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Identification of *Calotropis procera* L. as a potential phytoaccumulator of heavy metals from contaminated soils in Urban North Central India

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ABSTRACT

Lead and cadmium levels were monitored in soil at fifteen urban (riverbank, roadside, industrial and residential) sites in the north central part of India. *Calotropis procera*, a hardy xerophytic plant was identified and selected for remedial potential as it was seen growing well at all sites. Root and leaf samples were collected simultaneously with soil samples to assess the characteristics of accumulation and tolerance of Pb and Cd in *C. procera*. Chlorophyll and phenological studies were undertaken to investigate the health of plants. The overall trend of Pb and Cd content in soil and plant samples was in the order Industrial > Roadside > Riverbank > Residential. The highest uptake of both the metals was observed in plants from industrial sites. Sites with more anthropogenic disturbance like vehicular and machinery exhausts exhibited reduced chlorophyll levels, stunted growth as well as a delayed, shortened reproductive phase. The ratios of Pb in leaves to Pb in soil were in the range of 0.60–1.37; while similar ratios of Cd were in the range of 1.25–1.83. Highly significant correlation coefficients were determined between concentrations of Pb and Cd in the samples with R^2 values 0.839 for soil, 0.802 for leaf and 0.819 for root samples. The strong correlation between the degree of contamination and concentrations of Pb and Cd in plant samples identifies *C. procera* as an effective heavy metal remediator of contaminated lands coupled with environmental stress.

1. Introduction

All the compartments of the biosphere have been polluted by varied inorganic and organic contaminants due to anthropogenic causes that have altered the natural biogeochemical cycling. Sources of contamination include mining and refining of metals, energy and fuel production, application of fertilizers, etc. Lead (Pb) and cadmium (Cd) are persistent environmental contaminants since they cannot be degraded or destroyed. Pb is emitted 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 [1], 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 [2] and from vehicular exhausts and fertilizers.

Pb and Cd do not occur naturally in living organisms and have been reported not to have any known function in them [3], yet these toxic metals are absorbed by humans and animals through food, air and water and tend to accumulate. Lead poisoning damages all important organ systems notably the nervous system [4]. It can be harmful even at very low concentrations because it tends to bioaccumulate. Elevated levels of Cd can cause renal dysfunction, pulmonary emphysema and bone demineralization [5,6].

Agra (India) on the banks of the river Yamuna is a major international tourist attraction in the north-central region of India. Its satellite towns situated on the busiest national highway are subjected to heavy pollution levels due to the rapid pace of industrialization, urbanization and development.

The quantum of vehicular traffic has increased manifold in recent years. The river Yamuna located in the study area is also exposed to pollution from commercial and domestic waste discharged into the river as well as the heavy incoming and outgoing traffic. It is also exposed to the threats of agricultural runoff. The riverbed and riverbank thus, act like a sink for the pollutants that the river carries.

Several steps have been taken by the administration including industrial monitoring as well as traffic restriction in the vicinity of the Taj to combat and reduce further pollution, but simultaneous efforts to remediate and reclaim soil and water sources are urgently needed.

Several technologies exist for remediation of metal contaminated soils and water, but the emphasis is on 'green' phytoremedial systems nowadays. Phytoremediation is a practical, natural method that relies on plants to extract and concentrate pollutants includ-

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ing heavy metals from the environment into their tissues [7]. 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. It has been demonstrated [8–10] 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 lemongrass, Siam weed, wild grasses, vetiver, *Sesbania* spp., *Avena* and *Crotalaria* to name a few [11–17].

In the seasonal surveys carried out to identify the flora at the sampling sites, *Calotropis procera* L., a hardy plant that shows xerophytic adaptations was the dominant species at all sites. Other weeds were seasonal and too short-lived for practical remediation. *C. procera* also has the advantage of multiplying rapidly through root suckers so its above-ground parts can be repeatedly harvested without the need of reseeding. Therefore, this plant appeared to be of particular interest in this context, and thus, was selected for this study.

Stringent standards for maximum permissible limits have been set by agencies like the World Health Organization (WHO) and the United States Environment Protection Agency (USEPA). The levels of toxicants in urban soils have been monitored and well documented in developed countries of North America and Europe [18–23], but there is an acute paucity of data on heavy metal pollution and its remediation in developing countries. Phytoremediation is especially suited for countries where such labour, expertise and cost saving techniques are vital due to constraints of funds for other methods. The study was conducted to fill this gap by coming up with a comprehensive picture of Pb and Cd contamination in the semi-arid north-central urban zone in India as well as to identify potential candidate species for efficient, practical phytoremediation.

2. Materials and methods

2.1. Experimental design

Fifteen sites within the city limits of Agra and neighbouring towns were selected and a survey carried out to evaluate the existing levels of Pb and Cd in the soil. Along with soil samples, root and leaf samples of *C. procera* were collected to assess characteristics of accumulation and tolerance of Pb and Cd in the species. Chlorophyll and phenological studies were undertaken to investigate the overall health of plants in order to further evaluate their performance as a possible metal extractor species under prevalent soil conditions.

2.2. Description of sites

Agra, the city of Taj Mahal (27°10′N 78°02′E) is located in north-central India, in Uttar Pradesh and is connected to the national capital New Delhi, 200 km to the north by the National highway 2. Mathura and Shikohabad, the selected satellite sites are roughly 60 km north and east of Agra on the same highway. 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 of Rajasthan state. The fifteen sites selected in the target area as outlined in Fig. 1 characterize different urban localities *viz.*—riverbank (Sites 2, 3, 4) roadside (Sites 4, 5, 6, 7), industrial (Sites 8, 9, 10, 11) and residential (Sites 12, 13, 14, 15) zones. The riverbank sites represent the metal profile of the riverbanks of the river Yamuna. The roadside sites were at high traffic zone intersections along the busy National highway connecting the three cities. Industrial sites represent heavy machinery and leather goods industries equally. The residential sites were taken across the economic spectrum. Site 12 is a now-dismantled petroleum storage site. Soil from the botanical garden on the college campus was taken as control (Site 1).

