	1.56 ± 0.1^{b}	1.85 ± 0.1^{ab}	2.37 ± 0.15^{a}	3.26 ± 0.26^{a}	5.63 ± 0.105^{a}

The probability level of significant difference is at P< 0.05. Values were expressed as SD.

With increasing Zn levels, Cd uptake was decreased in the soil1 and increased in soil2. Somewhat, this was related to plant biomass. However, they had different ionic radii ($Zn^{+2} =0.074$ nm, $Cd^{+2}=0.097$ nm); this difference may play a role in plant selectivity for Zn. In other words, the reduced uptake of Cd as a result of the addition of Zn addition in the work might result from competitive transport and absorption interaction between these two ions (Moustakas *et al.,* 2011). Negative interaction heavy metals caused the reduced uptake in plant and its metabolism (Alloway, 2008). An antagonism between Zn and Cd. Their active absorption was also observed in lettuce roots (Costa and Morel, 1994).

Table 7: Cd uptake in plant tissue (µg Pot^{-1})

| Treatment | Soil1 | | | Soil2 | | |
	Root	Shoot	Total	Root	Shoot	Total
Zn$_1$	0.56 ± 0.006^{a}	7.327 ± 1.409^{a}	7.88 ± 1.41^{a}	1.85 ± 0.052^{a}	2.9 ± 0.0034^{b}	3.86 ± 0.055^{c}
Zn$_2$	0.559 ± 0.006^{a}	0.992 ± 0.262^{b}	1.552 ± 0.26^{b}	1.97 ± 0.024^{a}	3.16 ± 0.028^{ab}	5.13 ± 0.052^{b}
Zn$_3$	0.14 ± 0.0005^{b}	0.93 ± 0.083^{b}	1.07 ± 0.083^{b}	2.056 ± 0.16^{a}	4.25 ± 0.59^{a}	6.3 ± 0.43^{a}

The probability level of significant difference is at P< 0.05. Values were expressed as SD

Research Article

TF, also called the uptake factor, accumulation factor or concentration factor. The effectiveness of contaminant transfer from soils into plants was quantified by the TF (Cd[organ]/Cd[soil]) or which was the concentration of element of interest in the dried plant tissues divided by its concentration in the soils (Liang *et al.,* 2013). The TF value is shown in Table 8. The plants that had BCF>1, were accumulator and BCF<1 were excluder (Cluiz, 2004).

Table 8: Transfer factor (TF) value of sorghum in two soils

Treatment	Soil1			Soil2		
	Root	Shoot	Total	Root	Shoot	Total
Zn[1]	0.65 ± 0.012^b	6.25 ± 0.75^a	6.904 ± 0.76^a	2.7 ± 0.025^a	4.82 ± 0.26^a	7.52 ± 0.23^{ab}
Zn[2]	2.7 ± 0.27^a	5.95 ± 0.39^a	8.829 ± 1.38^a	3.6 ± 0.46^a	4.81 ± 0.075^a	8.45 ± 0.54^a
Zn[3]	1.37 ± 0.11^b	5.97 ± 0.66^a	7.112 ± 0.78^a	2.6 ± 0.27^a	3.566 ± 0.138^b	6.181 ± 0.14^b

