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Phytoextraction Potential of *Prosopis juliflora* (Sw.) DC. with Specific Reference to Lead and Cadmium

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Abstract Root and shoot samples of *Prosopis juliflora* were assessed for their heavy metal content to evaluate the species as a green solution to decontaminate soils contaminated with lead and cadmium. The highest uptake of both the metals was observed in plants from industrial sites. Sites with more anthropogenic disturbance exhibited reduced chlorophyll levels, stunted growth, delayed and shortened reproductive phase. The ratios of lead and cadmium in leaves to lead and cadmium in soil were in the range of 0.62–1.46 and 0.55–1.71, respectively. Strong correlation between the degree of contamination and concentrations of lead and cadmium in plant samples identifies *P. juliflora* as an effective heavy metal remediator coupled with environmental stress.

Keywords Lead · Cadmium · *Prosopis juliflora* · Phytoremediation

Human activity has continuously increased the level of heavy metals circulating in the environment. Heavy metal pollution of the biosphere has accelerated rapidly and soil toxicity poses major environmental problems. Lead (Pb) and cadmium (Cd) are persistent environmental contaminants since they cannot be degraded or destroyed. Pb is released into the environment during its mining and smelting activities, from automobile exhausts, by combustion of petro fuels with anti-knocking additives like tetraethyl and tetra methyl lead, old lead paints, batteries, etc. as well as from industrial and liquid domestic waste. Cadmium is released as a byproduct of zinc and lead refining (Duruibe et al. 2007) and from vehicular exhausts and fertilizers. Excessive metal concentration in contaminated soils might result in decreased soil microbial activity and soil fertility, yield loss and possible contamination of the food chain. Agra is a major international tourist attraction in the north-central region of India. The river Yamuna located in the study area is also exposed to pollution from commercial and domestic waste. The riverbed and riverbank thus, act like a sink for the pollutants that the river carries.

Several technologies exist for remediation of metal contaminated soils and water, but the emphasis is on 'green' phytoremedial systems nowadays. Phytoremediation is the use of living plants to extract metals from the soil (phytoextraction) or to render them harmless in situ by binding them (phytostabilisation) (Lombi et al. 2001). Many plants have been well documented as hyperaccumulators as well as indicators of high metal concentrations in soil, but these are slow growing, and not very widespread. In most cases their biomass yield is also quite low. The plants used in for successful phytoextraction should have substantial biomass and a significant translocation factor [TF]: the ratio of heavy metal concentration in the shoot to that in the root of the plant (Luo et al. 2005). It has been demonstrated that, wild native plants may be better phytoremediators for waste lands than the known metal bioaccumulators from literature. A range of native, well adapted plants have been tested and used widely for heavy metal bioindicator and phytoremedial purposes including *Lobelia chinensis*, *Solanum nigrum, Calotropis procera*

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and *Crinum asiaticum* (Peng et al. 2009; D'souza et al. 2010; Varun et al. 2010).

In the seasonal surveys carried out to identify the flora at the sampling sites, *Prosopis juliflora* (Sw.) DC., a hardy plant was seen growing well at nearly all sites. Other weeds were seasonal and too short-lived for practical remediation. *P. juliflora* (Honey Mesquite), a xerophyte well adapted to many soil types under a wide range of moisture conditions. The value of this tree lies in its exceptional tolerance of drought and marginal soils. It can reach deep within the soil and thus soil-root interaction is enhanced both in terms of quality and magnitude. Thus, this plant appeared to be of particular interest in this context and was studied.

Materials and Methods

Agra, on the banks of river Yamuna (27°10′N 78°02′E) is located in North-central India. A part of the great northern plains, the study region is considered a semi-arid zone as two-thirds of its boundaries are surrounded by the Thar Desert. Eight sites were selected in the target area from different zones *viz.*—riverbank (sites 2, 3), roadside (sites 4, 5), industrial (sites 6, 7) and residential (sites 8, 9) zones. Soil from the college's botanical garden was taken as control (site 1). A survey was carried out to evaluate the existing levels of Pb and Cd in the soil. Root and leaf samples of *P. juliflora* were collected to assess characteristics of accumulation and tolerance of Pb and Cd in the species.