2.3. Calotropis procera

Calotropis procera L. is a hardy xerophytic plant, which establishes well and quickly by producing root suckers. Commonly called milkweed or madar, it is an erect, spreading shrub, at times a small tree up to 4 ft. tall with copious milky sap. The leaves are large, oblong, glaucous, grey-green, and sessile with a pointed tip and two rounded basal lobes. Flowers are waxy white with 5 sepals, 5 petals tinted purple inside with a purplish crown-like structure at the centre that has the reproductive whorls. Flowers are carried in dense stalked clusters at the end of branches. The fruit is a recurved, inflated, grey-green follicle, 6–9 cm long containing numerous ovoid seeds with long tufts of silky white hairs at one end.

2.4. Collection of soil and plant samples

At each site, soil samples were collected from a depth of 5–15 cm using a core sampler. Five random samples were collected from each site to obtain a comprehensive profile of the 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.

2.5. Soil and plant analysis

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 [24] where 10 ml 1N 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 phosphate was determined by digital spectrophotometer (Systronics) [25], available potash by flame photometer (mediFlam) and available Nitrogen by micro-kjeldahl method [25].

For Pb and Cd content, soil, leaf and root samples from all the sites were digested for subsequent analysis. 0.5 g soil sample was digested using a wet digestion method with HNO_3 and $HClO_4$ (3:1 ratio) and boiled on a hot plate for 20 min. Plant samples were analyzed by dry ash method where the samples were ashed in a muffle furnace and 0.5 g cooled ash was dissolved in HNO_3 and boiled for 20 min on a hot plate. The filtrate in each case was analyzed for Pb and Cd content by Atomic Absorption Spectrophotometry (AAnalyst100, PerkinElmer, USA).

Chlorophyll content in *C. procera* 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 spectrophotome-

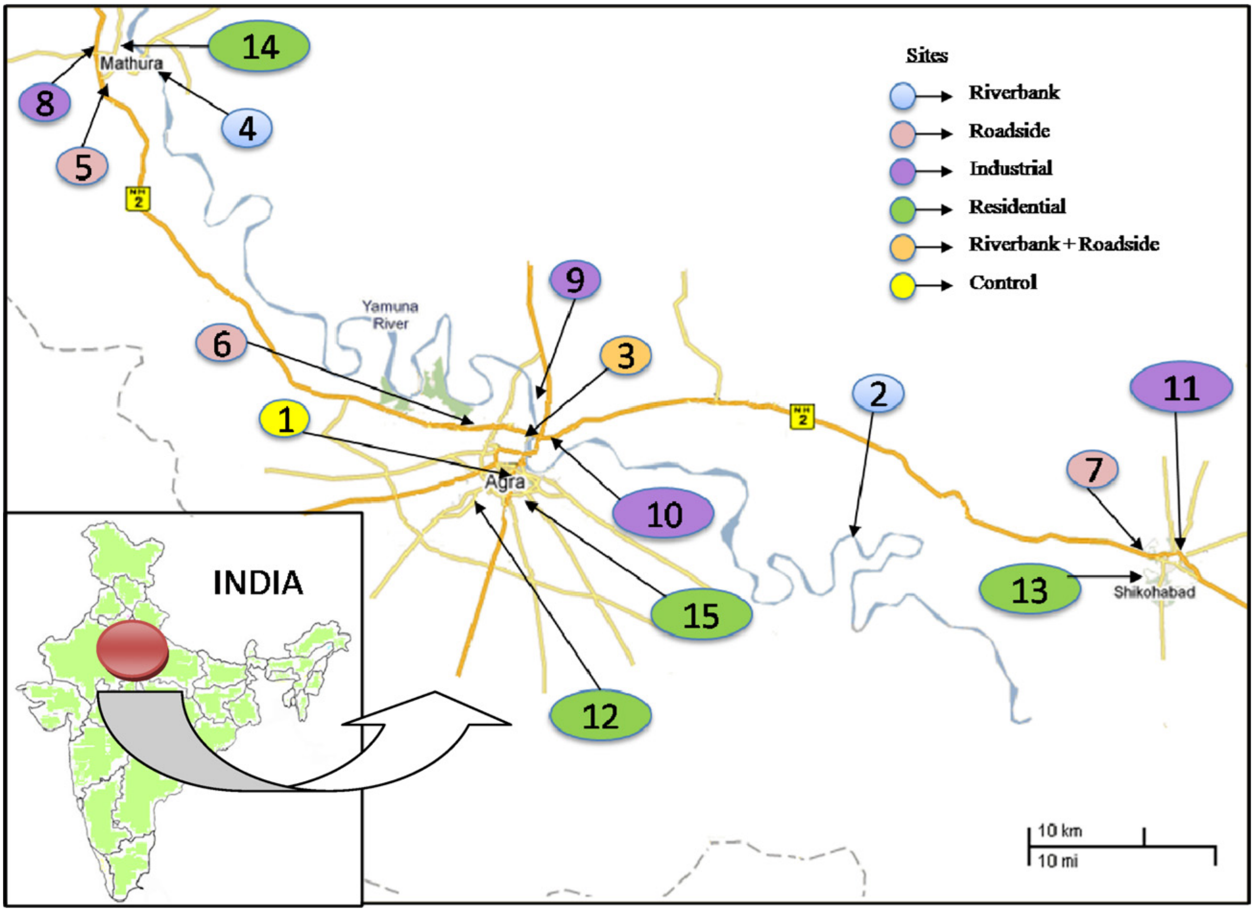

Fig. 1. Map of the study region indicating sampling locations.

ter at different wavelengths, i.e. 480 nm, 510 nm, 630 nm, 645 nm, 652 nm and 665 nm and chlorophyll content was calculated using relevant formulae [26].

