Annual Halophyte Chenopodium Botrys Can Phytoextract Cadmium from Contaminated Soils

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ABSTRACT

Phytoextraction technology has proven to be an efficient and innovative method to remediate soils contaminated with heavy metals. One major issue in phytoextraction is to select suitable hyperaccumulator plants for heavy metals. The objective of this study was to find out whether halophyte *Chenopodium botrys* L. can be used as a cadmium hyperaccumulator. Consequently, an extensive experiment was carried out to evaluate the phytoextraction abilities of one halophyte chenopodium species. The experimental soils were contaminated with Cd at 0, 5, 10, 20, 40, 60 and 100 mg kg^{-1} in the form of cadmium chloride. The results indicated that the Cd uptake was increased with cadmium contents in soil and the maximum accumulation has occurred in roots. The results also indicated that the average total Cd accumulated in plant (including root and shoot) was 108.5±64.2 mg kg^{-1} Cd and average total Cd removal by shoots was 120±44 g ha^{-1} (dry weight), with the average Cd level of 32 mg kg^{-1} in soil. The S/R ratio is more than one under 18.25 mg kg^{-1} soil Cd content. It shows that at moderate contamination (less than 18.25 mg kg^{-1}soil Cd content) the mobility of Cd in plant is much more than higher soil Cd concentrations, whereas most hyperaccumulating plants have lower S/R ratio than one. A high accumulation of cadmium in this plant shows that *chenopodium botrys* L. can be considered as a hyperaccumulator for cadmium.

KEY WORDS: Cadmium; Phytorextraction; *chenopodium botrys* L., halophyte.

1. INTRODUCTION

Presence of heavy metals in environment is a great concern because they cannot be reduced by degradation process (lasat, 2000). Cadmium is a toxic heavy metal for both plants and humans. Cadmium contamination in soils has become a global concern because it can be easily transferred to human food chain (Liu et al. 2009). This is particularly a major concern for developing countries with intensified industrial activities. Due to its health risks, Codex Alimentarius Commission, the international food standards organization, proposed a 0.1 mg kg–1 Cd on dry weight limit for cereals, pulses and legumes (Harris and Taylor, 2001).

Many agricultural soils in Iran are slightly or moderately contaminated by Cd due to long-term use of phosphatic fertilizers and sewage sludge application (malakouti and Homae, 2004). Increased Cd levels were also found in the surface soils near the metal processing industries all over the country (Abaspour et al. 2005). Therefore, it is important and urgent to develop methods to clean up Cd-contaminated soils. Remediating contaminated soils and waters require removal of toxic metals especially cadmium from contaminated areas (Henry 2000). Current technologies are soil excavation and either land filling or soil washing, followed by physical or chemical separation of the contaminants. The cost for physical or chemical soil clean up is very high and largely depends on the contaminants, soil properties, and site conditions (lasat, 2000).due to such high cost; there is an urgent need for less expensive clean up technologies. Phytoremediation, which means Plant-based environmental remediation technology, has been widely used in recent years for clean up of heavy metals from contaminated sites (Salt et al. 1995). Phytoremediation is evolving as an environment friendly, cost-effective alternative to high-energy and high-cost conventional methods (Henry2000). In addition, phytoremediation avoids landscape disturbance and preserves the ecosystem because it remediates the soil *in situ,* (lasat, 2000). In this technology, the so-called hyperaccumulative plants are used to remediate the contaminated soils.

The term "hyperaccumulator" was first introduced by Brooks et al. (1977) which refers to plants that can accumulate high concentration in their body. Beside this, there are several definitions for metal

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hyperaccumulation (Baker and Brooks, 1989; Baker et al. 2000; Lasat, 2000; Baker and Whiting, 2002). Shoot concentration defining hyperaccumulation being 0.01% (w/w) for cadmium (Baker et al., 2000) or capable to accumulate metals at levels 100-fold greater than those typically measured in common nonaccumulator plants .Thus a cadmium hyperaccumulator should be concentrate more than 100 ppm Cd on its dry matter (Lasat, 2000).