At each site, soil samples were collected from a depth of 5–15 cm using a core sampler. Four random samples were collected from each site to obtain a comprehensive profile of Pb and Cd levels at each site for statistical analysis. The samples were air dried, passed through 20 mesh sieve and stored in clean ziplock polythene bags until further use. Leaf and root samples were collected in similar bags from plants growing on spots from where soil samples were collected. In the lab, these were first washed with distilled water to remove adhering mud. They were further washed thoroughly 3–4 times with deionized water and allowed to drip dry completely in a dust free chamber.

Soil samples were tested for various parameters to obtain a clear profile of soils at the sites selected. Important soil properties are shown in Table 1. Soil pH was measured by a pH meter (Systronics μpH system 361) with a water soil ratio of 1:2.5. Soil conductivity was measured by a conductivity meter (Systronics) by preparing a 1:2.5 soil suspension in water. Organic carbon was measured by modified Walkley–Black rapid dichromate oxidation method (Piper 1966) where 10 mL 1 N potassium dichromate ($K_2Cr_2O_7$) and 20 mL conc. H_2SO_4 were added to 5 g soil. After 30 min. 200 mL distilled water was added along with 10 mL 85% phosphoric acid and 1 mL DPA indicator. This was titrated against N/2 ferrous ammonium sulphate. The value obtained was multiplied by the factor 1.724 to obtain organic matter content as reported. Available potash was estimated by flame photometer (mediFlam), available nitrogen by micro-kjeldahl and available phosphate was also determined using digital spectrophotometer (Jackson 1973).

Soil, shoot and root samples from all sites were digested using a microwave assisted wet digestion method with 3 mL HNO_3 + 9 mL HCl for 0.5 g soil, and 5 mL HNO_3 + 2 mL H_2O_2 for 0.5 g plant sample. The filtrate was analyzed for each metal by flame atomic absorption spectrophotometry or by graphite furnace atomic absorption spectrophotometry using a Solaar M2—Thermo Unicam instrument. For control of the results certified references (Virginia tobacco leaves CTA-VTL-2, Polish Certified Reference Material and NIST 2709—San Joaquin Soil) were used. The recovery rates and certified values for Pb and Cd analysed are 69%; (18.9 ± 0.5) and 92%; (0.38 ± 0.01), respectively. Limits of detection were 0.079

Table 1 Physico-chemical profile of Soil

Zones	Site	pH (1:2.5)	Electrical conductivity (dS/m) (1:2.5)	Organic matter (%)	Available nitrogen (kg/ha)	Available phosphate (kg/ha)	Available potash (kg/ha)
Control	Site 1	7.3	0.59	1.4	126	12.2	268
Riverbank	Site 2	7.2	0.33	0.46	56.15	45	140
	Site 3	7.57	0.42	0.66	94.8	51	303
Roadside	Site 4	7.7	0.44	0.24	39.4	14	210
	Site 5	8.4	0.48	0.34	53.6	2	383
Industrial	Site 6	7.15	0.66	0.15	66.3	18	366
	Site 7	7.66	0.86	0.21	87.3	30.2	640
Residential	Site 8	7.71	0.32	0.6	82.2	29.3	553
	Site 9	8.3	0.42	1.9	98.4	38.3	794

and 0.016 mg/L for Pb and Cd, respectively (using impact beads).

Chlorophyll content in *P. juliflora* leaf samples was determined on fresh weight basis. 40 mg fresh leaves were placed in 10 mL 80% acetone in a sealed, dark bottle in a refrigerator. After 5 days optical density of the solution was measured by a spectrophotometer at different wavelengths i.e. 480, 510, 630, 645, 652 and 665 nm and chlorophyll content was calculated using relevant formulae (Arnon 1949).

Pearson's coefficient for correlation of the data was statistically analyzed at a significance level of $p < 0.05$ and $p < 0.01$. One way Analysis of Variance (ANOVA) was calculated for data and means compared using Fisher's LSD method. For ANOVA, sites were jointly tested as zones for better comparison.