2.6. Phenological studies

The region experiences three seasons mainly — summer, monsoon and winter. The sites were surveyed on a bimonthly cycle for two years from October 2007 to September 2009 to compare the phenology of *C. procera* plants at the sites with each other as well as with the control plants in the college garden.

2.7. Statistical analyses

Pearson's coefficient for correlation and linear regression of the data were statistically analyzed at a significance level of $P < 0.05$ and $P < 0.01$ with SPSS 16.0 statistics software. Two way and One way Analysis of Variance (ANOVA) was calculated for data and means compared using Fisher's LSD method. For ANOVA, the sites were jointly tested as zones (Table 2) for better comprehension.

3. Results

3.1. Distribution of Pb and Cd in soil samples

The concentrations of Pb and Cd detected in soil samples are recorded in Table 2. Maximum Pb and Cd were found at Site 10 (urban industrial) with 134 mg/kg Pb and 3.6 mg/kg Cd. Levels of Pb and Cd were each recorded in the order Industrial > Roadside > Riverbank > Residential. 10–100 mg/kg 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 [27]. The high level of Pb and Cd at Sites 10 and 9 indicates pollution in the heavy industrial area. The high levels of the metals at Site 12 (residential) in comparison to other residential zones are due to storage of petro products at this erstwhile oil depot site though they were well below the toxic levels in soil set by WHO. This may be attributed to the fact that the site has now been a wasteland for quite a few years and *C. procera* was found to be the only plant flourishing. Site 3 had higher Pb levels than all other riverbank sites as expected, due to deposition of

Table 1
Physico-chemical profile of soil samples.

Zones	pH (1:2.5)	Electrical conductivity (dS/m) (1:2.5)	Organic matter (%)	Available phosphate (kg/ha)	Available potash (kg/ha)	Available nitrogen (kg/ha)
Control	8.10	0.54	1.1	17.5	393	112.896
Riverbank	7.20–7.57	0.39–0.47	0.49–0.69	55.0–65.0	178–371	66.057–87.808
Roadside	7.70–8.00	0.46–0.50	0.24–0.30	12.0–17.0	236–398	38.135–52.176
Industrial	7.25–7.39	0.72–0.98	0.17–0.25	16.7–32.5	456–780	56.19–77.808
Residential	7.81–8.20	0.34–0.50	0.78–1.6	21.3–40.0	560–713	87.716–96.182

Table 2
Pb and Cd content in soil and plant (*C. procera)* samples.

Zones	Sites	Pb content (mg/kg)			Cd content (mg/kg)		
		Soil	Leaf	Root	Soil	Leaf	Root
Control	Site 1	5.4 ± 0.5a	7.4 ± 1.0a	5.0 ± 0.4a	0.82 ± 0.2a	1.5 ± 0.4a	1.2 ± 0.9a
Riverbank	Site 2	11.2 ± 2.9b	11.4 ± 3.1a	9.2 ± 2.1b	1.2 ± 0.2a	2.0 ± 0.4a	1.4 ± 0.2a
	Site 3	26.4 ± 4.3c	18.4 ± 1.7b	14.1 ± 2.5c	1.4 ± 0.4a	2.2 ± 0.2a	1.6 ± 0.1b
Riverbank/roadside	Site 4	24.2 ± 4.2c	24.5 ± 2.6c	19.2 ± 3.0d	1.6 ± 0.2a	2.0 ± 0.3a	2.0 ± 0.2b
Roadside	Site 5	28.7 ± 1.6c	25 ± 2.4c	19.8 ± 2.6d	1.4 ± 0.1a	1.9 ± 0.2a	1.7 ± 0.1b
	Site 6	30.6 ± 2.3c	28.1 ± 2.0c	17.4 ± 0.9d	1.5 ± 0.1a	2.1 ± 0.1a	1.5 ± 0.1c
	Site 7	18.6 ± 1.6d	17.9 ± 1.1b	15.3 ± 0.3d	1.1 ± 0.1a	1.9 ± 0.2a	1.3 ± 0.1c
Industrial	Site 8	44.8 ± 4.5e	32.3 ± 1.4c	25.6 ± 0.9d	2.0 ± 0.3b	2.8 ± 0.1b	2.0 ± 0.2b
	Site 9	120.1 ± 18.6f	78.4 ± 9.5d	54.5 ± 3.6c	2.8 ± 0.4c	3.5 ± 0.1c	3.1 ± 0.2d
	Site 10	134 ± 6.2g	80.6 ± 3.9d	55.1 ± 4.0c	2.7 ± 0.4c	3.6 ± 0.3c	3.0 ± 0.3d
	Site 11	33.4 ± 3.9c	23.7 ± 2.1c	19.6 ± 2.4d	1.4 ± 0.3a	1.7 ± 0.3a	1.2 ± 0.1a
Residential	Site 12	34.4 ± 3.0c	26.2 ± 2.2c	20.8 ± 2.1d	2.0 ± 0.4d	2.6 ± 0.4d	2.2 ± 0.3b
	Site 13	7.6 ± 0.8a	8.1 ± 1.6a	5.5 ± 1.4a	ND	ND	ND
	Site 14	10.1 ± 0.6a	11.3 ± 0.9a	8.9 ± 1.2b	ND	ND	ND
	Site 15	16.3 ± 1.9h	14.5 ± 2.6b	12.8 ± 2.6b	0.93 ± 0.1a	1.3 ± 0.3a	1.1 ± 0.2a

Different letters in the same column denote significant statistical difference ($P < 0.001$) in mean Pb and Cd content at the sites selected.

the heavy metal arising from heavy traffic near it. Site 4 had similar conditions hence exhibited a minor difference in Pb content. In the case of Cd also, Sites 3 and Site 4 exhibited minor variations in concentrations.

