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RESEARCH ARTICLE

Assessment of Century Plant (*Agave americana*) for Remediation of Chromium Contaminated Soils

Sivakoti Ramana[1] · Sanjay Srivastava[1] · Ashis K. Biswas[1] · Ajay Kumar[1] · Amar B. Singh[1] · Dashrath Singh[1] · Poonam S. Rajput[1]

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Abstract *Agave americana* was evaluated for its tolerance to different levels of Cr (0, 25, 50, 100 and 200 mg kg^{-1} soil) and its suitability for the remediation of Cr contaminated sites. The pot culture experiment was carried out for 3 months in clay soil which was collected from 0 to 30 cm depth from the nearby agricultural field. The partitioning of Cr between roots and shoots and its uptake by the plant, bio-concentration factor, translocation factor, translocation efficiency etc. were used to determine the remediation potential of the crop. Overall, the plant could tolerate up to 200 mg Cr kg^{-1} soil, but a concentration of 81 mg Cr kg^{-1} soil caused a reduction in the dry weight of the plant by 50 %. The highest total uptake of Cr (2286 μg g^{-1} plant) and bio-concentration factor (6.59) was found at Cr 200 mg kg $soil^{-1}$. However, the translocation factor values were found to be <1 (0.18–0.13) indicating that Cr was mainly located in the roots exhibiting an exclusion mechanism. Based on these findings, it was concluded that *A. americana* could not be considered as a hyperaccumulator for Cr. Nevertheless, as shown by the accumulation ratios the plant has a massive potential for phytostabilization of Cr.

Keywords *Agave americana* · Bio-concentration factor · Chromium · Phytostabilization · Translocation efficiency · Translocation factor

✉ Sivakoti Ramana
sramana@iiss.res.in

[1] Indian Institute of Soil Science, Nabi Bagh, Berasia Road, Bhopal 462 038, India

Introduction

Pollution of soils with heavy metals is a global problem [1]. Among the heavy metals, Chromium (Cr) is an important pollutant that has been contaminating soil, sediment and ground water [2]. Contamination of Cr in soil mainly originates from its use in metallurgy, tanneries, leathers, dyes, textiles and wood preservation [3]. The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40 % of the total industrial use [4]. India is one of the largest producers of leather and nearly 80 % of the tanneries are engaged in the chrome-tanning process [5]. Considerable efforts have been made to develop suitable methods for the remediation of chromium-contaminated soils. However, these measures are very expensive. Phytoremediation has received considerable attention in recent years and it has been shown to be more preponderant than conventional technologies for remedying contaminated soils [6–8]. Till date, a number of plant species (400–450) have been identified that accumulate high amount of heavy metals in their above ground tissues on metal rich soils [9]. However, not all of these plants are ideal for phytoremediation, because most of them are slow growing (lichens, mosses or *Thlaspi* sp.) and/or some plants are too small to have a high biomass production [6]. For better land restoration or remediation, plant species used for the phytoremediation process must produce sufficient biomass while accumulating high concentration of the metal in question [10]. Thus, in order to overcome these limitations, attempts have been made to bring heavy metal tolerance from natural hyper accumulators like *Thlaspi* sp. into high biomass crop plant species like *Brassica juncea* (L.), *Helianthus annuus* L., *Zea mays* L. and *Brassica napus* L [11, 12]. Ecologically, use of edible crops for phytoremediation is not viable because the

heavy metals enter into food chain through consumption by human or animals. Therefore, researchers have proposed safe, economically feasible and eco-friendly approaches for phytoremediation using non-edible plants [13–19]. However, studies on use of non-edible plants for remediation of soils contaminated with Cr are limited [13, 20–22]. Further, the environmental conditions at polluted sites are expected to be harsh. An exhaustive screening of plants is therefore necessary for identifying the plants which can adopt these harsh conditions. It has been hypothesized that the plants which are adapted to dry/arid conditions may also tolerate heavy metal stress and play an important role in remediation of soils contaminated with heavy metals. It has been suggested that the biological and evolutionary significance of metal accumulation in plants is connected to drought resistance [23] and this mechanism may also indirectly contribute to heavy metal tolerance, as heavy metal stress is responsible for secondary water stress in plants in a way similar to salt stress [24].