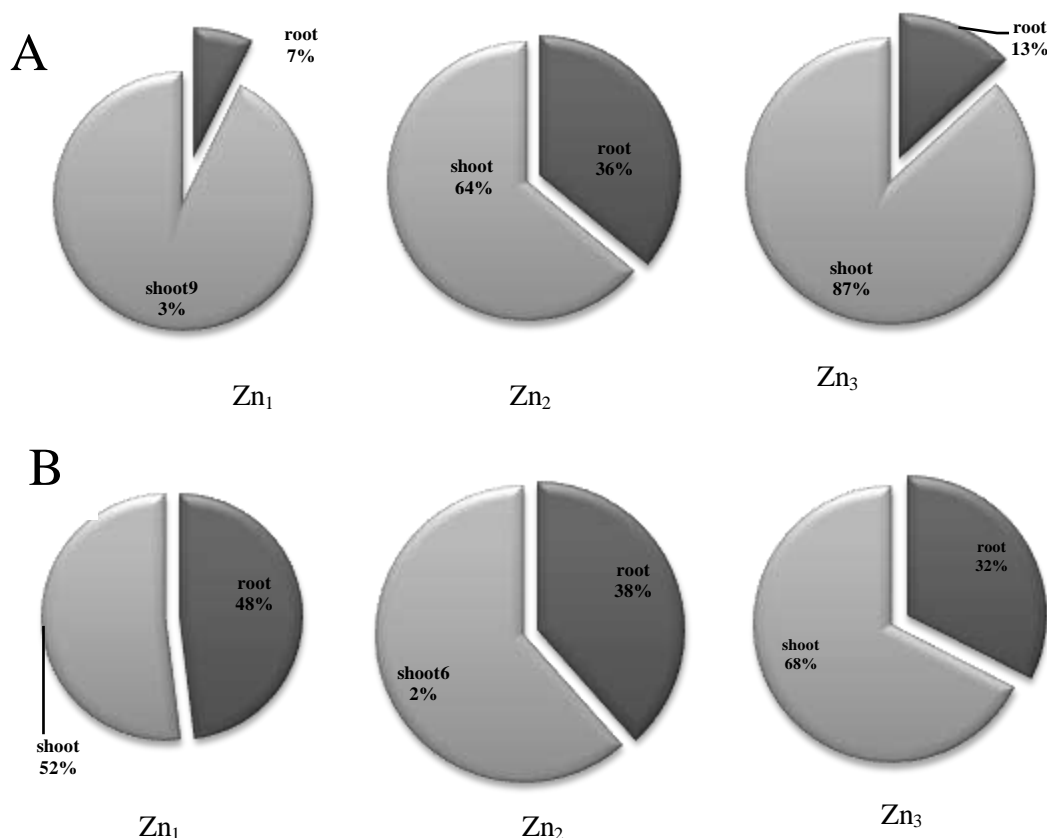*The probability level of significant difference is at P< 0.05. Values were expressed as SD*

Figure 3: The changing percentage of cadmium uptake in roots and shoots (A=soil1, B=soil2)
Increasing the Zn concentrations in soil (2) caused to reduce of the cadmium uptake percentage in roots relative to shoots. This is due to more competition in the roots than in shoots (Figure 3-A,B).

Removal Cadmium from Soil
Ashraf *et al.,* (2012) Observed that most of heavy metals such as cadmium are in residual and unavailable states in contaminated soil. For this reason, intensify pollution was decreased in the soil. The all zinc levels, the amount of HNO_3 extractable Cd, a considerable reduction in the rhizosphere of two soils (Table 9). Baneshi *et al.,* (2014) reported sorghum could removal pollutant from contaminated soil due to

Research Article

improvement of rhizosphere conditions. In according to, there was lower cadmium in soil1 before adding zinc sulphate, its reduced related to uptake by plant and transform to unavailable forms.

Table 9: Changes of extractable Cd concentrations (means ± SD) in soil (mg/kg dw) of bulk soil an rhizosphere

Time	Levels of Zinc	Soil1				Soil2			
		HNO$_3$	DTPA+TEA	Ca(NO$_3$)$_2$	H$_2$O	HNO$_3$	DTPA+TEA	Ca(NO$_3$)$_2$	H$_2$O
Initial of Planting	1	0.55±0.05	0.107±0.007	0.012±0.007	0.0025±0	0.7±0.06	0.35±0.02	0.017±0.007	0.0025±0.002
	2	0.7±0.025	0.192±0.02	0.065±0.02	0.004±0	0.98±0.1	0.33±0.007	0.017±0.0007	0.0025±0.002
	3	0.8±0.025	0.09±0.006	0.055±0.012	0.001±0	1.11±0.03	0.36±0.01	0.0115±0.0025	0.006±0.002
Seven weeks (Rhizosphere)	1	0.4±0	0.113±0.009	0.031±0.004	0.011±0.009	0.66±0.01	0.19±0.024	0.09±0.004	0.0125±0.0025
	2	0.19±0.014	0.072±0.022	0.0205±0.0005	0.035±0.001	0.55±0.05	0.28±0.1	0.081±0.06	0.015±0
	3	0.26±0.0125	0.105±0.013	0.0385±0.0035	0.0275±0.0125	0.9±0.03	0.312±0.018	0.04±0.0025	0.0245±0.0005

Discussion

Our results showed since HNO$_3$ extractable Cd constitute a large percentage of these forms, so its measuring due to more effect on the other species that can influence availability, is very important. Also, comparing between the uptake of Cd in plant with HNO$_3$ extractable Cd in the rhizosphere showed that with increasing this form of Cd, uptake amount in sorghum increase, too. The most changes percentage of HNO$_3$ extractable Cd is in soil1 at three and seven weeks. In soil2, these effects are taken at seven weeks. Indeed, rhizosphere changes occur delayed in heavy metals pollution soil. It may be related to decrease biological activity root and more intense competitive effects of heavy metals in this zone. Different mineralogy is one of important factors at form changes of cadmium in several consentration of zinc. So, investigation in this field is suggested to better understand this problem in future studies.

