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REMEDIATION OF CADMIUM BY INDIAN MUSTARD (*BRASSICA JUNCEA L.*) FROM CADMIUM CONTAMINATED SOIL: A PHYTOEXTRACTION STUDY

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Abstract

Cadmium is a toxic metal for living organisms and an environmental contaminant. Soils in many parts of the world are slightly too moderately contaminated by Cd due to long term use and disposal of Cd-contaminated wastes. Cost effective technologies are needed to remove cadmium from the contaminated sites. Soil phytoextraction is engineering based, low cost and socially accepted developing technology that uses plants to clean up contaminants in soils. This technology can be adopted as a remediation of cadmium from Cd-contaminated soils with the help of *Brassica juncea* plant. The objective of this work was to evaluate the cadmium (Cd) accumulate and the tolerance of *Brassica juncea*. The Cd accumulates in all parts of plants (roots, stems and leaves). It was found that accumulating efficiency increased with the increase in the concentration of applied cadmium metal solution. Maximum accumulation of cadmium was found in roots than stem and leaves. Phytoextraction coefficient and translocation factor were highest to show the validity of the *Brassica juncea* species for hyperaccumulation of the Cd metal. These results suggested that *Brassica juncea* has a high ability to tolerate and accumulate Cd, so it might be a promising plant to be used for phytoextraction of Cd contaminated soil.

Keywords: *Brassica juncea*, Cadmium, Phytoextraction, Translocation factor

Introduction

Biosphere Pollution by toxic metals has intensified rapidly since the onset of the industrial revolution, posing major toxic metal pollution of the environment and health problems. Heavy metals naturally occur at lower concentration in soils. However, they are considered soil contaminates due to their widespread occurrence, acute and chronic toxicity. Common heavy metal contaminants include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc, which present numerous health dangers to higher organisms and are also known to decrease plant growth, ground cover and have a negative impact on soil microflora (Zhang *et al.* 2009; Alizadah *et. al.* 2012). Among all the metal pollutants, cadmium is of major concern. It has been considered serious soil and environment pollutant due to its potential toxicity at low concentration. The cadmium toxicity is higher than 2-20 times than other heavy metals (Banavides *et. al.* 2005). The increasing contamination of Cd in the environment and its accumulation in the food chain from anthropogenic sources such as the non-ferrous metal industry, mining and smelter, use and disposal of batteries, metal-contaminated wastes and sludge disposal, application of pesticides and phosphate fertilizers leads to dispersion of Cd pollutant. Cadmium in soil can be bioavailable for plant uptake and subsequent human uptake, thus cadmium exhibits highly adverse effect on soil biological activity, plant metabolism and the health of humans and plant kingdom (Singh *et al.* 2009; Sao *et al.* 2006; ATSDR, 2008). According to ATSDR (2011) cadmium is seventh on the list of the "Top Twenty Hazardous Substances from the 2011 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances." This list is based on the frequency of occurrence, toxicity, and the potential for human exposure.

Humans can directly ingest Cd in many ways such as food, liquids or edible products that are in contact with Cd-coated containers. The human body absorbs about 1 up to 3 mg of cadmium per day through smoking (Zeneli, *et. al.*, 2009). The famous "itai-itai" or "ouch-ouch" bone disease of the Japanese in Toyama Inlet was the result of chronic exposure to Cd waste (Bernard, 2008). Cd is also taken up through the roots of plants to become concentrated in edible leaves, fruits and seeds. Cd builds up in animal milk and fatty tissues. On average, 25,000 to 30,000 tons of cadmium is released into the environment each year (ATSDR, 2008, CPCB, 2007). Cadmium is very toxic to most plants when present between 03 and 08 mg/kg soil. The most apparent visible symptoms of Cd toxicity in plants are retardation of plant growth, chlorosis and stunting (Banavides *et. al.*, 2005).

Few techniques are available for remediation of heavy metals from polluted soils, because heavy metals are non-degradable and generally strongly retained in the soil. For metal polluted soil, phytoextraction of heavy metals is an emerging technology that aims to extract metals in soils. The technology has attracted attention in recent years for the low cost of implementation and environmental benefits (Alizadeh *et al.*, 2012; Vassilev *et al.*, 2002). Moreover, the technology is likely to be more acceptable to the public than other traditional methods such as vitrification, electrokinetics, landfill of the top contaminated soil and excavation

(Liu *et al.*, 2010). It is a branch of the concept of phytoremediation based on plants ability to work as a solar driven pump, extracting and concentrating particular elements from the environment (Ghosh *et. al.*, 2005). The efficiency of this technique significantly depends on the choice of plants that are to be applied for the purpose of phyto-sorption of a given contamination from the soil into their aerial parts. A number of papers have been published that provided proofs that the solubility of metals in soil, and their subsequent uptake by plants and translocation in shoots may be considerably enhanced (Chinmayee *et. al.*, 2012).

