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Phytoremediation of Cd contaminated soils by ornamental cabbage (*Brassica oleracea*) species

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Abstract

Soil contamination by cadmium (Cd) is the main problem in the industrialized and developing countries. Phytoremediation is a simple and inexpensive technique to reduce the environment contamination. The present study evaluates the potential of ornamental cabbage varieties in removal of Cd from contaminated soils. Treatments were cultivars at two levels (songbird red and songbird white) and Cd concentrations at four levels (0, 10, 20 and 30 mg/kg). The experiment was laid out as a factorial based on randomized complete block design using three replicates. Roots and shoots Cd content, anthocyanin, proline, chl.a, chl.b, chl.a + b contents were measured. Results showed that the amount of Cd in shoots and roots of both species was increased with the increasing Cd concentrations. Higher accumulation of cadmium in roots and shoot of white cabbage was detected in comparison with red cabbage. The amount of chlorophyll and proline in white cabbage were not affected by different concentrations of cadmium. Proline production was found to be higher in red cabbage compared to white cabbage under cadmium treatment. Thus, two cabbage species can be used for phytoremediation of Cd-contaminated soils. However, if these species are used as hyper-accumulate, they should be burn after harvesting like dangerous waste.

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Introduction

Cd is considered as the most serious pollutant of the modern age (Singh *et al.*, 2009). Cd concentrations above the threshold limit values have been found to be carcinogenic, mutagenic and teratogenic for a large number of animal species (Degraeve, 1981). Cd has also been implicated as an endocrine disruptor (Awofolu, 2005). Although, many cleanup technologies have been developed to treat contaminated soil such as Mechanical and chemical methods, these technologies are usually expensive and soil disturbing. Consequently, a biologly-based emerging technology is gaining the attention of both soil remediation scientists and the general public—phytoremediation. Phytoremediation is a novel, less expensive, efficient, environment-andeco-friend lycleanup method with good public acceptance (Saier and Trevors, 2010; Revathi *et al.*, 2011). According to Pilon-Smits (2005), phytoremediation is natural processes by which plants and their microbial rhizosphere organisms sequester, degrade or immobilize pollutants for cleaning soils and water matrices contaminated with heavy metals or organic pollutants. Plant species vary significantly in the ability of accumulating metals from contaminated soils (Garbisu and Alkorta, 2001).

According to a study conducted on Cd uptake and accumulation in different parts of forage forages, it was found that Cd uptake by plants in samples with concentrations 50 and 100 ppm of cadmium was higher. The most uptakes occurred in clover. The concentration of Cd in alfalfa was higher in shoots than in roots. Sorghum had little ability to absorb cadmium ions (Izadiyar and Yargholi, 2010). In another study, Mohebbi (2012) evaluated capability of heavy metals absorption by corn, alfalfa and sunflower intercropping date palm who found sunflower is more suitable than corn and alfalfa for the removal of cadmium. Corn, with or without date palm was more suitable for the removal manganese than alfalfa and sunflower in either mono or dual culture. The present study was designed to examine the phytoextraction of Cd from simulated polluted soil by two varieties of ornamental cabbage.

Materials and methods

The present study was conducted during 2012-2013 in the greenhouse of the Department of agronomy , Agricultural and Natural Resources Campus, Islamic Azad University, Karaj, Iran(35° 45′ N, 51°6′ E, and 1313 m above sea level). The experiment was laid out as a factorial based on randomized complete block design using three replicates. Treatments were cultivars at two levels (songbird red and songbird white*)* and Cd concentrations at four levels (0, 10, 20 and 30 mg/kg). Cabbage seeds were sown in plastic trays and transferred to the appropriate pots (13×9.5×12.5) at 4-5 leaf stage. The pots were irrigated according to field capacity conditions by distilled water.

Cd was added in to the soil the form of cadmium nitrate (Cd $(NO_3)_2$). At the end of the growth stages, morphological characteristics were measured in two stages with a one month interval. These factors were leaf length, leaf width, number of leaves, plant diameter and length of the crown. Shoot fresh weight, root fresh weight, root size, root dry weight, shoot dry weight, the concentration of Cd in shoots and roots were measured at second stage. The samples were oven-dried at 72°C for 48 h and then weighed.

