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The remediation of lead from lead-contaminated soil using *Brassica juncea*: Implications for phytoremediation

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ABSTRACT

Remediation of heavy metal contaminated soil has been a major problem in all over the world and recently use of plants to clean such soils has been on the investigation. In this work, the ability of *Brassica juncea* to accumulate lead metal as well as its potential application for phytoremediation was assessed. *Brassica juncea* is an easily cultivated and high-biomass crop which could accumulate high concentrations of lead (Pb) from polluted soil. Lead is an environmental and food chain contaminant because of its potentially deleterious effect on living organisms. This research study was conducted in pot experiment with different concentration of lead treated soils. After 60 days of growing period, plants were harvested and divided into root, stem and leaves. The Pb 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 lead metal solution. The maximum concentration of lead metal was accumulated in the roots than those in the stems and leaves. The research indicates that *Brassica juncea is* a promising species for the metal tolerance and hyperaccumulation.

Introduction

Heavy metals are a natural constituent of the lithosphere, whose geochemical cycles and biochemical balances have been drastically altered by human activity (Almeida, et al., 2007). Therefore, pollution by Heavy metal is a global environmental and health safety issue. Soils may become contaminated by the accumulation of heavy metals and metalloids through disposal of metalliferous ores and metal scraps, electroplating, e-waste, application of fertilizer and pesticides, sludge dumping and generation of municipal waste and spillage of Petrochemicals (Wuana, et al., 2011). Heavy metals contamination in soil is a major problem of our environment and this is also one of the major contaminating agents of our food supply (Gholizadeh, et al., 2009). According to the European Environmental Agency (2007), the 32-member countries of the European Union have reported occurrences of 250000 polluted sites, which are contaminated by heavy metal and mineral oil. Common heavy metal contaminants include cadmium, chromium, copper, lead, mercury, nickel and zinc, which present numerous health dangers to Flaura and Fauna (Zhang, et al., 2009). According to World Health Organization the metals of most immediate concern are chromium, copper, zinc, iron, cadmium and lead (Mohapatra, et al., 2010).

Lead is one of the most dangerous and toxic metallic pollutant of our environment that can threat the life of living creatures in many ways. It originates from various sources such as mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Aghaz, et al., 2013). Like to other heavy metals, ions of lead are also potential carcinogenic agents that effects on human, animals and plants. Its ions also disturb intermediate metabolism, inhibits the exchange of saccharides in nerve tissue, affects the changes in metabolism of porphyrins and influences the inhibition of homeostasis. It mainly interferes with enzymes on their sulfhydryl and amide groups (Flora, et al., 2007; Hegedusova, et al., 2009). Uptake of lead produces damaging effects in the hematological dysfunction, renal, cardiovascular, central nervous system, respiratory system and gastrointestinal system (Ibrahim, et al., 2012; Sharma, et al., 2011). Lead accumulates in plant tissue and it toxicity leads to decrease in seed germination, root elongation, biomass, photosynthetic rates, carbon dioxide level and inhibition of chlorophyll biosynthesis (Shafiq, et al., 2008). It can also generate various kinds of active oxygen such as Superoxide and Hydrogen Peroxide which can disturb cell membrane activities of plants (Azad, et al., 2011). According to ATSDR (2011) lead is second 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."

Contamination of soil by lead poses a major problem that still needs an effective and affordable technological solution. Most of the conventional remedial technologies such as excavation and burial of the contaminated surface soil or 'washing out' of lead from the contaminated field are effective but often too expensive for a large scale remediation. The cost of the operation is around US$2 million/ha. The areas contaminated with a scattered pollution cannot be cleaned up by this method. Finally, very few sites are treated. Furthermore, this solution is not a sustainable one since it transfers the pollution to another site and constitutes a poisonous gift for the future generation (Salt, et al., 1995).