The present study was therefore conducted to investigate the tolerance of century plant (*Agave americana*) to different levels of Cr and its potential for the remediation (phytoextraction or phytostabilization) of chromium contaminated soils. The century plant was selected because it belongs to one of the underexploited plants that come up well on poor and degraded soils which are otherwise unsuitable for cultivation of crops. Further, it is a high biomass producing drought tolerant plant (one hectare of *Agave* annually yields 40 to 65 tonnes of biomass per hectare) which is noted for its strong and coarse fibre which is widely used for making ropes, cordage, twine, fishing nets, door mats and rugs etc. The plant exhibits exponential reproductive capacity and higher quality of biomass (62 % cellulose content and 2.4 % lignin content) and is an ideal feedstock for an integrated biorefinery [25].

Material and Methods

The pot culture experiment was carried out in a screen house at the Indian Institute of Soil Science, Bhopal, India. The climate is humid subtropical with average annual high and low temperatures as 31.7 and 18.6 °C respectively and the mean annual rainfall 1121 mm. Soil for the experiment was collected from 0 to 30 cm depth from nearby agricultural field. The soil was clayey in texture and classified as Typic Haplusterts. The soil was air dried, gently pounded and passed through 2 mm sieve and analysed for various physico-chemical properties as pH (7.7); CEC [55 c mol (p^+) kg^{-1} soil], OC (4.75 g kg^{-1} soil), available N (112 mg kg^{-1} soil), Olsen P (2.61 mg kg^{-1} soil) and available K (227 mg kg^{-1} soil). Later, 7 kg soil was transferred to plastic pots and spiked with Cr by adding a specific amount of stock solution (1000 mg ml^{-1}) of potassium dichromate and incubated for 4 weeks. Overall, there were 5 treatments including soil without chromium i.e., 0 (control), 25, 50,100 and 200 mg Cr kg^{-1} soils. The experiment was conducted in a completely randomized design (CRD) and there were five replications for each treatment. The data was analyzed statistically and the treatment means were compared using Critical difference (CD) at 5 % probability appropriate for CRD [25]. After 1 month, soil in each pot was taken out and mixed thoroughly and extractable Cr and fractions of Cr were determined (Table 1). Extractable Cr in soil samples was carried out by DTPA in accordance with standard methods [26]. For the extraction of hexavalent chromium in soil, 1 g soil was taken to which 10 ml of 1 M KH_2PO_4 was added. The samples were incubated for 48 h and were shaken for 2 h followed by centrifugation at 4000 rpm for 20 min. Later, the samples were filtered with 0.45 μm filter paper. Finally, chromium was determined colorometrically by reaction with 1,5 diphenyl carbazide in acid solution which produces a red-voilet colour with a maximum absorbance at 540 nm [19].

Determination of Growth Parameters and Tolerance Index

About 2 months old century plant (*A. americana*) procured from local nursery was transferred to each pot. Each plant contained eight leaves at the time of planting. The plants were watered whenever necessary but no fertilizers were applied. The plants were grown for 3 months after which they were harvested. Immediately following harvest, plants were shaken and washed with running tap water followed by distilled water. The clean samples were then separated into roots and leaves and dried in an oven at 70–80 °C till constant weight was obtained and the dry weights were recorded.

Tolerance index (TI) was calculated at different Cr concentrations by dividing dry weight of the plant exposed to different metal concentrations by that measured during growth in control. The following equation was used:

$$\mathrm{TI}(\%) = [(\text{Dry weight under metal treatment})/(\text{Dry weight in the control})] \times 100$$

Uptake of Cr and its Translocation

The dry roots and leaves were later ground and subsequently digested with 10 ml di-acid mixture (9 HNO_3: 4 $HClO_4$) [27]. The digested solution was filtered and then analysed for Cr concentration using Atomic Absorption Spectrophotometer (Perkin Elmer) and expressed as μg g^{-1} DW.

