Australian Journal of Basic and Applied Sciences, 7(7): 888-894, 2013
ISSN 1991-8178

Phytoremediation of Cadmium Contaminated Soil by Sunflower

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Abstract: A field experiment was conducted to investigate the capability of sunflower (*Helianthus annuus* L.) plants to remove cadmium (Cd) from a soil polluted with enriched sewage sludge at the experimental field station of King Abdulaziz University. The mobility of cadmium from root to shoot system as well as seed and its effects on studied agronomic traits were evaluated. The obtained results indicated that Cd had significant effects at ($p \leq 0.01$) on all studied traits. As Cd concentration in the sewage sludge amended soil increased, Cd in each plant part significantly increased. Cd concentrations were in root system > shoot > seeds. Moreover, sunflower uptake of Cd ranged between 57 to 72 % from the Cd content of the polluted soil without toxic effects on plants during the two successive growth seasons (2011-2012). Sunflower plants could be used as phytoemediators to decrease toxicity of heavy metal from polluted soils for human health.

Key words: Phytoremediation, Cd, Sunflower, Agronomic traits, Sludge, Heavy metals.

INTRODUCTION

Environmental pollution affects the quality of pedosphere, hydrosphere, atmosphere, lithosphere and biosphere. Great efforts have been made in the last two decades to reduce pollution sources and remedy the polluted soil and water resources (Lone *et al*., 2008). Toxic metal pollution of waters and soils is a major environmental problem, and most conventional remediation approaches do not provide acceptable solutions and they are typically invasive and expensive (David *et al*., 1995). Sewage sludge, in many countries, is used as soil additives to improve the soil physicochemical proprieties, growth conditions and as a good source of plant nutrients but this sewage sludge almost contain many toxics such as heavy metals, pesticides, toxic organics, hormone disruptors, detergents and various salts in addition to organic material (Singh and Agrawal, 2008). Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health (Sanita-diToppi and Gabrielli, 1999). The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja *et al*., 1990). Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, alterations in enzymes of the antioxidant defense system (Sandalio *et al*., 2001). Recently, the use of plants to remediate polluted soils, has appeared as an alternative more reliable (Arthur *et al*., 2005). Phytoremediation is used as a green technology and can be applied to both organic and inorganic pollutants present in soil water or the air (Gratao *et al*., 2005 and Salt *et al*., 1998). Pulford and Watson (2003) and Baker and Brooks (1989) suggested 100 mg kg^{-1} for Cd, 1,000 mg kg^{-1} for Ni, Cu, Cr, Co and Pb, and 10,000 mg kg^{-1} for Zn and Mn for any plant can be considered as hyperaccumulator for heavy metal without evident symptoms of toxicity. Plants such as sunflower (*Helianthus annuus* L.) show high tolerance to heavy metals and therefore, are used in phytoremediation studies (Pilon-Smits, 2005, Schmidt, 2003 and Tang *et al*., 2003).The finding obtained by Chhotu *et al*., (2008) and Rengel (1997) showed that the low doses of Cd, Pb and Ni applied stimulated the root and shoot elongation of sunflower plants but at higher concentrations, significantly reduced germination(%) and plant growth specially root and shoot elongation. The highest concentration of Cd in above-ground plant tissues was found to be 287 mg kg^{-1} in the leaves of *Lobelia chinensis* Lour, at the Datianwan site, followed by *Solamum nigrum* L. with 99 mg kg^{-1} Cd in the leaves, accordingly they might be potential Cd hyperaccumulators (Kejian *et al.*, 2006). Peng *et al.*, (2009) found that species Cd concentrations reached 36.9 and 41 mg kg^{-1} in *Solanum nigrum* leaves and *Lobelia chinensis* shoots, respectively under the polluted soil. Cadmium concentrations differ between the different genotypes under the polluted soil, and the plant root system accumulated the highest Cd concentrations in most plant species (Maxted *et al*., 2007, Gangrong, 2009, Chudhary and Singh, 2000, Hasan *et a*l., 2009 and Sylwia *et al.*, 2010).