Cd accumulation in roots and shoots of cabbage

To analysis Cd in plants, each plant part was washed in order to remove dust and soil particles. Then, plant parts were dried in an oven at 80°C for 48 hours. 1.0 g sample of the plant part was taken into a crucible. Then, the crucibles were kept into an oven with a temperature of 480 °C for 5 hours to be completely incinerated. The best ash is achieved when the material into crucible are white after 5 hours. The ash obtained was taken into a 100 mL Beaker and 5ml of 0.1 M HCl was added to it. The beaker containing the solute was heated on bain marie for 10 minutes until the digest became clear. The digest was cooled and then filtered through a Whatman filter paper and the

filtrate was transferred to a 100 volumetric flask. The balloon was filled with distilled water to a volume of 100 ml. The filtrate was used for the analysis of Cd by Atomic Absorption Spectrophotometer.

Measurement of anthocyanin

Anthocyanin was extracted and its concentration determined by the method of Wagner (1979) using acidified ethanol (ethanol: HCl 99: 1 v/v). 0.1 g of fresh leaf was homogenized in 10 ml acidified methanol and the content was kept at 25°C for 24 h in the dark conditions. The homogenate was centrifuged at 4000 rpm for 10 min and the absorbance of each supernatant was determined at 550 nm. The extinction coefficient of 33,000 (mol-1 cm-1) was used to calculate the amount of total anthocyanin and it was expressed as µ mol g-1 FW.

Measurement of proline

Proline was extracted and its concentration determined according to Bates *et al.* (1973) method. 0.5 g was homogenized in sulfosalicylic acid, and the homogenate was centrifuged at 3000 × g for 20 min. The supernatant was treated with acetic acid and acid-ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined.

Measurement of chlorophyll a, b and a + b

Chlorophyll a and b were estimated based on Brad, (1970) method. Accordingly, 0.1 g of fresh leaves and 0.5 g of sodium carbonate were mixed into a porcelain mortar. Then, 10 ml of pure acetone (100%) was gradually added and the contents were immediately transferred into a glass centrifuge tube and centrifuged at 2500 rpm for 2 min. A volume of 0.5 ml of solution and 4.5 ml of 80% acetone was injected into the spectrophotometer and Chlorophyll a and Chlorophyll b were detected at a wavelength of 663 and 645 nm, respectively. Absorbance data were placed in the formula and chlorophyll a and b contents (mg l -1) were separately determined.

Chlrophyll a= (0/0127×A663) - (0/00269×A645)

Chlrophyll b= (0/0229×A645) - (0/00468×A663)

Chlrophyll a+b= (0/0202×A643) + (0/00802×A663)

In which, chl.a, chl.b and chl.a + b are chlorophyll a and b contents and total chlorophyll in term of mg ml $^{-1}$, respectively. A is the rate of light absorbed by extract at the corresponding wavelengths.

Statistical Analysis

All data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2002). Treatment means were separated using Duncan test at $P < 0.05$. Graphs were plotted using Excel software.

Results and discussion

Cadmium content

A significant difference was found between white and red cabbage in terms of Cd content in shoots at % 1 level (Table 1). Higher accumulation of cadmium in roots and shoot of white cabbage was detected in comparison with red cabbage (Fig. 1 and Table 2). Gorska (2011) studied the potential of maize and pigweed contaminated soils with heavy metals lead, cadmium and zinc in greenhouse and outdoor, who found the average rate of metal uptake by pigweed was higher than in maize.