Correlation analysis was performed using univariate Pearson's correlation coefficient for all pairs of Pb and Cd concentrations (Table 3) to determine relationships between the two metal concentrations in soil, leaf and root samples from all the sites. Pb in soil showed significant correlation with Cd content in soil along with Pb and Cd content in *C. procera* leaf and root.

3.2. Distribution of Pb and Cd in C. procera

The results of analysis of *C. procera* leaf and root samples are also recorded in Table 2. Pb content values in *C. procera* leaf samples are within the range of 7.4–80.6 mg/kg whereas in roots they are in the lower range of 5.0–55.1 mg/kg. Levels of Pb in plant samples increased in proportion to its concentration in soil samples. Highest uptake values of Pb recorded were 80.6 mg/kg and 55.1 mg/kg in *C. procera* leaf and root samples, respectively, both from Site 10 which had the highest Pb levels of all sites.

Cd concentrations in leaf and root samples are in the range of 1.3–3.6 mg/kg and 1.1–3.0 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 samples detected was 3.6 mg/kg from Site 10, while that in root samples was 3.0 mg/kg detected at Site 9. Both sites showed the highest Cd levels in soil with a minor difference. The uptake ranges are encouraging when compared to reported toxic concentrations in plants as 30 mg/kg and 0.1 mg/kg for Pb and Cd, respectively [27].

4. Discussion

4.1. Uptake and sequestration of Pb and Cd in C. procera

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 [28,29]. This is attributed to the efficient translocation of heavy metals from roots to shoots [30] and is considered an advantageous strategy as the root system is the primary target in heavy metal toxicity [31,32]. Accumulation of potentially toxic metals is also thought to be a plant's defensive strategy against herbivores [33].

Significantly, all plant samples analyzed except Site 11 exhibited higher concentrations of Cd as compared to associated soil samples. This indicates good uptake and accumulation of Cd in *C. procera*. Cd in soil and root showed significant correlation with $R^2 = 0.848$. Leaf samples from only four Sites 2, 4, 13 and 14 showed higher Pb concentrations as compared to soil. As far as Pb uptake is concerned, lower values were recorded in all plant samples as compared to soil excepting Sites 2, 4, 13 and 14, where slightly higher values were obtained in leaf samples.

The ratios of Pb in *C. procera* leaves to Pb in soil are in the range 1.37–0.60 while similar ratios of Cd are in the range of 1.83–1.25. These ratios are an indication of good accumulation of Pb and Cd in *C. procera* as a ratio greater than 1 indicates higher accumulation of metals in the plant [34]. 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 [35]. Cd on the other hand is usually less adsorbed by soil and organic matter which makes it more available to plants [36–38].

From Table 3, where correlation coefficients are recorded, several interesting inferences can be drawn. Sequestration of Pb in leaf showed highly significant positive correlation with Pb content in root ($R^2 = 0.986$), and soil ($R^2 = 0.982$). Similarly, Cd content in leaf also exhibited positive correlation with Cd content in root ($R^2 = 0.858$), and soil ($R^2 = 0.882$).

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). On comparing means by Fisher's LSD test, a significant difference ($P < 0.001$) between mean Pb and Cd content in soil, leaf and root at sites in industrial zone vs. those of all other zones was obtained. No significant difference was obtained in mean Pb and Cd content in soil, leaf and root between control and residential zones Significant difference ($P < 0.001$) was seen in mean Cd content between *C. procera* leaf and root samples.

Table 3
Correlation coefficients among concentrations of Pb and Cd in soil and plant samples, soil pH and organic matter.

	Pb in soil	Pb in leaf	Pb in root	Cd in soil	Cd in leaf	Cd in root	Soil pH
Pb in leaf	0.982**						
Pb in root	0.968**	0.986**					
Cd in soil	0.837**	0.864**	0.877**				
Cd in leaf	0.867**	0.871**	0.869**	0.882**			
Cd in root	0.816**	0.830**	0.825**	0.848**	0.858**		
Soil pH	0.034	0.056	0.055	0.057	0.057	−0.090*	
Organic matter	−0.448*	−0.495*	−0.522**	−0.374*	−0.407**	−0.310*	−0.062

*Correlation is significant at 0.05 level (2-tailed).
**Correlation is significant at 0.01 level (2-tailed).

4.2. Soil pH and organic matter

In general soil pH seems to have the greatest effect of any single factor on the availability of metals in soil [39,40]. All sites had a soil pH in the range of 7–8 (Table 1). It has been demonstrated [41] 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 [42,43], thus reducing their bioavailability. As data in Table 1 show 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 as seen from Table 3.

4.3. Pb–Cd interaction

Highly significant correlation coefficients were determined between concentrations of Pb and Cd in soil, leaf and root samples. R^2 value for soil was 0.839 for soil (Fig. 2a). Correlation between Pb and Cd content in *C. procera* leaf and root samples was also found to be highly significant with $R^2 = 0.802$ for leaf (Fig. 2b) and 0.819 (Fig. 2c) for root. Pb and Cd levels in soil at the sites were synchronous barring a few samples. Site 10 with highest Pb level also had highest Cd level. The case with leaf and root samples was also the same. Thus, it is concluded that Pb did not inhibit the uptake and sequestration of Cd in *C. procera* samples and vice versa.