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MATERIAL AND METHODS

This study was carried out at the Agricultural Research Station, King Abdulaziz University during the two successive seasons of 2010/2011 and 2011/2012. Sunflower (*Helianthus annuus* L.) was used as a phytoremediator of cadmium heavy metal from highly polluted soil. Experimental soil was polluted with sewage sludge amendments (40 t of sewage sludge /ha) Four concentrations of Cd, were mixed with soil in each experimental plot before planting in both seasons.

Agricultural Practices:

The normal agricultural practices in this experiment were carried out from planting to harvesting. Planting dates were November 25, 2010 and November, 15 in 2011. Plants were sown at 40 cm as distance between plants. Drip irrigation system was used in this experiment. Plants were was fertilized with NPK fertilizer (20:20:18) with the rate of 600 kg/ha added in 3 equal doses, the 1^{st} dose after 25 days, 2^{nd} dose after 65 days and the 3^{rd} dose after 90 days from planting.

Initial Soil and Sewage Sludge Analysis:

Sewage sludge was collected from the wastewater and industrial treatment plant of the Industrial Development and Operating Co. (ICDOC) in Jeddah city, Saudi Arabia. Before adding sludge to soil, three random soil samples were collected at depth of 0.0 to 20.0 cm and analyzed. Three random samples of sewage sludge also were analyzed. Soil physical and chemical analysis were done according to Pansu and Gautheyrou (2006) (Table 1 and 2).

The Studied Treatments:

Sewage sludge was amended with 4 concentrations of cadmium (pb), as $3CdSO_4.8H_2O$, 0, 40, 80 and 120 mg/kg added in a rate of 40 t/ha for each plot. Each quantity of sewage sludge was amended with one concentration of Cd and incubated in its soil plot for 15 days before planting. Soil samples were taken from each plot for chemical analysis before planting with the same methods mentioned in the initial chemical analysis. Heavy toxic metals, Cd, Pb, Ni and Cr were determined in plant parts and soil using Inductively Coupled Plasma Atomic Emission spectrometry (ICP-AES).

Experimental Design:

4 X 4 Latin Square design was used in this experiment where the treatments were the 4 Cd concentrations. Plot size was 2 m length and 2.4 m as width.

Table 1: Initial properties of the experimental Soil.

pH	EC (mmos)			Organic Matter (%)		Organic Carbon (%)		Available Macro Nutrients (%)		
								N	P	K
7.72	1.28			0.8		0.47		0.33	0.073	0.86
Total Elements (mg/kg)										
Ca	Mg	Na	Fe	Mn	Cu	Zn	Cd	Pb	Ni	Cr
8220	7678	830	280	160.1	4.67	26.3	0.34	8.1	0.40	0.28
Sandy loam: Silt =13 Sand = 76 Loam = 11										

Table 2: Physio-chemical properties of the used sewage sludge.

pH	EC (mmos)			Organic Matter (%)		Organic Carbon (%)		Available Macro Nutrients (%)		
								N	P	K
6.22	7.1			58.63		34.08		2.1	1.66	2.63
Other Elements mg/kg										
Ca	Mg	Na	Fe	Mn	Cu	Zn	Cd	Pb	Ni	Cr
58260	38263	11037	1035	592	62.6	398	124.6	204.8	70.6	50.4

Data Recorded:

Agronomic Traits:

At harvesting 10 guarded random plants in each plot were tagged and plant root length (cm), plant height (cm) and seed weight/plant (g) were measured besides seed yield/ha(t) determined.

Toxic Metals in Plant Parts:

Plant Samples Collection and Preparation:

At harvesting, three guarded random representative sunflower plants /plot were harvested and separated into root system, shoot system and seeds. The plant samples were washed with tap and then with deionized water to remove any residual soil or dust and dried under room temperature for 10 days then in an oven at 70º C for 24 hours, and separately grinded with an electric mill to fine powder and saved as dried powder to be considered for chemical toxic metals analysis.