Results and Discussion

The concentrations of Pb and Cd detected in soil, leaf and root samples are recorded in Table 2. Maximum Pb and Cd were found at site 7 and site 6 (urban industrial) with 134 mg/kg Pb and 4.1 mg/kg Cd, respectively. Levels of Pb and Cd were each recorded in the order Industrial > Roadside > Riverbank > Residential. 10–100 and 0.06–3 mg/kg have been reported as ranges for Pb and Cd concentrations in soil, the lower value indicating normal and the higher value toxic level, respectively (Liphadzi and Kirkham 2005). The high level of Pb and Cd at sites 7 and 6 indicates pollution in the heavy industrial area. At river bank, site 3 had higher Pb levels than site 2 as expected, due to deposition of the heavy metal arising from heavy traffic near it. Cd was not detected in all the sample collected from site 8. Correlation analysis was performed using univariate Pearson's correlation coefficient, Pb and Cd in soil showed significant correlation (r = 0.689) as did Pb and Cd content in *P. juliflora* leaf (r = 0.793) and root (r = 0.745) ($p \leq 0.05$).

Table 2 Pb & Cd content in soil and plant (*P. juliflora*) samples

Zones	Sites	Pb content (mg/kg)			Cd content (mg/kg)		
		Soil	Leaf	Root	Soil	Leaf	Root
Control	Site 1	5.4^{a}	5.6^{a}	4.4^{a}	0.82^{a}	1.4^{a}	1.1^{a}
Riverbank	Site 2	11.2^{b}	16.4^{b}	8.7^{b}	2.7^{b}	2.3^{b}	1.6^{b}
	Site 3	22.3^{b}	23.1^{b}	14.6^{b}	2.4^{b}	2.6^{b}	1.7^{b}
Roadside	Site 4	30.6^{c}	34.2^{c}	15.3^{c}	3.2^{c}	3.5^{c}	2.1^{b}
	Site 5	23.4^{c}	26.7^{c}	17.6^{c}	2.9^{c}	3.0^{c}	1.8^{b}
Industrial	Site 6	120.1^{d}	76.4^{d}	58.2^{d}	4.1^{d}	4.3^{d}	3.1^{c}
	Site 7	134^{d}	83.1^{d}	62.6^{d}	3.7^{d}	3.9^{d}	2.6^{c}
Residential	Site 8	7.6^{a}	9.2^{a}	6.1^{a}	ND	ND	ND
	Site 9	10.1^{a}	10.8^{a}	8.3^{a}	0.93^{a}	1.5^{a}	0.98^{d}

Different letter in the same coloumn denote significant statistical difference ($p < 0.001$) in mean Pb and Cd content at the zones selected

Pb content values in *P. juliflora* leaf samples are within the range of 5.6–83.1 mg/kg whereas in roots they are in the lower range of 4.4–62.6 mg/kg. Levels of Pb in plant samples increased in proportion to its concentration in soil samples. Highest uptake values of Pb recorded were 83.1 and 62.6 mg/kg in *P. juliflora* leaf and root samples, respectively, both from site 7 which had the highest Pb levels of all sites. Cd concentrations in leaf and root samples are in the range of 1.4–4.3 mg/kg and 0.98–3.1 mg/kg, respectively. The sample plants accumulated higher levels of Cd with an increase in Cd concentration in soil at all sites, as seen in the case of Pb. The highest Cd level in leaf and root samples detected was 4.3 and 3.1 mg/kg respectively, both from site 6. The uptake ranges are encouraging when compared to reported toxic concentrations in plants as 30 and 0.1 mg/kg for Pb and Cd respectively (Liphadzi and Kirkham 2005).

All plant samples tested showed higher Pb and Cd accumulation in leaves compared to roots indicating greater allocation of metal to leaves. Similar studies have shown that leaves act as main sinks for heavy metals in hyperaccumulator plants (Psaras and Manetas 2001). This is attributed to the efficient translocation of heavy metals from roots to shoots (Küper et al. 2000) and is considered an advantageous strategy as the root system is the primary target in heavy metal toxicity. Accumulation of potentially toxic metals in leaves is also thought to be a plant's defensive strategy against herbivores (Liphadzi and Kirkham 2005). Significantly, all plant samples analyzed except site 6 and 7 exhibited higher concentrations of Pb as compared to associated soil samples. This indicates good uptake and accumulation of Pb in *P. juliflora*. Similarly, Cd was found too in higher concentration in plant samples compared to soil except site 2. Highly significant correlation was found between Pb in soil and leaf (r = 0.972) and soil and root (r = 0.976) ($p \leq 0.01$).