4.4. Chlorophyll patterns in C. procera leaf samples

Photosynthesis is perhaps the most basic aspect in plant metabolism assessment related to growth and survival in adverse conditions. Metals like Cd, Pb, Zn, Cr, etc. [44], when present 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 greater than chlorophyll 'b' content in *C. procera* leaves. Similar findings have also been reported in many arid zone plant species, weeds and crops [45,46]. Chlorophyll contents (Fig. 3) of leaf samples from Sites 2, 11, 12, 13, 14 and 15 do not exhibit marked variation from control, i.e. Site 1. Sites 2 and 11 were riverbank and industrial sites, respectively whereas all others were in the residential zones. Significantly, leaf samples from Site 12 with highest amongst residential Pb and Cd levels in soil and plant tis-

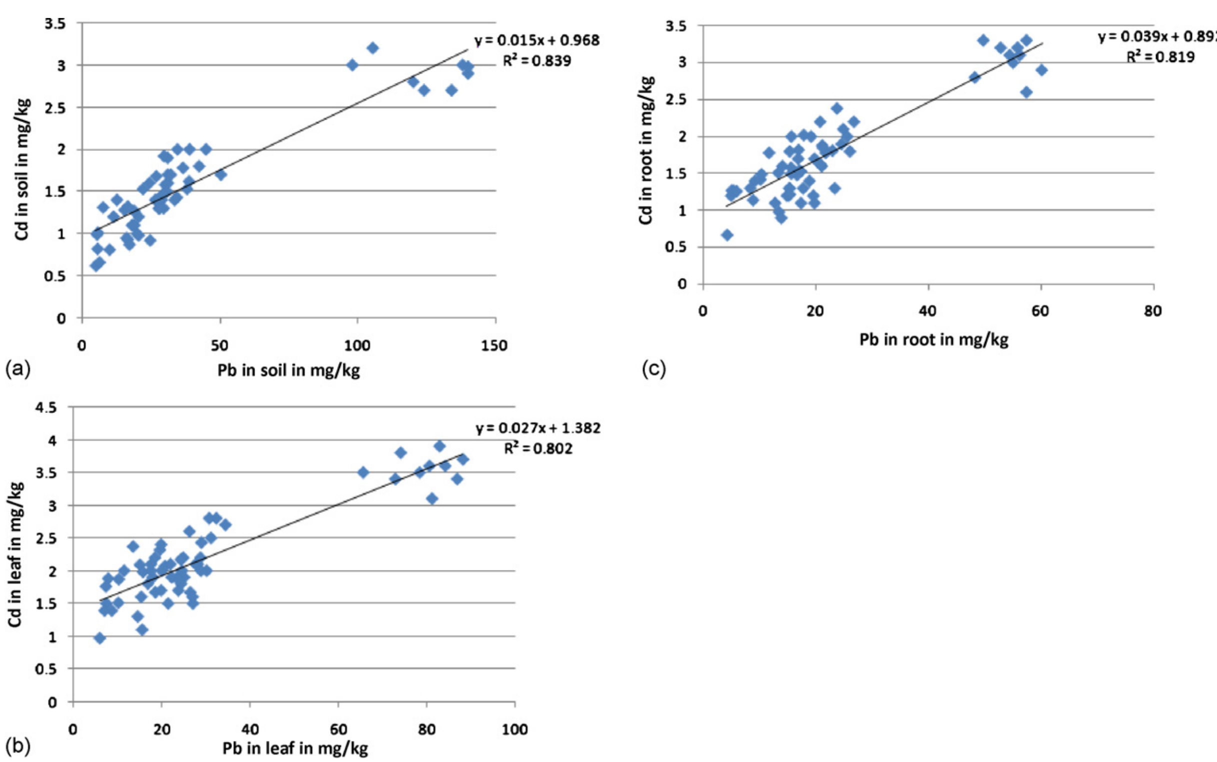

Fig. 2. Relationships between concentrations of Pb and Cd in soil (a), *C. procera* leaf (b) and root (c) samples ($n = 75$).

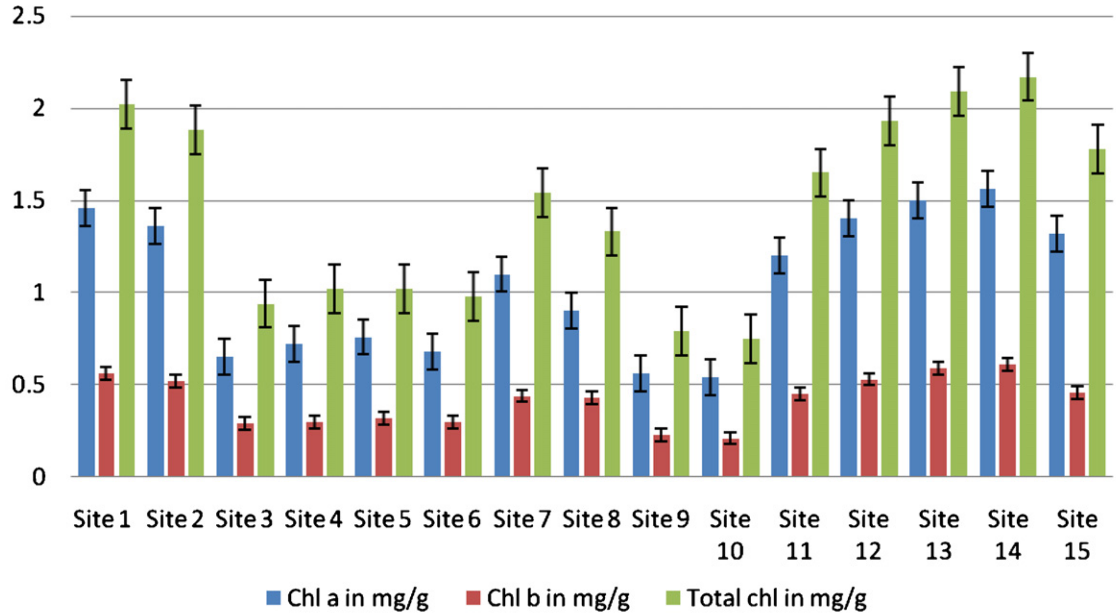

Fig. 3. Chlorophyll pigments in *Calotropis procera* leaf samples.