Determination of Toxic Metals in Plant Parts:

The grinded powder plant samples were prepared to chemical analysis as described by A.,O.,A.,C. (2000). Plant materials were analyzed for the concentrations of Cd, Pb, Ni and Cr (mg/kg) of dry weight using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

Final Soil Chemical Analysis:

Soil Cd, Pb, Ni and Cr concentrations in each plot were determined after harvesting using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). All safety rules were carried out according to European Cooperation in the Field of Scientific and Technical Research, COST 859, (2008).

Statistical Analysis:

The obtained data were statically analyzed according to the used design and the means were statistically compared using the Revised Least Significant Difference (RLSD) test at $p \le 0.05$ after application the analysis of variance assumptions (El-Nakhlawy, 2010) using SAS program (SAS, 2000).

Results:

Agronomic Traits:

Means of the studied agronomic traits under the 4 Cd concentrations were presented in Table (3). Statistical comparisons between each trait means under the Cd concentrations in the 2 successive seasons using RLSD at $p \le 0.05$ showed that as Cd concentrations increased plant height significantly decreased up to 80 mg/kg in the 2 seasons. No significant differences were detected in plant height between the 80 and 120 mg/kg Cd concentrations. The highest plant height was found under 0.0 mg Cd/kg with values of 109.5 cm and 107.5 cm in 2011 and 2012 seasons, respectively, while the shortest plants produced under the 120 and 80 mg/kg Cd in the 2 seasons with the value of 83.00,81.00 cm under 120 mg/kg in the 2 seasons, respectively and 84.7 and 82.75 cm under 80 mg/kg Cd during the 2011 and 2012, respectively. As for sunflower root length response to increasing Cd concentrations in sewage sludge amended soil, data in Table (3) showed gradually and significantly decreasing in root length from concentration to other in the 2 seasons. Root length ranged from 32.25 cm to 18.5 cm under 0.0 and 120 mg/kg Cd in 2011 season while in 2012 season, root length ranged from 27.50 cm under 0.0 Cd to 15.75 cm under 120 mg Cd/kg. The highest seed weight/plant in the 2 seasons were found under the concentrations of 0.0 and 40 mg/kg Cd without significant difference between each, and the lowest seed weight/plant were found under the 80 and 120 mg/kg Cd in the 2011 and 2012 seasons. Around 50% decreasing in seed weight /plant as Cd concentration increased from 0.0 to 120 mg/kg. Similar response was found for seed yield/ha under the studied Cd concentrations, where the 80 and 120 mg/kg Cd were the highest adversely affected the seed yield/ha in the 2 studied seasons. Dramatically reduction in seed yield/ha was showed under the 120mg Cd/kg comparing with yield under 0.0 mg Cd/kg.

Table 3: Means of plant height (cm), root length (cm), seed weight/plant (g) and seed yield (t/ha) of sunflower under the effect of Cd concentrations added to sewage sludge amended soil during 2011 and 2012 seasons.

Cd (mg/kg)	Means							
	Plant height (cm)		Root length (cm)		Seed weight/plant (g)		Seed yield/ha (t)	
	2011	2012	2011	2012	2011	2012	2011	2012
0.0	109.5[a]*	107.5[a]	32.25[a]	27.5[a]	154.25[a]	93.0[a]	1.65[a]	0.98[a]
40.0	91.5[b]	89.5[b]	26.25[b]	22.25[b]	120.0[ab]	72.25[ab]	1.26[ba]	0.70[ab]
80.0	84.75[c]	82.75[c]	21.5[c]	18.50[c]	103.5[bc]	62.5[bc]	1.08[bc]	0.68[bc]
120.0	83.00[c]	81.00[c]	18.5[d]	15.75[d]	72.75[c]	43.75 [c]	0.76[c]	0.41[c]

* Means followed by the same letter (s) are not significantly different according to R LSD at $p \le 0.05$.