The ratios of Pb in *P. juliflora* leaves to Pb in soil are in the range 1.46–0.62 while similar ratios of Cd are in the range of 1.61–0.85. These ratios are an indication of good accumulation of Pb and Cd in *P. juliflora* as a ratio greater than 1 indicates higher accumulation of metals in the plant (Barman et al. 2000). Pollutant bioavailability depends on the chemical properties of the pollutant, soil properties, environmental conditions and biological activity. Though Pb levels were higher than Cd levels in all soil samples, it is well documented that Cd is toxic to plants in far lower concentrations than Pb. Plants at all the sites studied showed a far higher uptake of Cd. This could be related to the fact that metals like Pb are mostly immobile in the soil, which reduces their bioavailability and subsequent uptake by the plant (Lombi et al. 2001). Cd on the other hand is usually less adsorbed by soil and organic matter which makes it more available to plants. Accumulation of Pb in leaf showed highly significant positive correlation with Pb content in root ($r = 0.976$), and soil ($r = 0.972$) ($p \leq 0.01$). Similarly, Cd content in leaf also exhibited positive correlation with Cd content in root ($r = 0.884$), and soil ($r = 0.857$) ($p \leq 0.05$). Analysis of Variance (ANOVA) showed significant difference ($p < 0.001$) for Pb and Cd content among zones (Control, riverbank, riverbank/roadside, roadside, industrial and residential) and medium (soil, leaf and root). Further, no significant difference was obtained in mean Pb and Cd content in soil, leaf and root between control and residential zones.

In general soil pH seems to have the greatest effect of any single factor on the availability of metals in soil (Ghosh and Singh 2005). All sites had a soil pH in the range of 7–8 (Table 1). It has been demonstrated (Pierzyenski et al. 1994) that at pH levels between 6 and 11, Lead (Pb^{2+}) changes to form $PbOH^{+}$ (solid phase). This could also account for lower uptake of Pb detected in plant samples in contrast to Cd. Organic matter content is known to influence the bioavailability of metals because some metals may form complexes with organic matter (Yoo and James 2002), thus reducing their bioavailability. Organic matter content was quite low at all sites tested as they were wastelands where only the hardiest of weeds were observed. So this parameter was not of much practical importance in this study. These parameters did not show any positive correlation with concentrations of Pb and Cd in various samples.

Presence of metals in high concentration in soil show potential toxic effects on overall growth and metabolism of plants. Chlorophyll, essential for photosynthesis, is directly influenced by environmental factors. Chlorophyll 'a' content was found greater than chlorophyll 'b' in *P. juliflora* leaves. Similar findings have also been reported in *C. procera* (D'souza et al. 2010). Lowest Chlorophyll content (Fig. 1) in leaf samples were reported from site 6 and 7 (industrial) with 0.73 and 0.62 mg/g respectively, this could be due to presence Pb and Cd in high concentration in soil disrupting the photosynthetic pigment synthesis in *P. juliflora.* Site 4 and 5 which is on roadside, also shows the reduction in chlorophyll values as compared to control (site 1), drop in chlorophyll levels could be attributed to the additional stress these plants were exposed to in the form of aerial deposition of various pollutants from vehicular emissions combined with elevated levels of Pb and Cd in the soil (Nabuloa et al. 2006). Plants at riverbank (sites 2 and 3) and residential (sites 8 and 9), do not exhibit marked variation from site 1 (control), due to sufficient availability of water & low metal pollution content at these sites.