sues had chlorophyll contents almost similar to the control, i.e. Site 1. This indicates that at the present ambient concentrations in soil, Pb and Cd did not disrupt the photosynthetic mechanism of *C. procera* drastically. Plants at riverbank Sites 3 and 4, roadside Sites 5 and 6 as well as industrial Sites 9 and 10 exhibited marked reduction in chlorophyll content as compared to Site 1 (control). It is significant to note that the above-mentioned riverbank and industrial sites were also exposed to heavy traffic. Hence the 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 [47–50]. Significant difference ($P < 0.003$) was obtained for total chlorophyll content among different zones studied. On comparing means by Fisher's LSD test, chlorophyll content in control vs. all zones except residential zone was significantly different ($P < 0.02$).

4.5. Phenological patterns in C. procera leaf samples

The growth calendar of *C. procera* as observed at the sites has been recorded in Fig. 4. As seen from phenological patterns, plants at roadside and industrial sites exhibited significantly delayed reproductive phases and poor overall health of the individuals. This could again be due to the deposition of vehicular emissions on the

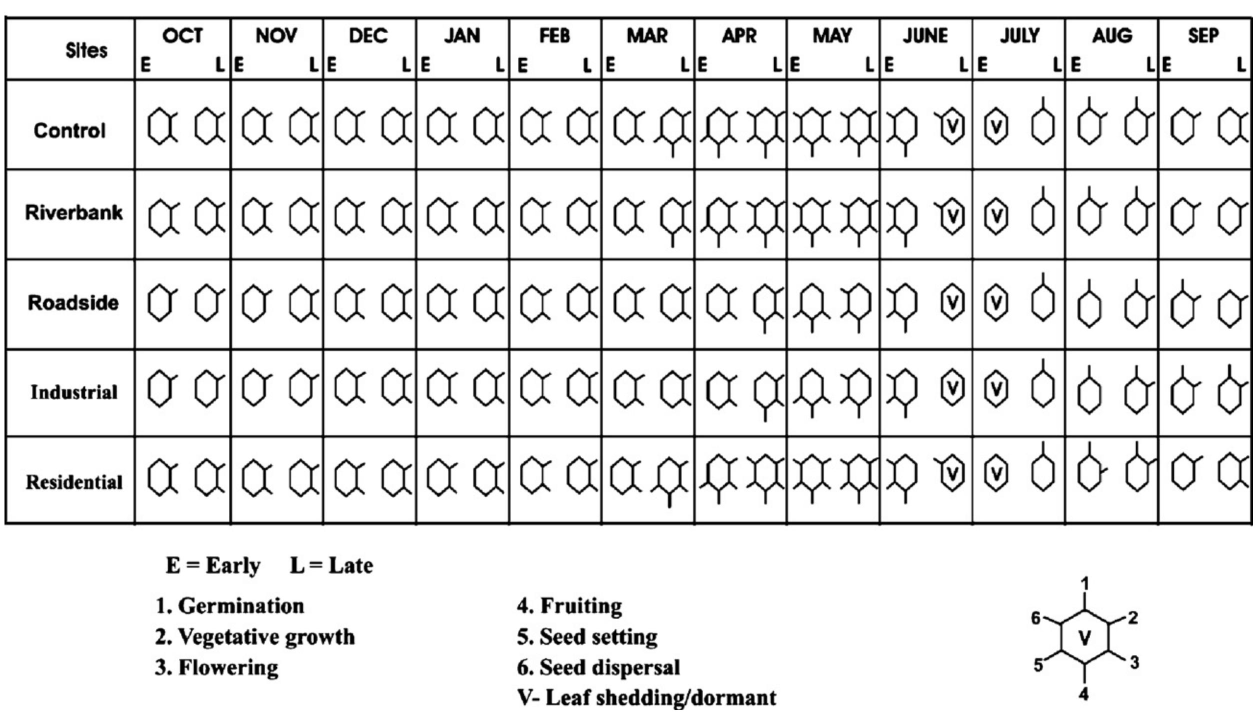

Fig. 4. Phenological observations of *Calotropis procera* at the test sites from October 2007 to September 2009.

plants combined with elevated levels of heavy metals as Sites 4, 5, 6 and 7 are along one of the busiest national highways in the region. Plants at 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 at Site 1 (control).

4.6. Prospects of Calotropis procera as a phytoextractor

Phytoextraction efficiency is determined by two key factors: metal hyperaccumulating capacity and biomass production [51]. Decontaminating a site in a reasonable number of harvests, thus, requires plants that are both high yielders of biomass and good metal accumulators by dry weight. Although known metal hyperaccumulators like *Thlaspi caerulescens* and *Alyssum bertolonii* are able to take up and accumulate appreciable amounts of metals in their tissues, their use in the field is limited because of their slow growth, shallow root system and low biomass production [9,52]. Hence, good phytoextractors, that are non-hyperaccumulators, with a greater growth potential compared with hyperaccumulators should be considered positively [53], in that they can compensate for their lower accumulation potential with their higher biomass. It is also an established fact that accumulation of heavy metals in leaves rather than roots is an advantageous strategy for a plant because the root system is the primary target of metal toxicity [31,32] as already stated earlier. The advantage of native species over non-native species has already been discussed [8–10]. On comparing the luxuriant vegetative growth and overall health of *C. procera* as indicated by near normal phenological and pigment patterns, the question of countering toxicity at root level appears to be of little consequence in this plant. Furthermore, this paper deals with the extraction potential of *C. procera in situ* without any aid. Studies to improve the uptake by altering soil pH, adding suitable fertilizers and soil amendments especially synthetic chelating agents to increase metal bioavailability and uptake are already in progress. The results obtained so far are quite promising, especially in the case of Pb.