One of the subdivision methods of phytoremediation is the so-called phytoextraction which is a commercially feasible technology. To remove contamination from the soil, this approach uses plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass. The most important factor of phytoextraction is selecting suitable species to remove heavy metals from soil.

There are two main phytoextraction strategies proposed to clean up toxic metals from contaminated soil. The first is the use of metal hyperaccumulator species (Baker et al. 1994) and the second is the use of high biomass and expanded root system plants (Ghosh and Singh, 2005 & Solhi et al. 2005). Since 1998, Brassicaceae was the only family which introduced as a cadmium hyperaccumulator (Robinson et al. 1998). Although cadmium hyperaccumulator plants such as brassicaceae family have been demonstrated to be potentially useful in soil remediation, their low biomass production limits their phytoextraction ability (Zhuang et al. 2007). For example, *Thlaspi caerulescens*, which can contain more than 1% Zn and 0.01% Cd in its shoots, is one of the best candidates for phytoextraction of metal-contaminated soils (Baker et al., 1994; McGrath and brooks, 1998). Greger (1999) has reported that Cd-removal by *Thlaspi caerulescens* is 35 ± 11 g ha^{-1} yr^{-1} and has recommended it for remediation of Cd-contaminated soils. But in conducted researches it is reported that dry matter biomass of *T. caerulescens* is very low. Very different values in the literature have been reported for dry mass potential of *T. caerulescens*. Robinson et al. (1998) estimated that dry biomass production of this species is about 2.6 t ha^{-1}. Keller and Hammer (2005) reported 0.9 ± 0.2 t.ha^{-1} dry biomass, and in field trials Kayser et al.(2000) also found it less than 1t.ha^{-1}. Vassilev et al. (2002) pointed that this species is not suitable for phytoextraction due to its low biomass and rosette characteristics. Ernst, (2005) also reported that *T. caerulescens* is not a suitable candidate for phytoextraction because of difficulties for its mechanical harvesting. Furthermore, it was mentioned that *T. caerulescens* has low resistance to hot and dry environments (Kayser et al., 2000). High-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) has been suggested instead of *T. caerulescens* (Brown et al., 1995). those plant species that have both high biomass production and can tolerate and accumulate high levels of contaminants are rated to be ideal for remediation. Such combinations are rarely possible since most of the hyperaccumulators are small and slow growing (Pulford and Watson, 2003). Keller et al. (2003) recommended that the higher biomass produced by crops could compensate their lower Cd contents when compared with hyperaccumulators but producing lower biomass.

In the other hand, as a general rule, native species are preferred to exotic plants, which can be invasive and endanger the harmony of the ecosystem. Iran has one of the largest collections of halophytes in the world which can tolerate high salinity levels. Some halophyte grasses such as saline grass has high biomass and expanded root systems . the main objective of this study was to investigate if such a salt tolerant species can be regarded as hyperaccumulators to remediate cd-contaminated soils. Consequently the halophyte *chenopodium botrys* was selected to find out its potential to phytoextract Cd from contaminated soils.

2. MATERIAL AND METHODS

An extensive experiment was established and conducted to evaluate the capability of halophyte *chenopodium botrys* for phytoextracting cd from contaminated soils. The experiment was conducted in a set of 28 microlysimeters were including seven treatments, each with four replicates. The lysimeters with 20cm high and 20cm diameter were carefully packed with sandy clay loam soils. All the experimental lysimeter were provided with drainage outlets at the bottoms, having a collector dish under the lysimeters to collect the drainage water. The balk density of the initial uncontaminated soil was 1.33 g/cm^3 which were applied for all lysimeters during the packing process. The soil Particle size distribution was determined by hydrometer method (Gee and Bauder, 1986). The experimental soil texture was sandy clay loam (50% sand, 26% silt and 24% clay) with initial pH of 7.98 (1:2 soil: water), electrical conductivity (EC) of 6.71 dS m^{-1} (1:2 soil: water), organic carbon of 0.7%, calcium carbonate of 7.5% and Cd concentration of 1.23 mg kg^{-1}. The latter was determined by the procedure proposed by Jacson (1975). The experimental soils were contaminated by implicating Cd concentrations of 0, 5, 10, 20, 40, 60 and 100 mg kg^{-1} soil, in the form of cadmium chloride (CdCl$_2$). No additives such as chelates or fertilizers were applied