Table 1 DTPA extractable Cr and its speciation at the time of transplantation of the plants in pots (1 month after incubation)

Total Cr(VI) (mg kg^{-1} soil) applied to the soil	DTPA extractable Cr(mg kg^{-1} soil)	Cr(VI) Remained (mg kg^{-1} soil)	Cr(III) (mg kg^{-1} soil) reduced	% Cr (VI) Reduction
0	<dl	<dl	<dl	–
25	5.2	0.82	24.18	96.72
50	23.7	4.32	45.68	92.18
100	45.5	18.72	81.28	82.82
200	94.3	68.62	131.38	68.16

<dl = below the detection limit (detection limit 3 μg L^{-1})

The uptake of Cr was computed by multiplying the concentration of Cr in plant tissue with dry weight of the plant tissue expressed as μg $plant^{-1}$.From the data on concentration and uptake, bio-concentration factor (BCF) [28], translocation factor (TF) [29–30] and translocation efficiency (TE) [31] were calculated. BCF was calculated by thefollowing formula[28].

$$BCF = [Cr]_{harvested\ tissue} / [Cr]_{soil}$$

where $[Cr]_{harvested\ tissue}$ is concentration of the target metal in the plant harvested tissue (roots, stem or leaves) and $[Cr]_{soil}$ is concentration of the same metal in soil.

$$TF = Cr_{shoots} / Cr_{roots}$$

$$TE\,(\%) = \left[\text{Cr content in the shoots}\left(\mu g\ plant^{-1}\right) / \text{Cr content in the whole plant}\left(\mu g\ plant^{-1}\right)\right] \times 100$$

The experiment was conducted in a completely randomized design (CRD) and there were five replications for each treatment. Data was analyzed statistically and the treatment means were compared using least significant difference technique (LSD) at 5 % probability appropriate for CRD [32].

Results and Discussion

Effect of Chromium on Plant Growth and Tolerance Index

The data on effect of chromium on biomass of roots, leaves, number of leaves per plant and tolerance index is given in Table 2. The plant could tolerate up to 200 mg Cr kg^{-1} soil, but beyond 50 mg kg^{-1} soil, Cr was toxic to the plant which did not show any growth at all. The number of leaves doubled in control and Cr 25 mg kg^{-1} soil. At Cr 50 mg kg^{-1} soil, there was a 40 % reduction in number of leaves $plant^{-1}$ over control. There was a significant reduction in biomass of roots and leaves by applying 50 mg Cr kg^{-1} soil. Reduction in the biomass was to the tune of 50 % at 50 mg Cr kg^{-1} soil, which increased further to 58 % at 200 mg kg^{-1}. Reduction of growth is a commonly observed response in a wide range of plants grown in metal-laden soils. The reduction in growth can be expressed as reduced growth rate, reduced leaf area and decrease in root biomass which further followed in reduction of the total biomass production. This reduction can be due to specific toxicity of metal to the plant, antagonism with other nutrients in the plant, or inhibition of the root penetration in soil [33].

In the present experiment, in order to determine phytotoxicity limit of Cr, phytotoxicity threshold concentration (PT_{50}) was also determined in the leaf tissue and soil. The phytotoxicity threshold concentration ($PT_{50\text{-leaf}}$) of Cr in the plant tissue is defined as the concentration of Cr in plant tissue that corresponds to 50 % growth retardation [34]. The ($PT_{50\text{-leaf}}$) value was found to be 88.5 mg kg^{-1} (Fig. 1). The $PT_{50\text{-leaf}}$ value varies between plant species and metal species. It has been reported that PT_{50} is 6.2 mg kg^{-1} for Indian mustard grown in Cr(VI) contaminated soils [35] and 55 mg kg^{-1} for Cr(III)/Cr(VI) contaminated soils. Similarly, $PT_{50\text{-soil}}$ is defined as concentration of Cr in soils (added) where Cr causes 50 % reduction in maximum yield [36]. Similar to $PT_{50\text{-leaf}}$, in the present study, $PT_{50\text{-soil}}$ was also found to be in the same range i.e., 81 mg kg^{-1} (Fig. 2). Further, the index of Cr tolerance (Fig. 3) decreased with increasing level of applied Cr. The data revealed that, the index of Cr tolerance decreased with increase in the level of applied Cr. Up to 50 mg Cr kg^{-1} soil, the index of tolerance was greater than 50 % and beyond that, it was <50 %. Chang et al. [34] has reported that a value of 50 % is the minimum desired limit for the plants growing in a metal contaminated site for Cr tolerance.