More recently, Phytoremediation has therefore emerged as a new technology that use plants for removing pollutants from contaminated area. It is a cost effective, environmentally and aesthetically friendly method of immobilizing/stabilizing, degrading, removing, or detoxifying contaminants, including

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metals, pesticides and hydrocarbons (Ahmadpour, et al., 2012; Pilon-Smits, 2005). For effective phyoremediation of metals they must be translocated and accumulated in the aerial parts of plants. There have been few reports about lead hyperaccumulating plants. Pumpkin was studied by Poniedziałek *et al*. (2010) for Pb accumulate in Pb contaminated soils. It was able to accumulate up to 7.4 % Pb in shoots. Investigation on the ability of plants in removing heavy metals from contaminated soil has been intensified using in *Pisum sativum* (Kevresan, et al., 2001), *Zea mays* (Małkowski, et al., 2002), citronella (Cymbopogon winterianus), Mentha sp. and Ocimum basilicum (Gupta, et al., 2013). This research work concerned on *Brassica juncea*

Brassica juncea (Indian mustard) belongs to family brassicaceae and is a very important oil crop. Mustard oil is one of the major cooking oils in India. It is a fast growing plant which produces a high biomass even in heavy metal polluted soils. It was expected that this plant could be effective hyperaccumulator for remediation of lead polluted soil.

The objectives of this work were to study the accumulation of lead in different plant organs (roots, stem and leaves) of *Brassica juncea* and efficiency of lead removal of this plant.

Materials and Methods

Seeds and chemicals

The *B. juncea* (Pusa Bold Dir-50 variety) seed used in the experiment was collected from local market of Morena of Madhya Pradesh, India. 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. All chemicals and reagents used were of AR grade**.**

Soil Preparation

Soil was collected at 0-30 cm from agricultural land in Morena region which was transported to the laboratory and air dried, finely ground with a mortar and pestle, and sieved through a 2-mm mesh sieve prior to determine the physicochemical characteristics (Table 1)

Pot experiment

This research work was conducted in the pot during Nov-Jan 2012-2013 cropping season and carried out at Department of chemistry, Govt. P. G. College Morena, of Madhya Pradesh, India. This area located in north of India ($25^0$22' N to $26^0$52' N latitude and $77^0$10' E to $78^0$42' E longitude). Firstly, *B. juncea* seeds were germinated on filter paper in Petri dishes, then transferred into earthen pot with untreated soil (control) and treated soil to which lead metal as lead nitrate ($Pb(NO_3)_2$)had been applied with different concentration i.e. 0.01M, 0.03,0.05,0.07 and 0.09 M. Each pot contained 8 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 Pb metal solution added, which could be greater accumulation of metals in plants. The composition of Hoagland nutrition's solution was 0.3 mM $Ca(No)_3.4H_2O$, 0.5 mM KNO_2, 0.03 mM KH_2PO_4, 0.02 mM $FeCl_3.6H_2O$, 0.2m M $MgSO_4.7H_2O$, 10.0µM H_3BO_4, 0.5 µM $ZnSO_4.7H_2O$, 0.2 µM $CuSO_4.5H_2O$, 0.05 µM $Na_2MoO_4.2H_2O$ and 0.02 µM $MnCl_2.4H_2O$. All pots were watered daily in the morning.

Lead in plant analysis

The *B. juncea* plants were harvested after 60 days growing period. During the growing period, the physical parameters like heights, numbers of leaves and toxicity symptoms were also observed. Each harvested plant was cut into leaves, stems and roots and washed with deionized water and then put in oven at 70^0C for 48 hours. Leaves, stems, and roots were weighed separately. Lead was extracted after wet digestion with HNO_3-$HClO_4$ mixture. The digest was cooled, diluted, filtered through whatman No.42 filter paper and made up to 50 ml. Lead was measured by atomic absorption spectroscopy (Perkin Elmer100 A-analyst).

Two plants from each replicate of a pot was analysed for its various parameters and the average was calculated. These mean values were statistically analysed by a single factor of analysis of variance (ANOVA) using Microsoft excel -2007. Significant differences were made using the Tukey test ($P \leq 0.05$).

Results and discussions

Growth and Biomass yield

Reduction in root length, shoot height and biomass was observed with increasing lead concentration which is presented in table 2. Decline in root length ranged from 7.79 % (0.01M Pb) to 38.96 % (0.07 M Pb). Similarly shoot height declined from 8.36 % (0.01 M Pb) to 23.91 (0.07M Pb). Plants grown in untreated soil (control) produced nearly twice the biomass of the plants (19.03 g/pot) receiving the 0.07 M (9.02 g/pot) application. Highest reduction show in treatment of 0.07M Pb in which total biomass 9.07 g / pot. While in treatment of 0.09 M Pb, the biomass of the plant was 9.82 g/pot which were greater than 0.07M Pb treatment. The main reason of the reduction in 0.07 M lead treatment was growth of one replicate out of three was very slow in which height of *B. juncea* at 27^{th} day was 21.20 cm as compared to other treatment on the same day. While, the heights of other plants were observed between 50 and 65 cm at 27^{th} day. There were no noticeable phytotoxic effects of Pb on plants except for the reduction in plant growth and biomass. It is known that metal phytotoxicity causes stress to the plant resulting in a reduction in biomass eventual death (in some cases) (Cunnigham, et al., 1996). Similar results were found by several studies at the evaluated Pb concentration on dry biomass is significantly reduced in *Pisum sativum* (Kevresan, et al., 2001), *Zea mays* (Małkowski, et al., 2002) and *Solarium melongena* (Yilmaz, et al., 2009).