5. Conclusion

Calotropis procera has good phytoextraction potential as shown by the accumulation ratios under natural conditions. It is well adapted and proliferates freely through seeds and root suckers even in adverse conditions with practically no agronomic input. Luxuriant growth was observed throughout the year at most of the sites selected. Since it is not consumed by humans or livestock, it is a safe choice to be used as an instrument of phytoremediation.

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References

[1] S.H. Badawy, M.I.D. Helal, A.M. Chaudri, K. Lawlor, S.P. McGrath, Soil solid-phase controls lead activity in a soil solution, J. Environ. Qual. 31 (2002) 162–167.
[2] J.O. Duruibe, M.O.C. Ogwuegbu, J.N. Egwurungu, Heavy metal pollution and human biotoxic effects, Int. J. Phys. Sci. 2 (5) (2007) 112–118.
[3] Lenntech Water Treatment and Air Purification. Water Treatment, 2004, published by Lenntech, Rotterdamseweg, Netherlands (www.excelwater.com/thp/filters/Water-Purification.htm).
[4] M.O. Ogwuebgu, W. Muhanga, Investigation of lead concentration in the blood of people in the copperbelt province of Zambia, J. Environ. 1 (2005) 66–75.
[5] M.H. Bhattacharya, B.D. Whelton, D.P. Peterson, B.A. Carnes, E.S. Moretti, J.M. Toomey, L.L. Williams, Skeletal changes in multiparous mice fed a nutrient-sufficient diet containing cadmium, Arch. Toxicol. 50 (1988) 193–204.
[6] D.E. Salt, I.J. Pickering, R.C. Prince, D. Gleba, S. Dushenkov, R.D. Smith, I. Raskin, Metal accumulation by aquacultured seedlings of Indian mustard, Environ. Sci. Technol. 31 (1997) 1636–1644.
[7] I. Raskin, R.D. Smith, D.E. Salt, Phytoremediation of metals: using plants to remove pollutants from the environment, Curr. Opin. Biotechnol. 8 (2) (1997) 221–226.
[8] G. Porębska, A. Ostrowska, Heavy metal accumulation in wild plants: implications for phytoremediation, Pol. J. Environ. Stud. 8 (6) (1999) 433–442.
[9] S. Saraswat, J.P.N. Rai, Phytoextraction potential of six plant species grown in multimetal contaminated soil, Chem. Ecol. 25 (1) (2009) 1–11.
[10] K.V. Nedunuri, C. Lowell, W. Meade, A.P. Vonderheide, J.R. Shann, Management practices and phytoremediation by native grasses, Int. J. Phytorem. 12 (2010) 200–214.
[11] B. Yang, W. Shu, Z. Ye, C. Lan, M. Wong, Growth and metal accumulation in Vetiver and two Sebania species on lead/zinc mine tailings, Chemosphere 52 (2003) 1593–1600.
[12] S. Uraguchi, I. Watanabe, A. Yoshitomi, M. Kiyono, K. Kuno, Characteristics of cadmium accumulation and tolerance in novel Cd-accumulating crops, *Avena strigosa* and *Crotalaria juncea*, J. Exp. Bot. 57 (12) (2006) 2955–2965.
[13] S.J. Salami, E.A. Akande, D.M. Zachariah, Level of heavy metals in soils and lemongrass in Jos, Bukuru and Environs, Nigeria, Glob. J. Pure Appl. Sci. 13 (2) (2007) 193–196.
[14] I.A. Ololande, A. Ologundudu, Concentration and bioavailability of cadmium by some plants, Afr. J. Biotechnol. 6 (16) (2007) 1916–1921.
[15] G.V. Awolola, A.O. Odeinde, O.O. Dosumu, Assessment of heavy metals concentration in soil and Siam weed, Niger. J. Soil Sci. 17 (2007) 130–132.
[16] G.K. Handique, A.K. Handique, Proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.), J. Environ. Biol. 30 (2) (2009) 299–302.
[17] V.P. Gadepalle, S.K. Ouki, T. Hutchings, Remediation of copper and cadmium in contaminated soils using compost with inorganic amendments, Water Air Soil Pollut. 196 (2009) 355–368.
[18] T. Chirenje, L.Q. Ma, M. Reeves, M. Szulczewski, Lead distribution in near surface soils of two Florida cities: Gainesville and Miami, Geoderma 119 (2004) 113–120.
[19] D. Crnković, M. Ristić, D. Antonović, Distribution of heavy metals and arsenic in soils of Belgrade (Serbia and Montenegro), Soil Sediment Contam. 15 (2006) 581–589.
[20] L. Madrid, E. Dĭaz-Barrientos, F. Madrid, Metals in urban soils of Sevilla: seasonal changes and relations with other soil components and plant contents, Eur. J. Soil Sci. 55 (2004) 209–217.
[21] M. Imperato, P. Adamo, D. Naimo, M. Arienzo, D. Stanzione, P. Violante, Spatial distribution of heavy metals in urban soils of Naples city (Italy), Environ. Pollut. 124 (2003) 247–256.
[22] L. Tijhuis, B. Brattli, O.M. Saether, A geochemical survey of topsoil in the city of Oslo, Norway, Environ. Geochem. Health 24 (2002) 67–94.
[23] J. Chronopoulos, C. Haidouti, A. Chronopoulou-Sereli, I. Massas, Variations in plant and soil lead and cadmium content in urban parks in Athens, Greece, Sci. Total Environ. 196 (1997) 91–98.
[24] C.S. Piper, Soil and Plant Analysis, Interscience Publisher, N.Y., Bombay, 1966.
[25] M.L. Jackson, Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi, 1973.
[26] D.I. Arnon, Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*, Plant Physiol. 24 (1949) 1–15.
[27] M.S. Liphadzi, M.B. Kirkham, Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment, S. Afr. J. Bot. 71 (1) (2005), 24-37.s.
[28] D.E. Salt, M. Blaylock, N.P.B.A. Kumar, D. Viatcheslav, B.D. Ensley, Phytoremediation a novel strategy for the removal of toxic metals from the environment using plants, Biotechnology 13 (1995) 468–474.
[29] G.K. Psaras, Y. Manetas, Nickel localization in seeds of the metal hyperaccumulator *Thlaspi pindicum* Hausskn, Ann. Bot. 88 (2001) 513–516.
[30] H. Küper, E. Lombi, F.J. Zhao, S.P. McGrath, Cellular compartmentation of cadmium and zinc in relation to other elements in hyperaccumulator *Arabdiopsis halleri*, Planta 212 (2000) 75–84.
[31] D.L. Godbold, W.J. Horst, J.C. Collins, D.A. Thurman, H. Marschner, Accumulation of zinc and organic acids in roots of zinc-tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*, J. Plant Physiol. 116 (1984) 59–69.
[32] A.J.M. Baker, R.D. Reeves, A.S.D. Hajar, Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (*Brassicaceae*), New Phytologist 127 (1994) 61–68.
[33] M.S. Liphadzi, M.B. Kirkham, Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment, S. Afr. J. Bot. 71 (1) (2005) 24–37.
[34] S.C. Barman, R.K. Sahu, S.K. Bhargava, C. Chatterjee, Distribution of heavy metals in wheat, mustard and weed grown in field irrigated with industrial effluents, Bull. Environ. Contam. Toxicol. 64 (2000) 489–496.
[35] E. Lombi, F.J. Zhao, S.J. Dunham, S.P. McGrath, Phytoremediation of heavy metal contaminated soil: natural hyperaccumulation versus chemically enhanced phytoextraction, J. Environ. Qual. 30 (2001) 1919–1926.
[36] M.J. McLaughlin, R.E. Hamon, R.G. McLaren, T.W. Speir, S.L. Rogers, Review: A bioavailability based rationale for controlling metal and metalloid contamination of agricultural in Australia and New Zealand, Aust. J. Soil Res. 38 (2000) 1037–1086.
[37] K. Perronnet, C. Schwartz, E. Gerard, J.L. More, Availability of cadmium and zinc accumulation in the leaves of *Thlaspi caerulescens* incorporated into soil, Plant Soil. 227 (2000) 257–263.