Uptake and Translocation of Cr by *Agave americana*

In general, the concentration of Cr in plant tissues (roots and leaves) increased with increasing addition of Cr to soil (Table 3). Significantly greater accumulation of Cr occurred in roots than in leaves. On an average the concentration of Cr was about seven times higher in roots than in leaves. The concentration of Cr in roots ranged from 122 μg g^{-1} dw at Cr 25 mg kg^{-1} soil to 1318 μg g^{-1} dw at 200 mg kg^{-1} soil. On the other hand, concentration of Cr

Table 2 Effect of different levels of Cr on some physiological parameters in *Agave americana*

Treatment (mg Cr kg^{-1} soil)	Dry weight (g $plant^{-1}$)		Number of leaves $plant^{-1}$
	Root	Leaves	
0	3.75	16.88	18.30
25	3.43	14.65	17.00
50	1.87	10.12	11.30
100	1.63	7.02	8.00
200	1.59	7.21	8.00
CD(0.05)	0.89	2.27	0.70

CD critical difference

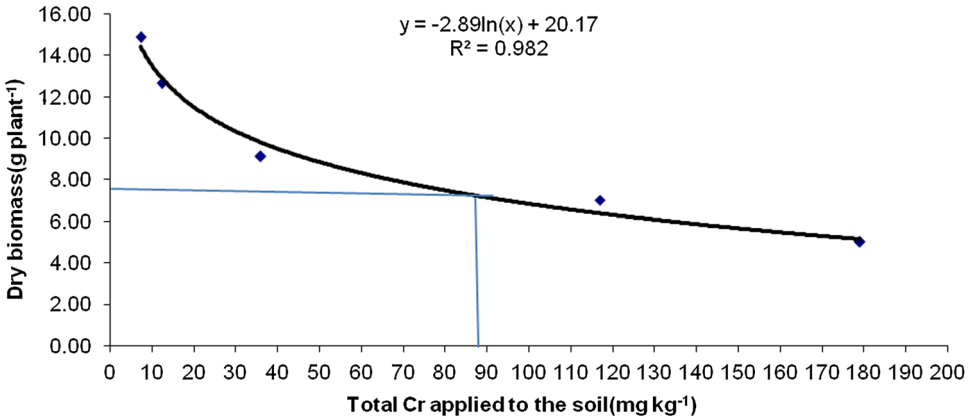

Fig. 1 $PT_{50\text{-leaf}}$ in *Agave americana*. ($PT_{50\text{-leaf}}$ is the concentration of Cr in the leaf tissue where there was 50 % reduction in the dry weight of the leaf)