Metal Tolerance

The potential of *Brassica juncea* to tolerate Pb stress was evaluated using an index of tolerance (TI) which is shown in Figure 1. The index of tolerance is expressed in percentage and smaller the percentage greater the effect of added substance. An index of tolerance of 50% that means is considerable to be the minimum desirable biomass for plants growing in a metal contaminated soil (Ximenez-Embun, et al., 2002). TI is an important indicator that reflects the heavy metal tolerance of plants (Wang,et al., 2011). The index of tolerances of 0.01M, 0.03M, 0.05M, 0.07M and 0.09M lead are 79.63 %, 68.50 %, 68.03 %, 46.73 % and 50.88 % respectively. The index of tolerance of lead treated *Brassica juncea* was reduced by between 47 % and 80% as compared to control (100%). The smallest index of this study is 46.73 % found in 0.07 M lead treatment which highest effected by the biomass of *B. juncea*. As evidence in this case, the height of the plant was also reduced. While, TI of other treatments was found more than 50%, that means highly stress to lead metal. The reason for low tolerance of plant at 0.07 M lead treatment might be due to changes in the physiological mechanism of the plant growth. The highest tolerant to lead also reported previous study such as Han et al. (2008) reported that the index of tolerance (IT) on *I. lactea* and *I. tectorum* in which found among 0–8 mmol l^{-1} Pb treatments of *I. lactea* were not different, while those of *I. tectorum* at 6 mmol l^{-1} Pb were decreased. The results indicated that *I. lactea* var. *chinensis* was more tolerant to Pb than *I. tectorum*. Other studies have also been reported to show high tolerance to lead metal on *i.e. C. Comosum (Wang, et al., 2011), R. communis* L. (Romeiro, et al., 2006) plants.

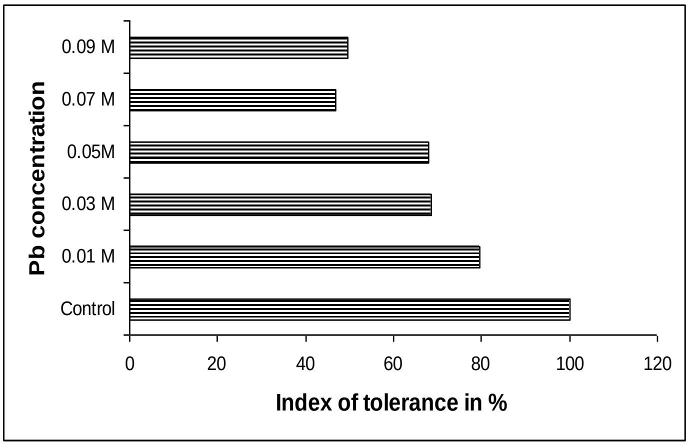

Figure 1: Index of tolerance of *B. juncea*

Pb accumulation and distribution in different organs of Plant:

The accumulation of lead metal by *Brassica juncea* has been conducted in pot at different concentrations for a period of 45 days treatment and 60 days growing. The amount of lead in all organs of plants showed significant increase with increase in metal concentrations. Once metal ions are absorbed, they can be accumulated in the roots or be exported to the aerial parts (Stems and leaves) via the transpiration (Jadia, et al., 2008).