to the experimental soils in order to resembling Cd uptake in natural conditions. The CdCl$_2$ was first dissolved in distilled water and then thoroughly sprayed on the 9 kg soils for each pot. The contaminated soils were carefully compacted in four cm increments at 1.33 g/cm^3 bulk density. Appropriate amount of water was added to the experimental lysimeters to hold the water content at field capacity. The lysimeters were then left at field capacity for eight weeks to reach equilibrium with the applied cadmium. Thereafter, a seedbed was prepared and seeds were carefully seeded in the experimental pots. The experimental plants were irrigated by a tape irrigation system to avoid any drought stress. The drainage water was carefully collected and re-applied to the pots in order to prevent any Cd loss, resulting from soil leaching. This way, the cadmium taken up plants was the only source of cadmium removal from the experimental soils. when plants were fully developed, three representative plants were remained in each pot, harvesting the rest. This was done because *chenopodium botrys* L. has an expanded root system as well as high biomass. The plants were harvested at maturity growth stages 120 days after the seeding. The plants daily transpiration was measured not only to record the transpired water, but also to calculate the required applied water for the next irrigation interval. The fresh and dry weights of plants were obtained to evaluate the effect of cadmium on *chenopodium botrys* L growth and produced biomass. After harvesting, the roots were also separated from the soil and collected by soaking soil of each pot for 3 days in some tubs. The collected plants samples were washed with distilled water, oven-dried in oven at 70 °C for 48 hours. The dried samples were milled and digested in concentrated HNO$_3$–HClO$_4$-H$_2$SO$_4$ (40-4-1) acids and analyzed for Cd by ICP apparatus (JY138 ULTRACE). Some soil samples were also taken from each lysimeter. These collected soil samples were air dried, mixed, passed through 2 mm sieve to measure their cadmium contents in both total and soluble forms. These were done by following the procedure proposed by Gupta (2003), using atomic absorption apparatus (SpectrAA-200 –Varian). The designated statistical experimental design was randomized block design, having four replicates for each treatment. After collecting the required data, analysis of variance (ANOVA) was performed using SPSS software.

3. RESAULTS AND DISCUSSION

Some selected physical and chemical properties of the experimental soils and the applied irrigation water are presented in Table 1. The initial concentration of soil Cd, Perimeter shoots‹ fresh and dry weights of grown *chenopodium botrys* L. are also presented in table 2. The presented results in this table indicates that the fresh yield of *chenopodium botrys* was ranged from 2866 to 1223, g m^{-2} with Cd levels varying from 1.57 to 94.6 mg kg^{-1}. The produced dry shoot weight was 2.8 t ha^{-1} in uncontaminated treatment. As can be seen, the fresh plants and dry matter yields of *chenopodium botrys* L. was affected up to 4.75 mg Cd kg^{-1} soil. Figure 1 shows the relation between the transpired water and yield as a function of soil Cd concentration. These curves explain that the relative transpiration and the relative yield are decreased by increasing soil Cd concentrations. Figure 2 shows the Cd concentration in roots and shoots of *chenopodium botrys* L. at different soil Cd contaminated levels. The Cd concentration in both roots and shoots were increased by increasing soil Cd content (Fig. 2). The Cd concentration in roots was more than that of shoots in soil Cd level above 18.25 mg kg^{-1} in most cases and after that the accumulations followed the order root > shoot (Fig. 2). This observation is in agreement with the findings of Clarke and Brennan (1980) where reported by increasing soil Cd, the Cd concentration in all tissue fractions increases significantly. Generally, the Cd followed the roots > leaves > stems sequence for *populous tremuloides* (Clarke and Brennan, 1980).