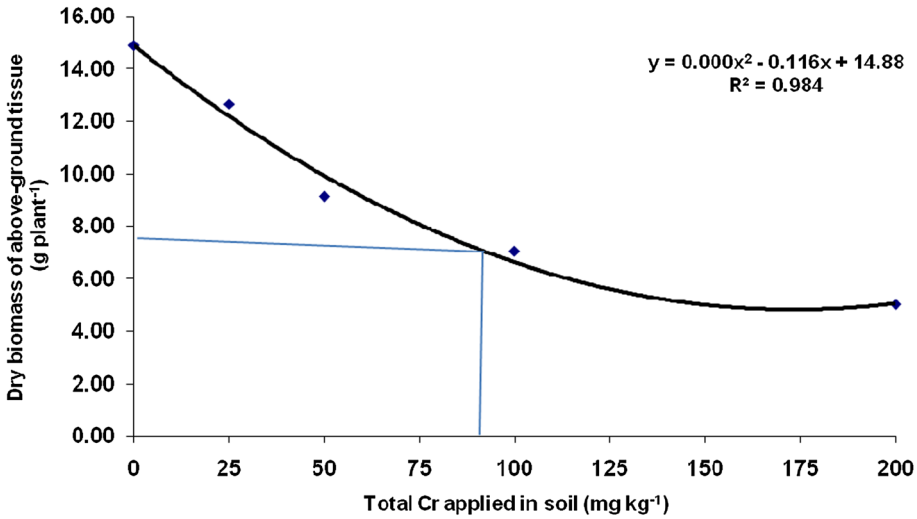

Fig. 2 $PT_{50\text{-Soil}}$ in *Agave americana*. ($PT_{50\text{-Soil}}$ is the concentration of Cr in the soil where there was 50 % reduction in the dry weight of the leaf)

in leaves was less than 300 μg g^{-1} DW, the criterion set for hyperaccumulation of Cr [37]. The concentration of Cr in the leaves ranged from 22 μg g^{-1} dw at Cr 25 mg kg^{-1} soil to 179 μg g^{-1} dw at 200 mg kg^{-1} soil. Therefore, *Agave americana* could not be classified as an hpyeraccumulator for Cr. The big difference between root and shoot concentrations indicated an important restriction of the internal transport of Cr from roots to shoots. This sequestration of heavy metals in roots enables plants to continue growing uninhibited and it is an important means of heavy metal tolerance [38, 39]. Golovatyj et al. [40] have also shown that Cr distribution in crops had a stable character and the maximum quantity was always contained in roots and a minimum in the vegetative and reproductive organs. Chaney et al. [10] suggested that it is extremely unlikely that soil Cr will be remediated by

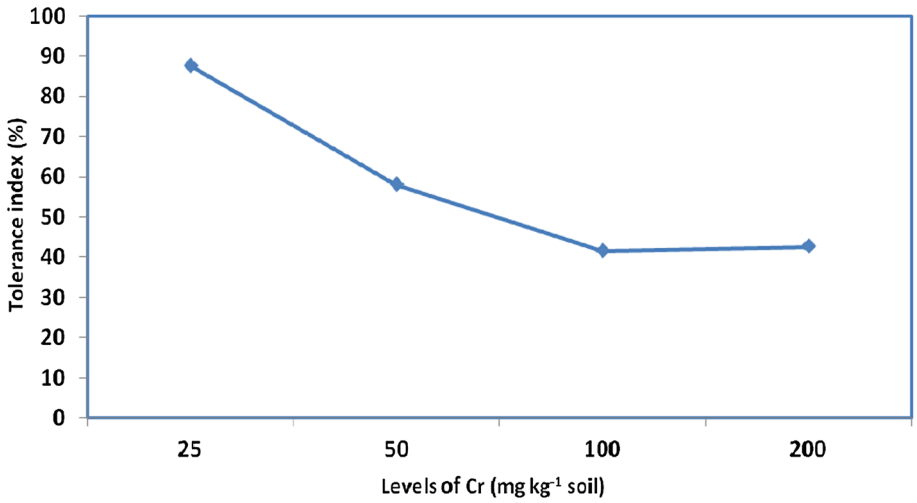

Fig. 3 Tolerance index of Cr in *Agave americana*

Table 3 Partitioning, uptake, translocation factor and translocation of Cr in *Agave americana*

Treatment (mg Cr kg^{-1} soil)	Concentration of Cr in the tissue (μg g^{-1} DW)		Uptake of Cr (μg g^{-1} plant)			% Cr removal	TF	TE (%)	BCF
	Root	Leaves	Root	Leaves	Total				
0	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	–
25	122	22	417	327	745	0.47	0.18	44	4.88
50	253	40	473	364	890	0.24	0.16	41	5.06
100	567	87	924	417	1535	0.21	0.15	27	5.67
200	1318	179	1568	718	2286	0.16	0.13	31	6.59
CD(0.05)	128	19	253	144	259	0.12	0.02	–	–

TF translocation factor, *TE* translocation efficiency (%), *BCF* bio concentration factor, *CD* critical difference, <dl = below the detection limit (detection limit 3 μg L^{-1})

hyperaccumulator plants. Till date only a few plants (*Spartina argentinensis* [41] and *Leersia hexandra* [42] have been identified as hpyeraccumulators for Cr.