Toxic Metals Contents in Sunflower Plant:

The statistical comparisons between the four toxic metal means in sunflower root, shoot and seed under the effects of the 4 Cd concentrations using RLSD test at $p \le 0.05$ were presented in Table (4).

Root System:

As the results of analysis of variance, Cd concentrations only in the root system were significantly different. Significant increasing in Cd concentrations was found as Cd added into soil increased in the 2 seasons. The lowest Cd concentrations were 41.03 and 44.6 mg/kg under the 0.0 Cd in the 2 successive seasons, respectively increased by around 70% under 40.0 mg/kg Cd, and by 58% under the 80 mg/kg. The highest Cd concentrations were 79.55 and 80.25 mg/ kg under the highest Cd level in the 2 successive seasons, respectively. As for the response of Pb, Ni and Cr in root to increasing of Cd into soil. The results showed no significant differences between their concentrations under the difference Cd levels. Pb concentrations ranged from 50.61 to 52.18 mg/kg in 2011 season, and from 52.23 to 53.97 in 2012 season under the 0.0 and 120 mg/kg of Cd, respectively. Ni ranged from 19.37 to 19.44 mg/kg in the 1st season and from 19.38 to 19.45 mg/kg in the 2nd season and the Cr concentrations ranged from 24.22 to 24.88 mg/kg in 2011 season and from 24.23 to 24.99 mg/kg in 2012 under the 0.0 and 120 mg/kg Cd levels amendment to the soil.

Shoot System:

Sunflower shoot system Cd contents under the 4 Cd levels (Table 4) illustrated significantly increasing in Cd content as Cd in the soil increased. Shoot Cd contents ranged from 31.77 to 70.55 mg/kg under 0.0 and 120.0 mg/kg in 2011 season and from 33.08 to 72.73 mg/kg under the lowest and highest Cd levels, respectively in 2012 season. Shoot Cd contents were less than the root Cd contents under the 4 Cd treatments in the 2 seasons. No significant differences were showed between the concentration means of Pb, Ni and Cr metals in shoot system as affected by Cd in soil.

Seed:

Comparisons of Cd, Pb, Ni and Cr contents in seed under the 4 Cd levels amended to soil (Table 4) showed that the highest Cd concentrations in seed were under 120 mg/kg Cd with values of 39.39 and 38.28 mg/kg in 2011, and 2012 seasons, respectively, while the lowest values were found under the 0.0 Cd levels with means of 26.95 and 27.63 mg/kg in the 2 seasons, respectively. Generally, seed Cd, Pb, Ni and Cr contents were the lowest comparing with Cd concentrations in root and shoot systems.

Table 4: Means of Cd, Pb, Ni and Cr (mg/kg) in root, shoot system and seeds of sunflower under the effect of Cd concentrations added to sewage sludge amended soil during 2011 and 2012 seasons.