Brassica juncea was identified as a potential phytoextraction species for the remediation of Cd toxic metal from Cd-contaminated soils, because of its high biomass yield under elevated Cd levels and its ability to translocate high amounts of Cd into its stems and leaves. Therefore, making *Brassica juncea* part of a proper crop rotation can help control various toxic metal levels and minimize the toxic load deposited into soils (Ginneken *et al.*, 2007). *Brassica juncea* is belongs to the family of brassicaceae and also one of the important oil crops of India. The main objective of this study was to evaluate the effectiveness of *Brassica juncea* as a phytoextraction species.

Materials and Methods

1. Seed and chemicals

Seeds of *Brassica juncea* used were obtained from the local market of Morena of Madhya Pradesh, India. This area is located in north India ($25^0$22' N to $26^0$52' N latitude and $77^0$10' E to $78^0$42' E longitude). Seeds with uniform size, colour and weight were chosen for experimental purpose and surface sterilized with 0.1 percent mercuric chloride solution and washed thoroughly with tap water and then with distilled water. The soil was collected from agriculture land of Morena, India. It could be crumbed to pass 4 mm sieve for pot experiment and 2 mm sieve for analysis of physicochemical properties. Some physicochemical properties are reported in Table 1.

2. Experiment setup

Firstly, *B. juncea* seeds were germinated on filter paper in Petri dishes, then transferred into earthen pot with untreated soil (control) and treated soil to which cadmium metal as cadmium nitrate ($Cd(NO_3)_2 . 4H_2O$) was applied with different concentrations i.e 0.01M, 0.03M, 0.05M, 0.07M and 0.09M Cd. Each pot contained 4 kg soil. Two seeds were grown in each pot with three replicates. The 5 ml solution of each concentration was applied to soil in alteration with 10 ml nutrition solution (Hoagland nutrition's solution) for 45 days. The next 15 days, the plants were grown without Cd metal solution, which could be greater accumulation of metals in plants. All pots were watered daily in the morning.

3. Analytical methods

The *B. juncea* plant was harvested after 60 days growing period. During growing period, the physical parameters like heights, no. of leaves and toxicity symptoms were observed. Each part of plant (roots, stems and leaves) was washed with deionized water and then put in oven at 70^0C for 48 hours and digested with HNO_3- $HClO_4$ mixture. The digest was cooled, diluted, filtered through Whatman No.42 filter paper and made up to 50 ml. The samples were analyzed by Atomic Absorption Spectrophotometer (Perkin Elmer100 A-analyst).

4. Data analysis

The potential of *Brassica juncea* to translocate heavy metals from the roots to the harvestable parts (stems and leaves) defined as translocation factor, TF (Mattina *et al.*, 2003). The phytoextraction coefficient was described as the heavy metal concentration in plant divided by heavy metal concentration in the soil (Zu *et al.*, 2005). The mean values were statistically analysed by single factor of analysis of variance (ANOVA) using Microsoft excel -2007. Significant differences were made using the Tukey test ($P \leq 0.05$)

Table 1: Physical and chemical properties of the soils

Parameters		Values	Analytical Method
Moisture content (%)		22.73	Gravimetrically method
pH		7.83	1:1 soil/water slurry
Conductivity (dSm^{-1})		0.90	1:2 soil/water slurry
Total N (%)		0.23	Kjeldahl
Organic Matter (%)		1.20	Walkly-Black Method
CEC (mequ/100g)		20.12	Ammonium Replacement Method
Soil Texture	Sand %	35.64	Hydrometer method
	Silt %	18.00	Hydrometer method
	Clay %	46.36	Hydrometer method
	Soil type	Clay	
Metal concentration in soil (mg/kg)	Zn	3.24	Atomic Absorption spectrophotometer
	Cu	4.32	
	Cd	ND	
	Pb	ND	