In order for the phytoextraction process to be effective, substantial amounts of the Cr taken up by root must be translocated to the harvestable plant parts so that it can be completely removed from the contaminated site. Therefore, translocation factor (TF) was calculated. It indicates the efficiency of the plant in translocating the accumulated heavy metals from roots to shoots. TF of >1 shows that the accumulation of heavy metals in the shoots is higher than in roots. Higher the TF value stronger is the phytoextraction ability [43]. However, in the present study, the TF values were found to be <1 and a linear decrease in TF values were observed with increasing Cr concentration (0.18–0.13) (Table 2). This may be one of the mechanisms for plants to survive in high Cr contaminated soils [36]. The reason for decrease in TF values with increase in the applied Cr is that, at lower concentrations, Cr has higher transfer mobility from roots to leaves and when roots take up more Cr from soil, transfer efficiency from roots to leaves decreases. The TF values obtained in the present study are comparable to those obtained in common Australian fern species i.e., (0.08–0.97) [44] and the results of previous studies with *Euphorbia milii* (0.59) [22]. Similarly, the translocation efficiency (TE %) decreased from 44 % at 25 mg Cr/kg soil to 27 % at 100 mg Cr/kg soil which is contrary to previous study with *E. milli* [16] in which TE of over 80 % was obtained. The lower translocation efficiency (<50 %) in the present study demonstrates that only a small proportion of Cr has been translocated to the harvestable biomass of plant. Both these factors i.e., TF < 1 and TE < 50 % supports authors argument that the *Agave americana* cannot be considered for phytoextraction of Cr from contaminated soils. The uptake of Cr was calculated by multiplying the total amount of biomass produced (root and leaves) with respective concentration of Cr in roots and leaves to give an indication of the efficiency of metal accumulation by the plant. The total accumulation of Cr by the plant ranged from 814 μg/plant at 25 mg Cr kg^{-1} soil to 2286 μg/plant at 200 mg Cr kg^{-1} soil. This is higher than that in calendula, dahlia, chrysanthemum and aster [20] and comparable to *Euphorbia milli* [22]. Further, when the amount of the Cr removed from the total soil Cr was calculated, it was observed that only a minimal fraction of the

applied Cr was removed by the plant (Table 3). The highest value was observed at 25 ppm (0.47 %) which declined rapidly with increase in the concentration of applied Cr (0.16 % at 200 mg Cr kg^{-1}soil).

Baker and Walker [45] categorized plants into three groups according to their strategies for growing on metal-contaminated soils: (1) excluders (2) indicators and (3) accumulators or hyperaccumulators. From the data on concentration of Cr in the above ground tissue (<300 μg g^{-1} DW), TF values <1, century plant was found to exhibit exclusion mechanism. The exclusion mechanism also has a major role in phytoremediation which involves two broad techniques i.e., phytostabilization and phytoextraction. The main difference between the two is that the former is used by the plants that can accumulate high concentration of metals in the roots than that in shoots [46] while the latter is contrary [47]. Therefore, the present study has clearly demonstrated that the plant could not be classified as a hyperaccumulator of Cr. Hence, it can not be considered for phytoextraction of Cr, however it could be considered as a potential candidate for phytostabilization of Cr contaminated soils. The present results are in accordance with the findings of Salt et al. [48] and Bluskov et al. [49].

Conclusions

The century plant (*A. americana*) exhibited great tolerance and had stronger ability to accumulate Cr in the roots. However,it could not be classified as a Cr hyperaccumulator as the concentration of Cr in the shoots is <300 μg g^{-1} DW and TF values is <1. Therefore, century plant has got great potential to be used for phytostabilization of soils contaminated by Cr.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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