Cd. (mg/kg)	Means (mg/kg)							
	Cd		Pb		Ni		Cr	
	2011	2012	2011	2012	2011	2012	2011	2012
Root System								
0.0	41.03^{d}*	44.6^{d}	50.61^{a}	52.23^{a}	19.37^{a}	19.38^{a}	24.22^{a}	24.23^{a}
40.0	58.49^{c}	59.58^{c}	52.25^{a}	54.06^{a}	19.49^{a}	18.5^{a}	24.18^{a}	24.18^{a}
80.0	70.23^{b}	71.43^{b}	51.52^{a}	53.24^{a}	19.51^{a}	19.65^{a}	25.13^{a}	24.28^{a}
120.0	79.55^{a}	80.25^{a}	52.18^{a}	53.97^{a}	19.44^{a}	19.45^{a}	24.88^{a}	24.99^{a}
Shoot System								
0.0	31.77^{d}*	33.08^{d}	45.90^{a}	45.94^{a}	15.27^{a}	15.54^{a}	23.24^{a}	23.96^{a}
40.0	50.44^{c}	47.15^{c}	45.66^{a}	46.15^{a}	15.55^{a}	15.20^{a}	23.71^{a}	23.54^{a}
80.0	63.48^{b}	65.75^{b}	46.01^{a}	46.01^{a}	15.14^{a}	14.77^{a}	23.12^{a}	23.99^{a}
120.0	70.55^{a}	72.73^{a}	45.26^{a}	44.85^{a}	15.48^{a}	15.12^{a}	23.55^{a}	34.58^{a}
Seeds								
0.0	26.65^{d}*	27.63^{d}	20.60^{a}	21.78^{a}	13.18^{a}	13.64^{a}	14.55^{a}	14.21^{a}
40.0	28.08^{c}	30.28^{c}	21.06^{a}	21.35^{a}	13.13^{a}	13.27^{a}	14.98^{a}	14.71^{a}
80.0	34.74^{b}	34.93^{b}	20.51^{a}	20.58^{a}	12.54^{a}	13.39^{a}	14.94^{a}	14.68^{a}
120.0	39.39^{a}	38.28^{a}	19.88^{a}	20.70^{a}	12.24^{a}	13.30^{a}	14.09^{a}	14.24^{a}

* Means followed by the same letter for each metal in each plant part are not significantly different according to RLSD at $p \leq 0.05$.

Toxic Heavy Metals in Soil:

Toxic metals (Cd, Pb, Ni and Cr) were determined in soil after incorporating Cd with sewage sludge amended soil before planting, also after harvesting sunflower plants.

Mean Comparisons:

Data in Table (5) showed that Cd concentrations in soil under the 4 Cd treatments after harvesting ranged from 42.06 and to 43.68 mg/kg under 0.0 Cd in 2011 and 2012 seasons up to 43.34 and 45.35 mg Cd/kg under 120.0 mg Cd/kg treatments without significant differences between the 4 Cd treatments in each season comparing with 97.2 and 104.96 under 0.0 Cd in 2011 and 2012, respectively to 153.76 and 166.05 under 120 mg Cd/kg in the 2 seasons, respectively before planting. Also no significant differences were found between the concentration means of Pb, Ni and Cr after sunflower harvesting in the 2 seasons.

As a positive effect for using sunflower plants as a phytoremediant for higher levels of the toxic metals (Cd) in the soil, the results of Table (5) showed that Cd concentrations decreased by 58% , 62% , 68 % and 72% from the Cd concentrations in the soil before planting under 0.0,40.0,80.0 and 120.0 mg/kg Cd incorporated into soil as a result of growing sunflower for one season in the contaminated soil with Cd.

Comparing Cd contents in sunflower plants after application of the phytoremediation technology with the phytotoxic standards Table (6) , it was showed that the Cd contents in the aerial plant parts were more than the phytotoxic limits, hence, the recommendation is removing the phytoremediator plant according to COST (2008) without using in feed of animal or food of humans or using in edible industrial products.

Table 5: Means of Cd, Pb, Ni and Cr (mg/kg) in soil before planting and after harvesting sunflower under the effect of Cd concentrations added to sewage sludge amended soil during and after phytoremediation during 2011 and 2012 seasons.

Cd (mg/kg)	Means (mg/kg)															
	Cd				Pb				Ni				Cr			
	Before planting		After harvesting		Before planting		After harvesting		Before planting		After harvesting		Before planting		After harvesting	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0.0	97.2[d]*	104.96[d]	42.06[a]*	43.68[a]	133.82[a]	134.36[a]	75.96[a]	76.54[a]	72.85[a]	73.68[a]	29.19[a]	29.78[a]	82.17[a]	82.30[a]	42.75[a]	43.58[a]
40.0	110.67[c]	119.45[c]	42.61[a]	44.25[a]	133.99[a]	134.92[a]	75.84[a]	75.67 a	74.29[a]	75.21[a]	29.09[a]	29.47[a]	82.22[a]	82.35[a]	42.78[a]	42.66[a]
80.0	134.67[b]	145.43[b]	43.19[a]	44.85[a]	134.32[a]	135.28[a]	76.21[a]	75.14[a]	73.46[a]	74.34[a]	28.11[a]	29.76[a]	83.77[a]	84.08[a]	43.74[a]	43.66[a]
120.0	153.76[a]	166.05[a]	43.34[a]	45.35[a]	134.64[a]	134.55[a]	76.63[a]	75.65[a]	73.7[a]	74.58[a]	28.71[a]	29.37[a]	83.98[a]	84.31[a]	43.10[a]	42.63[a]