Statistically significant ($p < 0.05$) difference in accumulation of lead in different part of *Brassica juncea* has been shown in Figure 2 and table 3. Results of this study show that the lead content of root, stem and leaves of this plant increased with increased in lead treatment in the soil. The highest level of lead of all treatments was found in roots followed by leaves and stems. It is about 1-1.5 times more than was found in the stem and 4-5 times more than in leves. When considering whole plant (roots, stems, and leaves) of different treatment, the highest amount was found in which the amount of lead in roots, stems and leaves were higher then those of the same organs of another treatment, in which lead concentrations were found 124.92, 90.52 and 72.20 mg/kg respectively. While The lowest amounts of lead was 46.12 (in root) , 34.38 (in stem) and 21.07 (in leaves) for *B. juncea* treated with Pb at 0.01 M. Showed this experiment, increasing lead level in the soil, increased the uptake and accumulation of lead amounts in root, stem and leaves of *B. juncea*. Similar observation as stated by Beladi et al (2011) in *Lathyrus sativus* , *Cho*-Ruk *et al. (2006) in A. philoxeroides and* Sinegani et al (2008) *in Helianthus annuus* . Significant correlations were also found highly positive correlation between the level of Pb in soil and root (r^2= 0.988), in soil and stem (r^2= 0.991) and in soil and leaves (r^2= 0.998) from figure 2. The strong correlation between Pb in soil and accumulated by plant parts was reported by other studies (Si et al. 2006, Soleimani et val 2009).These significant correlation implies that the bioavailability of Pb in soil had a marked influence on the accumulation of Pb by *Brassica juncea* roots and aboveground tissues.

The amount of lead concentration was significantly increased from 0.01M to 0.09M. The distribution of lead metal in different organs (roots, stems, and leaves) of *Brassica juncea* is reported in figure 4. The same results were found in *Zea mays* (corn) and *Pisun sativum* (Huang et al., 1997) and Helianthus annuus, Nicotiana tabacum, Vetiveria zizanioides (*Boonyapookana, 2005*),. The results show that the maximum uptake of lead by the *Brassica juncea* was retained in roots. Lead level in plant decreased in the order: roots > stems > leaves.

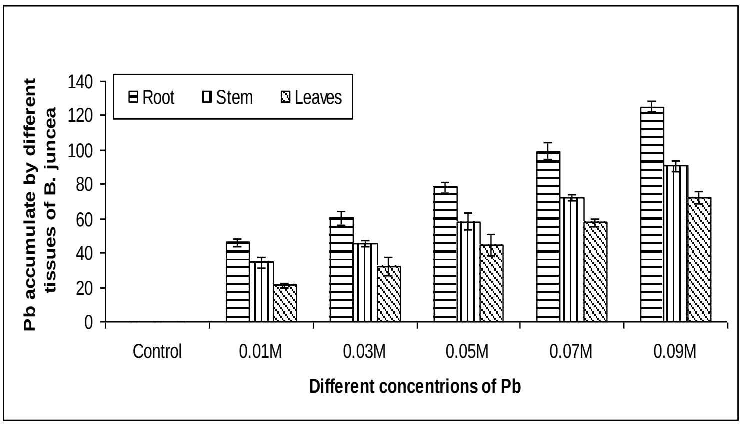

Figure 2: Lead accumulates by tissues of *B. juncea* at different concentrations of lead

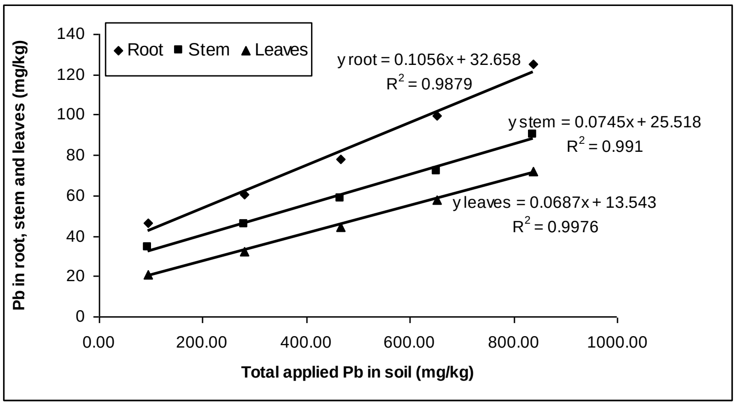

Figure 3: Relationship among Pb concentration in soils with roots, stem and leaves of *B. Juncea*.

This result indicated that the strong retention of lead observed in the root of *Brassica jun*cea that means the response of this plant highly stress to heavy metal. This is supported by the results of Hamadouche et al. 2012 who state that the amount of accumulated Pb was higher in roots compared to the stem and leaves in Raphanus sativus L. Similar evidence was also found by Soleimani et al. (2009) in Cynodon *dactylo* plant. Our results are in agreement with the findings of Cunningham *et al.*, 2000) who reported larger accumulation of Pb in roots could be assumed due to its strong binding to ion exchangeable sites on the compartment of the cell walls of the roots.