[38] M. Mejare, L. Bulow, Metal binding proteins and peptides in bioremediation and phytoremediation of heavy metals, Trends Biotechnol. 19 (2001) 67–73.

[39] J.R. Henry, An Overview of Phytoremediation of Lead and Mercury, NNEMS Report, Washington D.C. (2000) 3–9.

[40] M. Ghosh, S.P. Singh, A review of phytoremediation of heavy metals and utilization of its byproducts, Appl. Ecol. Environ. Res. 3 (1) (2005) 1–18.

[41] G.M. Pierzyenski, J.L. Schnoor, M.K. Banks, J.C. Tracy, L.A. Licht, L.E. Erickson, Vegetative remediation at superfund sites, in: R.E. Hester, R.M. Harrison (Eds.), Mining and its Environmental Impact, Royal Society of Chemistry, Cambridge, UK, 1994, pp. 49–69.

[42] M. Gardner, Dissolved phase speciation of Zn in the Hunber estuary, Chemosphere 38 (1999) 2117–2124.

[43] M.S. Yoo, B.R. James, Zinc extractability as a function of pH in organic waste-amended soils, Soil Sci. 167 (2002) 246–259.

[44] V. Agrawal, K. Sharma, Phytotoxic effects of Cu, Zn, Cd and Pb on *in vitro* regeneration and concomitant protein changes in *Holarrhena antidysentrica*, Biol. Plant. 50 (2006) 307–310.

[45] M.S. Paul, Seed patterns and adaptation of plants in Indian desert, Ph.D. thesis (1989) Univ. of Jodhpur, Jodhpur, India.

[46] J.P. Singh, An investigation on the effect of environment complex on *Parthenium hysterophorus* L., its adaptive strategies and management through non-chemical practices. Ph.D. thesis (2002) Univ. of Allahabad, Allahabad, India.

[47] R. Asghar, Badr-uz-Zaman, N. Nadir, Q. Nawaz, The effect of autoexhaust pollution on the growth of road side plants in twin cities, Rawalpindi and Islamabad, Sarhad J. Agric. 20 (3) (2004) 415–418.

[48] A. Banerjee, R.K. Sarkar, A. Mukherjee, Reduction in soluble protein and chlorophyll contents in a few plants as indicators of automobile exhaust pollution, Int. J. Environ. Stud. 20 (3–4) (1983) 239–243.

[49] L. Han, G. Zhuang, S. Cheng, Y. Wang, J. Li, Characteristics of re-suspended road dust and its impact on the atmospheric environment in Beijing, Atmos. Environ. 41 (35) (2007) 7485–7499.

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

[51] S.P. McGrath, F.J. Zhao, Phytoextraction of metalsand metalloids from contaminated soils, Curr. Opin. Biotechnol. 14 (2003) 277–282.

[52] M.E. Saifullah, M. Qadir, P. de Caritat, F.M.G. Tack, G. Du Laing, M.H. Zia, EDTA-assisted Pb phytoextraction, Chemosphere 74 (2009) 1279–1291.

[53] T. Vamerali, M. Bandiera, G. Mosca, Field crops for phytoremediation of metal-contaminated land—a review, Environ. Chem. Lett. 8 (2010) 1–17.