* Means followed by the same letter for each metal are not significantly different according to RLSD at $p \leq 0.05$.

Comparing soil contents from the 4 toxic elements after the application of phytoremediation technology with sunflower plants, with the phytotoxic limit standards, Cd was higher than the standard limit of toxicity. Accordingly, another plant phytoremediation cycle(s) must be done until the Cd concentration in the polluted soil reach the safety limits.

Table 6: Range of the toxic elements in the studied crop plant parts and soil and the photoxicity standards.

Cd	Plant and soil heavy metal detected concentrations (mg/kg)				Standards	
	Root	Shoot	Seed	Soil	Plant *	Soil**
	41.03-80.25	31.77-72.73	26.65-39.39	42.06-45.35	5-30	3

* Kitagishi (1981), Chaney (1989) and WHO/FAO. (2007),

** European Union Standards (EU,2002).

Discussion:

Decreasing in sunflower traits as increasing the Cd amendment to the soil might been due to the adversely effects of the highest Cd concentrations absorbed by sunflower roots and its translocation into shoot system, accordingly decreased the efficiency of dry matter synthesis and leaf photosynthesis, besides it inhibited the seed filling and decreased the dry matter accumulation in seed and finally, the plant produced small seed number and size, which reflected in low seed yield. These findings were confirmed with the results obtained by Prabhahar *et al.,* (2011) , Babak *et al.,* 2008 , Chhotu *et al*., (2008), Dinesh *et al*., (2007) and Madejon *et al*., (2003).

Cd concentrations accumulated in sunflower shoots were lower than those reported by Maxted *et al*. (2007) which ranged from 131 to 425 mg/kg under the effect of different mobilizing agent and different treatment concentrations of cadmium. Concerning the seed content of cadmium, it was the lowest part and the study results were similar with the results obtained by Andersen and Hansen (1984), Mathew *et al*.,(2002) and Murakami *et al*., (2008). Using sunflower to clean the polluted soil with heavy metals especially Cd confirmed with the results obtained by Zou *et al*., (2008). The sunflower studied agronomic traits negatively responded to the increasing of Cd concentrations in their soil before planting. This adverse effect on yield and yield components might been due to the adversely effects of the highest accumulated Cd on the photosynthetic rate, inhibition the biosynthesis of the plant metabolites and effects on the cell membrane and cell pressure adjustment. On the other hand, sunflower plants absorbed high Cd amount from the soil and translocate into shoot and seed, causing high concentrations of Cd accumulation in root, shoot and/or seed. These results might been due to the uptake, translocation and metabolism mechanisms of this species and the interaction with the soil pollution. These results were confirmed with Ewais (1997), Gangrong and Qingsheng (2009), Prabhahar *et al*., (2011) and Rana *et al*., (2011).

Conclusion:

Sunflower can be used as a phytoremediator for cadmium removal from the high polluted environmental sites. Sunflower could remove around (57% to 72%) Cd from the initial soil concentrations of Cd metal. This technology is low cost effective and ecofriendly for pollutants removal, such as heavy metals from contaminated soil, sludge, sediment and/or ground water. The phytoremediator sunflower plant must be not used in feed and food. Standards procedures must be used to recycle the phytoremediator plants.

ACKNOWLEDGMENT

This work was funded by the King Abdulaziz City for Science and Technology under grant no. (A-S-10-0109). The author therefore acknowledge with thanks to KACST technological and financial support.

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