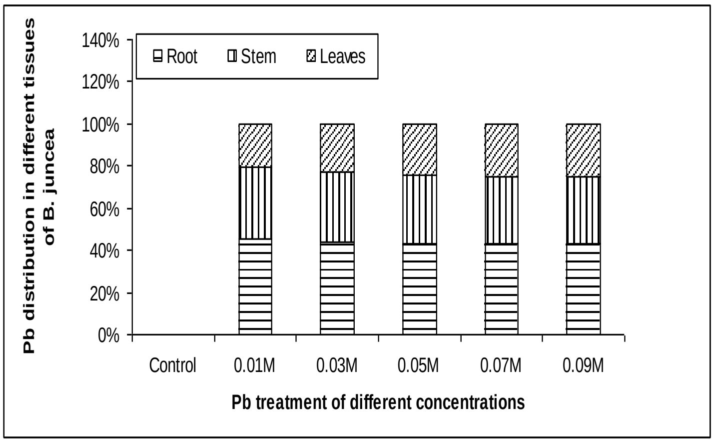

Figure 4. Lead distributions in tissues of *B. juncea* at different concentrations of lead

Table 1. Physicochemical properties of soil

Parameters		Values	Analytical Method
Moisture content (%)		22.73±2.20	Gravimetrically method
pH		07.83±3.41	1:1 soil/water slurry
Conductivity (dSm^{-1})		00.90±2.96	1:2 soil/water slurry
Total N (%)		00.23±1.82	Kjeldahl
Organic Matter (%)		01.20±4.31	Walkly-Black Method
CEC (mequ/100g)		20.12±2.50	Ammonium Replacement Method
Soil Texture	Sand %	35.64±1.09	Hydrometer method
	Silt %	18.00±2.07	Hydrometer method
	Clay %	46.36±3.17	Hydrometer method
	Soil type	Clay	
Metal concentration in soil (mg/kg)	Zn	3.24±0.17	Atomic Absorption spectrophotometer
	Cu	4.32±0.21	
	Pb	ND	

Values are three replicates ± SD , ND: Not Detect

Table 2. Effect of different Lead concentrations on growth and biomass production of *B.juncea*

Applied concentration	Root Length (cm)	Shoot Height (cm)	Biomass (g/pot)
Control (0)	15.4±0.7	84.9±1.0	19.03±0.3
0.01 M	14.2±0.4	77.8±0.3	15.37±1.2
0.03 M	12.0±1.7	75.7±1.6	13.22±0.6
0.05 M	11.7±0.8	72.7±1.0	13.13±2.0
0.07 M	09.4±1.1	64.6±0.7	09.02±1.2
0.09 M	10.9±1.3	70.2±0.5	09.82±6.0

Values are three replicates ± SD

Table 3. Lead accumulates (mg/kg in dry weight) by root, stem and leaves of *B. juncea*.

Treatments	Root	Stem	Leaves	Total uptake of lead by B. juncea
Control	ND	ND	ND	0
0.01M	46.12±2.10	34.38±3.18	21.07±1.09	101.57
0.03M	60.33±4.17	45.77±1.66	32.03±5.65	138.13
0.05M	78.35±3.21	58.37±4.52	44.65±6.31	181.37
0.07M	99.38±5.20	72.13±1.59	57.80±2.33	229.31
0.09M	124.92±3.12	90.52±3.03	72.20±3.43	287.64

Values are three replicates ± SD, ND: Not Detect

Another explanation Roots are the first organs contact with the metal ions, and generally roots accumulate significantly higher amounts of metal ions than the aerial parts of plants. Larger deposits of lead in this compartment have been reported by numerous researcher (Glinska,et al., 2002; Szarek-Lukaszewska et al., 2004)

Conclusions

The present study shows that a *Brassica juncea* plant has high potential to accumulate the toxic metal like lead. Total accumulates capacity of lead by this plant was ranging from 101.57 (0.01 M) to 287.63 (0.09 M) mg/kg. This may be important for soil remediation technologies based on metal extraction from plants in phytoremediation. It was also observed that by increasing lead concentration in soil, its uptake in different plats organs (root, stems and leaves) increased. Metal tolerance index results shows that this plant higher stress at higher concentration of lead metal. The data obtained in this study suggest that *B. juncea* could be used as a heavy metal hyperaccumulator in heavy metal polluted sites of soils.

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