Table 1 Selected physical and chemical properties of the experimental soil and water

Soil Parameters	Unit	
Soil texture		Sandy Clay Loam
pH(1:2)		7.6
Bulk density	(gr/cm^3)	1.334
EC(Soil)	(dS/m)	6.71
Initial Cd (soil)	mg kg^{-1}	1.22
N	%	0.07
CaCO$_3$	%	7.5
OM	%	0.7
Plant biomass(Shoot DW)	Kg ha^{-1}	4380
EC(Applied water)	(dS/m)	1.2
Initial Cd(Applied water)	mg kg^{-1}	0.013

The reductions in plant yield were 53%, 72%, 60%, 75% and 28% in fresh shoots, fresh roots, dry shoots, dry roots and perimeter shoot at Cd concentration of 94.75 mg kg^{-1} (Table 2). The Cd$_{50}$ value, Cd at which the yield is reduced by 50%, for *Chenopocium botrys* shoots was 78 mg kg^{-1}. In case of cucumber (*Cucumis sativus* L.), a crop earlier proposed for phytoremediation by An et al (2004), an effective concentration of Cd for 50% reduction in shoot was observed to be 88 mg kg^{-1} soil which is very close to our findings.

The injury symptoms in *chenopodium botrys* were clearly observed only at 94.6 mg kg^{-1} of soil cadmium contamination. When the Cd concentration was 94.6 mg kg^{-1}, the leaf chlorosis was observed and all leaves especially young leaves became smaller. The injury symptoms were obviously more extensive at early growth stages than the middle growth stage.

Table 2: Initial concentration cadmium (soil), Perimeter, wet and dry matter of *chenopodium botrys*

Initial(SOIL)		Perimeter (Cm)	Dry matter yield (gr/m^2)		Fresh plant(gr/m^2)	
Applied	Total Cd	shoot	Root	shoot	wet root	wet shoot
0	1.575	113.75	34.8	438.1	168.7	2887
5	4.75	108.7	32.7	411	168	2753.6
10	8.68	99.4	28.7	351	160.3	2465.1
20	16.25	102.1	19.5	321.1	129.6	2328.2
40	35	98	17.3	268.1	101.7	2183.7
60	54.26	90.5	14.1	187.3	94.3	1988
100	94.75	81.6	12	130.5	45.8	1222.4
Mean	30.75	99	22.7	301.	124.	2261.8
SD	33.9	9.4	9.7	64	46.1	435.4

One important issue for Cd phytoextraction is the amount of cadmium removal by plants from the contaminated soils. Figure 2 shows the Cd concentration in roots and shoots of *chenopodium botrys* at different soil Cd levels. as can be seen in this figure, the Cd concentration in roots and shoots increased by increasing soil Cd concentration . The Cd concentration in roots was more than that of shoots in soil Cd level above 18.25 mg kg^{-1} in most cases. It is important to notice that some heavy metals such as Pb, Cr and Cu are usually accumulated in root tissues, whereas Cd can easily translocated to aerial tissues (Polford and Watson, 2003). Figure 3 shows the relation between root/shoot ratio and total soil Cd content (mg kg^{-1}). As can be seen, the S/R ratio is more than 1 under 18.25 mg kg^{-1} soil Cd content. It shows that at moderate contamination (less than 18.25 mg kg^{-1} soil Cd content) the mobility of Cd in plant is much more than higher soil Cd concentrations, whereas most hyperaccumulating plants have lower S/R ratio than 1. In this study, the S/R ratio was decreased by increasing Cd concentration in root as a result of increasing soil cadmium content (Fig. 3).