ND: Not detect

Results and Discussions

The effect of Cd on biomass is presented in table 2. In this study, the biomass yield was significantly affected with increasing concentration of Cd solution in soil. *Brassica juncea* had high biomass production, which increased from 0.01M to 0.03M (11.10 to 11.58 g/pot) as compared to control treatment (9.44 g/pot). After 0.03M treatment, the biomass was decreased from 0.05M to 0.07M (10.72 to 10.06 g/pot), which higher biomass than control. Lowest biomass which was 8.93 g/pot observed at 0.09 M treatment. There were no phytotoxicity symptoms during 60 days growing period of all treatments indicating that *Brassica juncea* plants could tolerate the Cd concentration in soil. The decrease in biomass at 0.09 M was caused by reduced plant height and number of leaves. Similar result was found in *L. albus* in which 38 and 15 % reduction of biomass noted on 18 and 45 µM Cd treatment (Zornoza *et al.,* 2002).

Cd hyperaccumulation is defined as plant species capable of accumulating more than 100 mg Cd /kg dry matter in shoots (leaves and stems) dry weight (Baker *et al.,* 2000). While maximum plant species, Cd concentration is generally lower than 3 mg Cd /kg, but may reach 20 mg Cd /kg or more in Cd enriched soils. A plant concentration of >100 mg Cd /kg may be regarded as exceptional, even in Cd contaminated soil (Yang *et al.,* 2004).

The accumulation of Cadmium metal by *Brassica juncea* at various concentrations has been conducted in pot experiments for 45 days treatments of cadmium metal and 60 days growing period. The results obtained from statistical comparison of average accumulative cadmium in roots, stems and leaves indicate the highest cadmium absorption in roots (Table 2). It was further observed that by increasing the Cd concentration in the soils, its accumulation in plant organs also increased. Once metal ions are absorbed, they can be accumulated in the roots or be exported to the arial part (Stems and leaves) via the transpiration (Ximenez *et al.,* 2002). The maximum amount of Cd was found in roots rather than stems and leaves of *Brassica juncea*. Our results contrast with those of Zheng *et al.,* (2010) who reported that normally cadmium ions are mainly retained in the roots and only small amounts are transported to the shoots. Roots of the plant act as a barrier against heavy metal translocation and this may be a potential tolerance mechanism operating in the roots (Bonnet *et al.,* 2010).

The highest amount of Cd was found 45.23, 31.95 and 12.72 mg Cd /kg in roots, stems and leaves respectively when *Brassica juncea* plants was exposed to 0.09M. The same results have been found for *Amaranthus tricolor (*Watanabe *et al.,* 2009*), Brassica chinensis* (Liu *et al.* 2007), *L. Albus (*Zoronza *et al.,* 2002*).* The significant correlation coefficients were found between the amount of Cadmium in roots and aerial organs. The figure 1 indicated that the level of Cd in root of *B. juncea* is proportional to stem (r^2 = 0.979) and leaves (r^2 = 0.977) which was positive. This confirmed that plant accumulate cadmium and distribute it between tissues proportionally to its level in the soil. Cadmium accumulation in *B. juncea* was related to uptake efficiency. The uptake efficiency of cadmium by *B. juncea* was about 7-15% which shows that *B, juncea* is good hyperaccumlator for toxic Cd element.

The Phytoextraction coefficient and transport factor of cadmium metal by *Brassica juncea at* 0.01, 0.03, 0.05, 0.07 and 0.09M given in Table 3. Phytoextraction coefficient is a useful tool of metal accumulation power of plants at metal concentration. It is bound to get reduced with increase in metal concentration in soil. The Phytoextraction coefficient of cadmium varied from 0.57 to 0.23 at various concentration of cadmium. The phytoextraction coefficient was found high at 0.01M (0.57) and low at 0.07 M (0.21). Results indicate that *Brassica juncea* has excellent potential for Cd phytoextraction because of its high biomass production and high Cd phytoextraction coefficient. Several reports are in agreement with the findings of this study (Robinson *et al.,* 2000; Singh *et al.,* 2009).