The amount of Cd in shoots and roots of both species was increased with the increasing Cd concentrations. An analysis of metal in roots and shoot of the species showed that more Cd was accumulated in the root when compared to shoot. Species tend to absorb cadmium and also translocation of Cd from root to shoot was increased with increasing Cd levels, and therefore the contents of cadmium in roots and shoots were increased. These findings are in agreement with those of Khodaverdiloo and Homaee (2008), who reported there was a direct relationship between Cd concentrations in the shoots and soil so that Cd accumulation in the root was higher than shoot with the increasing Cd concentrations in the soil. Mohamadipour and Kapourchal (2012) found that Cd concentration within the roots and shoots was increased to 19.3 and 34 times, respectively with cadmium concentration.

Cadmium accumulation in the white and red cabbage was different under cadmium concentrations. The maximum concentration of Cd in shoot and roots of white cabbage occurred at 30 mg Cd /kg. No accumulation of Cd in root and shoo of species occurred under control treatments (Fig. 4 and 5). These results are inconsistent with Aghaz and Bandehagh (2013) findings who reported no interaction was found among dill *(Anethom graveolens)* ecotypes in term of lead uptake.

Table 1. Analysis of variance of cadmium for roots and shoots of white and red cabbage.

SOV	df	F	
		Root	Shoot
Rep	2	0.67	1.37
Cultivar	1	6.41*	10.57**
Cd concentration	3	152.87**	104.37**
Cultivar* Cd concentration	3	11.4**	6.96**
E	14	14	
CV (%)		9.22	11.35

* and ** significant at 1% and 5%, respectively.

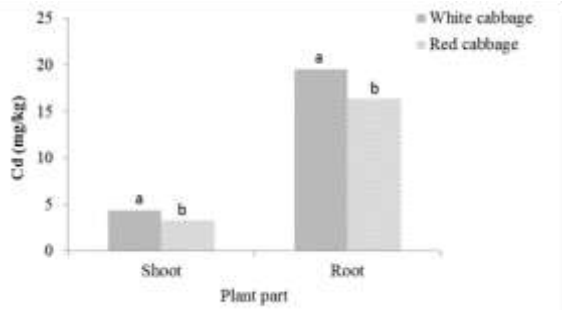

Fig. 1. The concentration of cadmium in white and red cabbage.

Table 2. Mean comparison of effect of cadmium on root and shoot weight of cabbage varieties.

Treatments	Average	
	Root	Shoot
a1	4.361^{a}	19.243^{a}
a2	3.377^{b}	16.36^{b}
b4	7.519^{a}	34.31^{a}
b3	4.406^{b}	20.038^{b}
b2	3.551^{c}	16.857^{c}
b1	0^{d}	0^{d}
a1b1	0^{d}	0^{e}
a1b2	3.572^{bc}	11.31^{d}
a1b3	4.679^{bc}	16.618^{cd}
a1b4	9.194^{a}	37.511^{a}
a2b1	0^{d}	0^{e}
a2b2	3.53^{c}	22.404^{bc}
a2b3	4.134^{bc}	23.459^{bc}
a2b4	5.844^{b}	31.109^{ab}

a1= white cabbage, a2= red cabbage; b1, b2, b3 and b4=0, 10, 20 and 30 mg/kg Cd, respectively.

Means within each column sharing the same letter(s) are not significantly different based on Duncan test at p = 0.05.

Anthocyanin chlorophyll and proline contents

The effect of different concentrations of cadmium on anthocyanins and proline contents of red cabbage was different (Table 3). Mean comparison showed that proline content of red cabbage was increased with the increasing Cd concentrations in the soil (Table 4). The content of anthocyanin in red cabbage was decreased at 10 and 20 mg Cd/kg, while it was increased at 30 mg Cd/kg (Table 4). Stress factors such as biotic and abiotic stress at the cellular level causes changes in the metabolic pathway. The type and content of proteins required for the plant change under stress conditions. The resistance varieties show a defensive mechanism under Ion toxicity and cabbage species biosynthesis higher levels of anthocyanin. These finding these findings are in contradiction to Aghaz and Bandehagh (2013) findings who reported the contents of Chlorophyll a, total chlorophyll and anthocyanin in dill *(Anethom graveolens)* plants were increased under Pb exposure but the chlorophyll b and carotenoids contents were decreased.