REFERENCE

Abreu CA, Angela AM, Furlani C, Furlani PR, Abreu MF And Bataglia OC (2006). Quest of Water Extract Analysis of Micronutrients in Soilless Organic Substrates. *Communications in Soil Science and Plant Analysis* **37** 2327–2338.

Alloway BJ (2008). *Zinc in Soils and Crop Nutrition* (International Zinc Association. Brussels, Belgium) 135.

Ashraf MA, Maah MJ and Yusoff I (2012). Chemical Speciation and Potential Mobility of Heavy Metals in the Soil of Former Tin Mining Catchment. *The Scientific World Journal* 11.

Baneshi MM, Rezaei Kalantary R, Jonidi Jafari A, Nasseri S, Jaafarzadeh N and Esrafili A (2014). Effect of bioaugmentation to enhance phytoremediation for removal of phenanthrene and pyrene from soil with Sorghum and Onobrychis sativa. *Journal of Environmental Health Science and Engineering* **12** 24.

Bouyoucos CJ (1962). Hydrometer method improved for making particle-size analysis of soil. *Agronomy Journal* **54** 464-465.

Chang AC, Warneke JE, Page AL and Lund LJ (1984). Accumulationof heavy metals in sewage sludge- treated soils. *Journal of Environmental Quality* **13** 87-91.

Chen L, Yang X, Raza W, Li J, Liu Y, Qiu M, Zhang F and Shen Q (2010). Tricho-derma harzianum SQR-T037 rapidly degrades allelochemicals in rhizospheres of continuously cropped cucumbers. *Applied Microbiology and Biotechnology* **89** 1653–1663.

Cluis C (2004). Junk-greedy greens: phytoremediation as a new option for soil decontamination. *Biotechnology Journal* **2** 60–67.

Research Article

Costa G and Morel JL (1994). Water relations gas exchange and amino acid content in Cd treated lettuca. *Plant Physiology and Biochemistry* **32** 561–570.

Dechun S, Jianping X, Weiping J and Woonchung W (2009). Cadmium uptake and speciation changes in the rhizosphere of cadmium accumulator and non-accumulator oilseed rape varieties. *Journal of Environmental Sciences* **21** 1125–1128.

Ejeta G and Axtell J (1990). Development of hard endosperm high lysine sorghums. In: *Proceedings of an International Conference on Sorghum Nutritional Quality, Purdue University, West Lafayette, IN, USA,* 126-142.

Fageria NK and Moreira A (2011). The Role of Mineral Nutrition on Root Growth of Crop Plants. In: *Advances in Agronomy* edited by Donald L Sparks (Burlington: Academic Press) **110** 251-331.

Grant CA, Buckley WT, Bailey LD and Selles F (1998). Cadmium ac-cumulation in crops. *Canadian Journal of Plant Science* **78** 1–17.

Guiwei Q, de Varennes A, Martins LL, Mourato MP, Cardoso AI, Mota AM, Pinto AP, Gonçalves ML (2010). Improvement in soil and sorghum health following the application of polyacrylate polymers to a Cd-contaminated soil. *Journal of Hazardous Materials* **173**(1-3) 570-5.

Jingchun, L, Chongling Y, Ruifeng Z, Haoliang L and Guangqiu Q (2008). Speciation Changes of Cadmium in Mangrove (*Kandelia candel*(L.)) Rhizosphere Sediments. *Bulletin of Environmental Contamination and Toxicology* **80** 231–236.

Kabata-Pendias A and Pendias H (1992). *Trace Elements in Soils and Plants* (CRC Press, Boca Raton, FL) 2nd edition.

Kabata-Pendias A and Pendias H (2000). *Trace Elements in Soils and Plants* (CRC Press, BocaRaton, FL) 3rd edition 143– 157.

Khoshgoftarmanes AH, SanaeiOstova A, Sadrarhami A and Chaney R (2013). Effect of tire rubber ash and zinc sulfate on yield and grain zinc and cadmium concentrations of different zinc-deficiency tolerance wheat cultivars under field conditions. *European Journal of Agronomy* **49** 42– 49.

Kuang YW, Wen DZ, Zhong CW and Zhou GY (2003). Root exudates and their roles in phytoremdiation. *Chinese Journal of Plant Ecology* (in Chinese) **27**(5) 709-717.

Liang Zh, Ding Q, Wei D, Li J, Chen Sh and Ma Y (2013). Major controlling factors and predictions for cadmium transfer from the soil into spinach plants. *Ecotoxicology and Environmental Safety* **93** 180–185.

Lindsay WL and Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42** 421-428.

McBride M, Sauve S and Hendershot W (1997). Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *European Journal of Soil Science* **48** 337-346.