Transport factor shows transport metals through root to stems and leaves in plants, which become functional to understand the transport mechanism of heavy metals in plants organs (roots, stems and leaves). Better translocation is advantageous to phytoextraction: (i) it can reduce metal concentration and thus reduce toxicity potential to the root, and (ii) translocation to the stems and roots is one of the mechanisms of resistance to high metal concentration (Gonzalez *et al*, 2012; Wani *et al.*, 2011). *Brassica juncea* showed maximum translocation in this work. Highest rate of transport factor was found in root to stems of 70.72 % (0.09M), while in root to leaves was 32.06% (0.07M) thus lower transport factor was 63.34% (0.01M) in root to stem level and 22.03 % (0.03M) in roots to leaves. On the basis of the TF values of Cd, *B. juncea* was also found to be Cd hyperaccumulator because Cd treatments of this soil show that the TF values were found highest at all treatments. This result is in agreement with the results of (Rosa *et al.*, 2004) who have also shown that *S. kali* uptakes and translocates cadmium easily to shoot organs as a potential Cd-hyperaccumulator. Another previous study showed that *Zea Mays* (Stingu *et al.*, 2011) and *Aeluropus littoralis* (Rezvani *et al.*, 2011) were potential Cd hyperaccumulator.

Table 2. Biomass (g/pot in dry weight) and Cadmium uptake (mg/kg in dry weight) by *Brassicajuncea*

	Root (mg/kg)	Stem (mg/kg)	Leaves(mg/kg)	Average metal uptake by per pot (mg/kg)	Biomass (g/pot)
Control	ND	ND	ND	ND	9.44 ± 1.49
0.01M	13.55±06.56	8.08 ±02.43	3.62±02.69	25.25±11.15	11.10 ± 2.14
0.03 M	21.73 ±12.28	11.83±02.98	5.23±01.99	38.80±17.79	11.58 ± 0.93
0.05 M	29.28 ±18.36	17.40±06.71	7.03±02.12	53.72±32.16	10.72 ± 0.92
0.07M	33.45±09.97	20.85±07.55	9.53±02.29	63.83±13.05	10.06 ± 0.62
0.09M	45.23 ±14.04	31.95 ±12.81	12.72±08.56	89.90±34.58	8.93 ± 2.05

Table 3. Phytoextraction coefficient and transport factor of *Brassica Juncea*

Concentration	Transport Factor (TF) in %		Phytoextrction coefficient (PEC)
	Root to Stems	Root to leaves	
Control	ND	ND	ND
0.01M	63.34±14.97	24.82 ±17.30	0.57±0.25
0.03M	64.72±27.36	23.47±06.64	0.29±0.13
0.05M	68.82±22.54	22.03 ±17.58	0.24±0.15
0.07M	65.99±29.10	32.06±18.72	0.21±0.04
0.09M	70.72±18.16	26.99±23.96	0.23±0.09

Values are averages of three replicates ± S.D

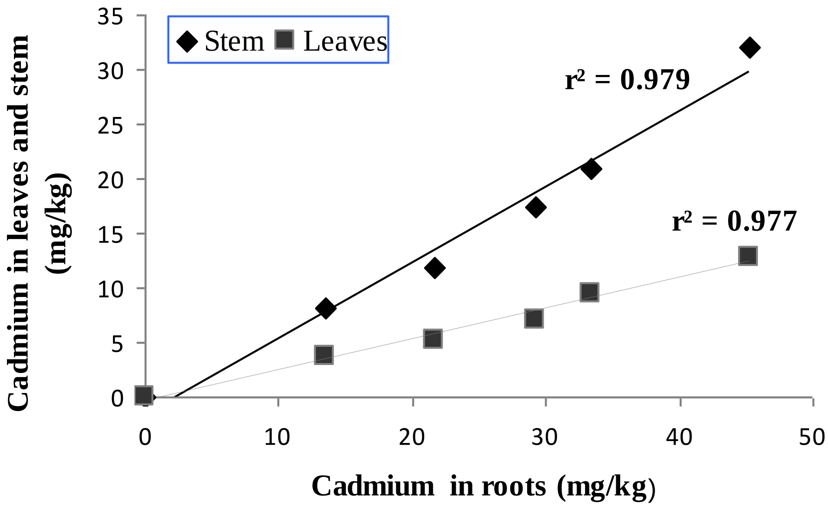

Figure. 1. Relationship among Cd concentrations in roots-stem and roots-leaves of *B. Juncea* in cadmium contaminated soil

Conclusion

In this research study, we have concluded that the *Brassica juncea* plant had strong tolerance and accumulation ability to cadmium. The distribution order in the *Brassica juncea* was root > stem > leaves. The growing period of 60 days shows that the highest total accumulates of cadmium was 89.90 mg/kg in whole plants at 0.09M treatment. The highest Phytoextraction coefficient and transport factor were 0.57 and 70.72 % respectively. Therefore *Brassica juncea* is an effective accumulator plant for phytoextraction of cadmium from cadmium contaminated soil.

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