Figure 4 represents the total Cd removal (g ha^{-1}) by plant at different soil Cd concentrations. It is obvious that total Cd removal increased by increasing soil Cd content with Cd level from 1.58 to 18 mg kg^{-1}. In 18 mg kg^{-1} soil Cd contamination, which is a moderate soil Cd contamination, the Cd removal by shoots was 178.25 mg kg^{-1}. Above 18 mg kg^{-1} soil, the Cd removal was decreased because of decreasing shoot biomass and yield. The rate of cadmium removal from soil during the growth period was equivalent to the area under the curve in Fig. 4. These Cd-removals are comparable to plants such as Thlaspi (35 ± 11 g ha^{-1} yr^{-1}) that is being recommended for remediation of Cd-contaminated soils (Greger, 1999). Zhuang et al (2007) reported that *R. crispus*, could extract 160 gr Cd per hectare when the total cadmium on soil was only 7.2 mg kg^{-1}. However, our results indicate that the Cd removal at 18.25 mg kg^{-1} soil Cd was 178.2 g ha^{-1}.
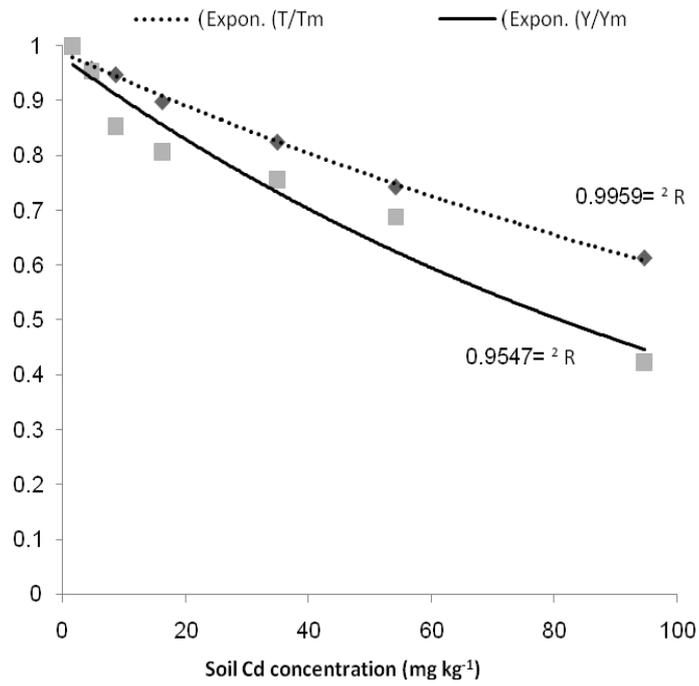
Figure 1, Relative transpiration and yield as function of soil Cd concentration
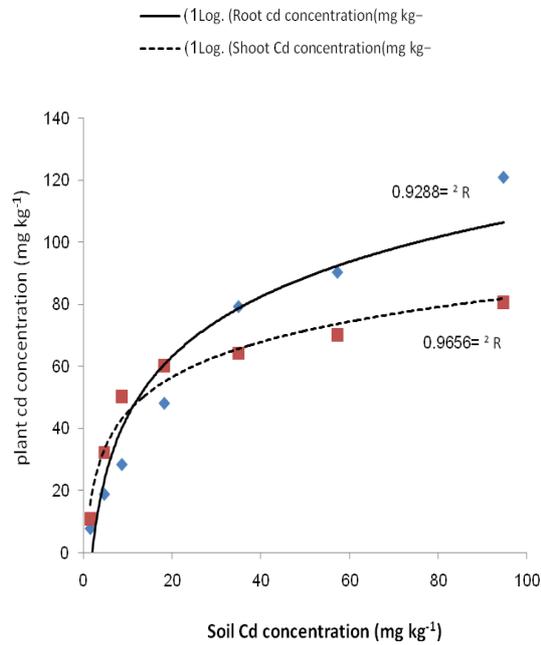