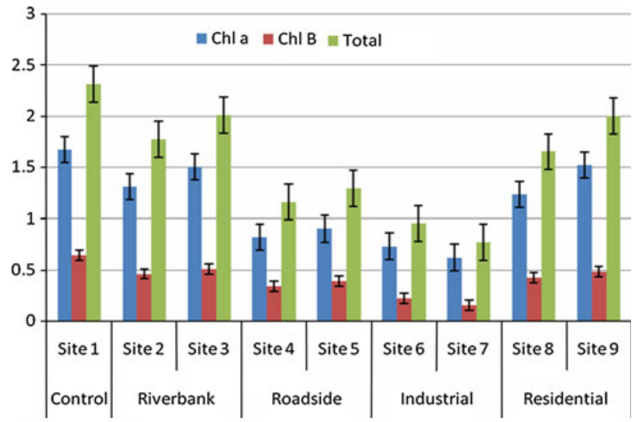

Fig. 1 Chlorophyll pigments (mg/g) in *P. juliflora* (leaf)

The sites were surveyed on a bimonthly cycle for 2 years from November 2008–October 2010 to compare *P. juliflora* plants at the sites with each other as well as with the control plants in the college garden. Plants from roadside and industrial sites exhibited delayed reproductive phases and significantly poor overall health of the individuals. This could again be due to the deposition of vehicular and industrial emissions on the plants combined with elevated levels of heavy metals as sites 2 and 3 are along the busiest national highways in the region. Plants from riverbank and residential sites were not much affected by the ambient levels of Pb and Cd in soil and their health was comparable to plants present at site 1 i.e. control.

Phytoremediation in developing countries like India, offers a feasible and economic alternative to achieve remediation of contaminated soils. *P. juliflora* has a good phytoextraction potential as shown by the accumulation ratios under natural conditions. Luxuriant growth was observed throughout the year at most of the sites selected. It proliferates freely through seeds even in adverse conditions with practically no agronomic inputs. The value of this tree lies in its exceptional tolerance to drought, saline soils and seasonal waterlogging. *P. juliflora* has been planted successfully on soils with acid to alkaline reactions. Since it is not consumed by humans or livestock, it is a safe and effective choice to be used as a tool of phytoremediation.

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References

Arnon DI (1949) Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Barman SC, Sahu RK, Bhargava SK, Chatterjee C (2000) Distribution of heavy metals in wheat, mustard and weed grown in field irrigated with industrial effluents. Bull Environ Contam Toxicol 64:489–496

D'souza RJ, Varun M, Masih J, Paul MS (2010) Identification of *Calotropis procera* L. as a potential phytoaccumulator of heavy metals from contaminated soils in Urban North Central India. J Hazard Mater 184:457–464

Duruibe JO, Ogwuegbu MOC, Egwurungu JN (2007) Heavy metal pollution and human biotoxic effects. Int J Phys Sci 2(5): 112–118

Ghosh M, Singh SP (2005) A review of phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18

Jackson ML (1973) Soil chemical analysis. Prentice Hall of India Pvt. Ltd, New Delhi

Küper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in hyperaccumulator *Arabdiopsis halleri*. Planta 212:75–84

Liphadzi MS, Kirkham MB (2005) Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment. S Afr J Bot 71(1):24–37

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal contaminated soil: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30:1919–1926

Luo C, Shen Z, Li X (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59:1–11

Nabuloa G, Origa HO, Diamond M (2006) Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. Environ Res 101:42–52

Peng KJ, Luo CL, Chen YH, Wang GP, Li XD, Shen ZG (2009) Cadmium and other metal uptake by *Lobelia chinensis* and *Solanum nigrum* from contaminated soils. Bull Environ Contam Toxicol 83:260–264

Pierzyenski GM, Schnoor JL, Banks MK, Tracy JC, Licht LA, Erickson LE (1994) Vegetative remediation at superfund sites. In: Hester RE, Harrison RM (eds) Mining and its environmental impact. Royal Society of Chemistry, Cambridge, United Kingdom, pp 49–69

Piper CS (1966) Soil and plant analysis. Interscience Publisher, New York

Psaras GK, Manetas Y (2001) Nickel localization in seeds of the metal hyperaccumulator *Thlaspi pindicum* Hausskn. Ann Bot 88:513–516

Varun M, D'souza RJ, Kumar D, Paul MS (2010) Bioassay as monitoring system for lead phytoremediation through *Crinum asiaticum* L. Environ Monit Assess. doi:10.1007/s10661-010-1696-9

Yoo MS, James BR (2002) Zinc extractability as a function of pH in organic waste-amended soils. Soil Sci 167:246–259