Table 3. Analysis of variance of anthocyanin and proline contents in red cabbage.

SOV	df	F	
		Anthocyanin	Proline
Rep	2	0.63	4.25
Cd concentration	3	33.42**	15.84**
E	6	14	
CV (%)		8.66	6.34

* and ** significant at 1% and 5%, respectively.

Table 4. Mean comparison of effect of cadmium on root and shoot weight of cabbage varieties.

Treatments	Average	
	Anthocyanin	Proline
b1	1.133[a]	21.51[d]
b2	3.377[b]	49.92[c]
b3	7.519[a]	84.37[b]
b4	4.406[b]	119.79[a]

a1= white cabbage, a2= red cabbage; b1, b2, b3 and b4=0, 10, 20 and 30 mg/kg Cd, respectively.
Means within each column sharing the same letter(s) are not significantly different based on Duncan test at p = 0.05.

The amount of chlorophyll and proline in white cabbage were not affected by different concentrations of cadmium (Table 5). Proline production was found to be higher in red cabbage compared to white cabbage under cadmium treatment, indicating red cabbage was more affected by cadmium for the production of propylene. Heavy metal inhibits Chl biosynthesis by inhibiting α-aminolevulinic acid dehydrogenase and proto-chloropyllide reductase activity and breakdown of pigment or their precursors (Stobart *et al.*, 1985). According to Sharma and Dubey (2005) heave metals can replace the central magnesium atom of chlorophyll and consequently this substitution reduces the light received by chlorophyll and reduces photosynthesis. Shariat *et al.* (2011) found that with increasing concentrations of Cd, proline content of *Eucalyptus occidentalis* was increased but the amount of pigments decreased.

Table 5. Analysis of variance of Chlorophyll and proline contents in white cabbage.

SOV	df	F	
		Chlorophyll	Proline
Rep	2	2.02	0.39
Cd concentration	3	2.98[ns]	4.16[ns]
E	6	14	
CV (%)		2.33	3.24

NS, not significant at 5% level.

Correlation

Pearson's correlations coefficients between the evaluated traits in white cabbage showed that the maximum correlation (0.973) was obtained between the root and shoot Cd. A significant positive correlation was detected between proline content with root and shoot cadmium (0.754 and 0.720, respectively). There was no significant correlation between chlorophyll content and other factors. Thus, the chlorophyll content and have no effect on cadmium and protein contents, vice versa, proline and cadmium have significant positive impacts on each other (Table 6).

In red cabbage, the highest correlation (0.975) was found between the amount of cadmium in root and shoot. A significant positive correlation was observed between proline with root and shoot contents like white cabbage (0.910 and 0.834, respectively). Significant correlation was found between anthocyanin content with proline, root and shoot contents. This means that the increase in anthocyanin content in cabbage can reduce the amount of proline and cadmium (Table 7).

Table 6. Pearson's correlation coefficients between Cd, chlorphyll and proline in white cabbage.

	Cd (shoot)	Cd (root)	chlrophyll	proline
Cd (shoot)	1			
Cd (root)	0.973**	1		
anthocyanin	-0.209	-0.177	1	
proline	0.720**	0.754**	0.109*	1

Table 7. Pearson's correlation coefficients between Cd, anthocyanin and proline in red cabbage.

	Cd (shoot)	Cd (root)	Anthoc-yanin	proline
Cd (shoot)	1			
Cd (root)	0.975**	1		
anthocyanin	-0.775**	-0.824**	1	
proline	0.910**	0.834**	-0.592*	1

Conclusion

The present study demonstrated that cadmium accumulation in the white and red cabbage was different under cadmium concentrations. The amount of Cd in shoots and roots of both species was increased with the increasing Cd concentrations. The capability of white cabbage in Cd uptake was higher than red cabbage. In both species, a greater accumulation of cadmium occurred in roots than shoots. Thus, two cabbage species can be used for

phytoremediation of Cd-contaminated soils. However, if these species are used as hyper-accumulate, they should be burn after harvesting like dangerous waste.

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