Figure 2, the relation between Shoot-root Cd content and soil Cd concentration

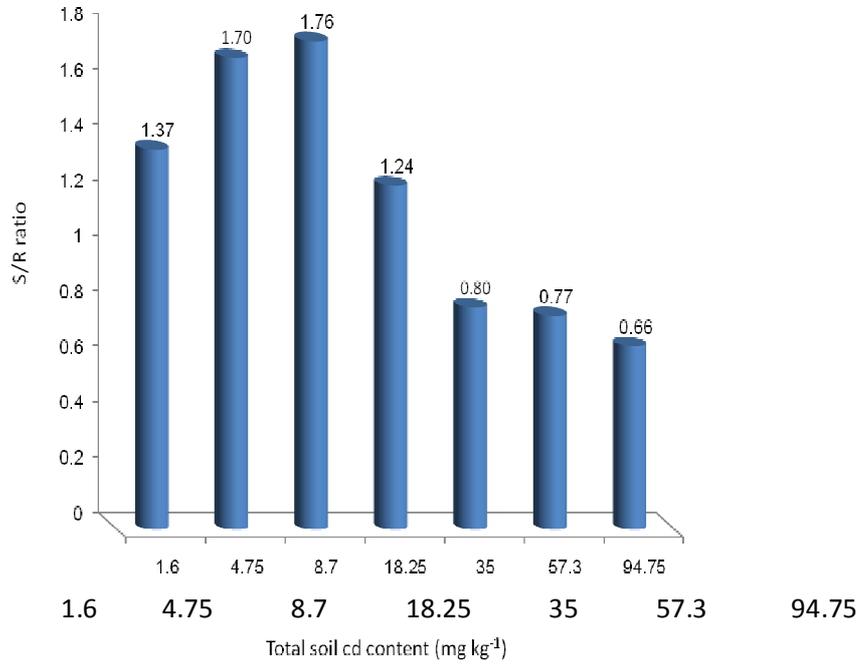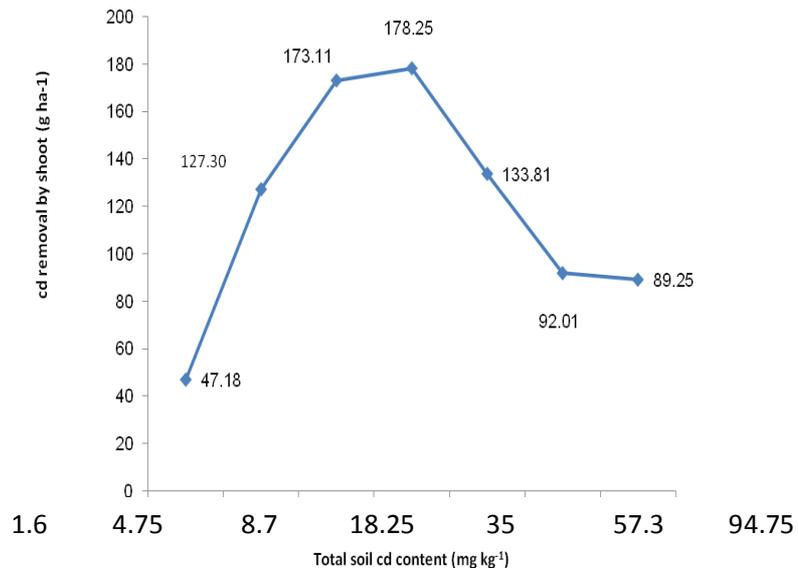**Figure 3, Shoot to root(S/R) accumulated Cd retio**

Figure 4, Cd removal by shoots (g ha^{-1})

4. CONCLUSION

The main idea behind this research was to find out whether some halophyte species as environmental resistance stress plants can also take up large amount of cadmium from contaminated soils. For this purpose, the halophyte *chenopodium botrys* as a high resistance environmental stress species which could resist dry climate and very high soil salinity was selected. The overal results obtained in this study indicate that halophyte *Chenopodium botrys* has the ability to take up and accumulate Cd both in its roots and shoots. Due to high root/shoot Cd ratio of *chenopodium botrys* L., which was larger than 1 in moderate Cd concentration and also because of high amount of Cd concentrations and high cadmium removals (132.1±44 g.ha^{-1}), we report that this species can be regarded as a Cd hyperaccumulator plant. Thus, this plant can be used to phytoextraction cadmium from the contamination soils.

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