McCatcheon SC and Schnoor JL (2003). *Phytoremediation, transformation and control of contaminants* (Wiley- Interscience).

McLaughlin MJ, Tiller KG, Naidu R and Stevens DP (1996). Review: the behaviour and impact of contaminants in fertilizers. *Australian Journal of Soil Research* **34** 1–54.

Mehmood S, Gulfraz M, Rana NF, Ahmad A, Ahring BK and Minhas N, *et al.,* (2009). Ethanol production from Sorghum bicolor using both separate and simultaneous saccharification and fermentation in batch and fed batch systems. *African Journal of Biotechnology* **8** 2857–65.

Mench M, Vangronsveld J, Didier V and Clijsters H (1994). Evaluation of metal mobility, plant availability and immobilization by chemical agents in a limed-silty soil. *Environmental Pollution* **86** 279-286.

Mimmo T, Hann S, Jaitz L, Cescoa S, Gessa CE and Puschenreiter M (2011). Time and substrate dependent exudation of carboxylates by Lupinus albus L. and Brassica napus L. *Plant Physiology and Biochemistry* **49** 1272–1278.

Research Article

Moustakas NK, Akoumianaki-Ioannidou A and Barouchas PE (2011). The effects of cadmium and zinc interactions on the concentratio n of cadmium and zinc in pot marigold (Calendula officinalis L.). *Australian Journal of Crop Science* **5**(3) 277-282.

Nelson DW and Sommers LE (1982). Total carbon, organic carbon, and organic matter. In: *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties* edited by Page AL *et al.,* 2nd edition (Agronomy monograph no.9, SSSA and ASA, Madison, WI. USA). 539-580.

Rao CRM, Sahuquillo A and Lopez Sanchez JF (2008). A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water, Air & Soil Pollution* **189** 291-333.

Rengel Z and Marschner (2005). Nutrient availability and management in the rhizosphere: exploiting gentypic differences. *New Phytologist* **168** 305-132.

Rhoades JD (1996). Salinity: Electrical conductivity and total dissolved solids. In: *Methods of Soil Analysis, Part 3: Chemical Methods* edited by Sparks DL (SSSA. Book series no. 5. Madison. WI. USA).

Rooney WL, Blumenthal J, Bean B, John E Mullet (2007). Designing sorghum as a dedicated bioenergy feedstock. *Biofuels, Bioproducts & Biorefining* **1** 147–157.

Savonina EY, Chernova RK, Kozlova LM and Fedotov PS (2005). Fractionation and determination of different lead species in contaminated soils. *Journal of Analytical Chemistry* **60**(9) 874-879.

Séguin V, Gagnon C and Courchesne F (2004). Changes in water extractable metals, pH and organic carbon concentrations at the soil–root interface of forested soils. *Plant and Soil* **260** 1–17.

Sheppard SC, Grant CA, Sheppard MI, De Jong R and Long J (2009). Risk indicator for agricultural inputs of trace elements to Canadian soils. *Journal of Environmental Quality* **38** 919-932.

Sposito G, Lund LJ and Chang AC (1982). Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Cd, and Pb in solid phases. *Soil Science Society of America Journal* **46** 260-264.

Thomas GW (1996). Soil pH and soil acidity. In: *Methods of Soil Analysis, Part 3: Chemical Methods* edited by Sparks DL (SSSA. Book series no. 5. Madison. WI. USA).

Vivas A, Biró B, Ruí z-Lozano JM, Barea JM and Azc ó n R (2006): Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhi -zal efficiency under Zn-toxicity. *Chemosphere* **62** 1523–1533.

Waling I, Van vark W, Houba VJG and JJ Der lee (1989). *Soil and Plant Analysis: A Series of Sillabi* Part 7 (Plant analy proc. Wageningen Agricultural University).

Wang Zh, Shan X and Zhang Sh (2002). Comparison between fractionation and bioavailability of trace elements in rhizosphere and bulk soils. *Chemosphere* **46** 1163-1171.

Wei-Hong Xu, Huai LIU, Qi-Fu MA, Zhi-Ting and Xiong (2007). Root Exudates, Rhizosphere Zn Fractions, and Zn Accumulation of Ryegrass at Different Soil Zn Levels. *Pedosphere* **17** 389-396.

Wenzel WW (2009). Rhizosphere processes and management in plant-as sisted biorem ediation (p hytoremedi ation) of soils. *Plant and Soil* **321** 385–408. doi: 10.1007/s11104-008-9686-1.

Zhuang P1, Shu W, Li Z, Liao B, Li J and Shao J (2009). Removal of metals by sorghum plants from contaminated land. *Journal of Environmental Sciences* **21**(10) 1432-7.