Conference Paper

Ricinus communis and *Calotropis procera* As Putative Plant Species for the Phytostabilization of Tannery Contaminated Soil: A Dynamic Approach

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Abstract

The present study involves the assessment of four metals (Cr, Pb, Cu, and Mn) and their mobility (primary and dynamic translocation and bioconcentration factors) in *Ricinus communis* and *Calotropis procera* growing in tannery contaminated soil (TCS) and control soil (CS). The area is moderately to strongly contaminated with Cr. Except for Cr, all the analyzed metals were found within the critical range in TCS and in both plants. The assessment of both primary and dynamic translocation and bioconcentration factors showed TF $<$ 1 and BCF $>$ 1 for both plants, which demonstrates the major transfer and accumulation of Cr from soil to root. As these plants are not grazed upon by grazing animals, the ecological metal transfer risks from these plants are quite low. Moreover, the high commercial importance of these plants (biofuel production and medicinal value) further enhances their utilization for the phytostabilization of moderately Cr-contaminated sites.

Keywords: chromium, *Ricinus communis*, *Calotropis procera*, dynamic factors, tannery industry

1. Introduction

Population explosion and rapid urbanization have resulted in the establishment of different industries and introduced the problem of heavy metal pollution, which has raised critical concerns about human health and the ecosystem. Among all the industries, the chrome tanning industry is one of the most potent, carcinogenic and toxic industries. It is remunerative and used in many part of the world to make high-quality products (leather) [1]. However, the direct discharge of their untreated, heavy-metal-loaded effluent (especially Cr VI) into the environment is matter of concern: concentrations as low as 0.5 mg/kg in solution and 5 mg/kg in soil can be toxic to plants [2]. Heavy metals are toxic, non-degradable and persist in the environment for a long time, which produces adverse effects on human health and other living biota.

Plants growing in and around tannery contaminated soil (TCS) accumulate significant concentrations of heavy metals such as chromium (Cr), lead (Pb), copper (Cu) and manganese (Mn) in their tissues. Cr (VI) is highly toxic for plants and causes DNA and membrane damage and the inhibition of seed germination, root tip cell division and photosynthesis [3]. Prolonged intake of Cr via plants, vegetables and crops has long been considered the predominant pathway for human exposure, which leads to the contamination of the environment and food chain and causes many diseases, disorders and cancer [1, 4].

Phytoremediation is an eco-friendly, cost-effective and resource-generating technology that is gaining attention across the world as a means for using tannery contaminated fallow and agricultural lands for resource generation [1]. *Ricinus communis* and *Calotropis procera* are two potentially important plant species that have been found to be suitable for bioenergy/biofuel production: they also have medicinal and commercial value. The present research primarily investigates the status of the heavy metal contamination of TCS. Secondly, the metals' mobility and uptake by two plants species (*R. communis* and *C. procera*) was also evaluated using primary and dynamic translocation factors in order to check the potential of phytoremediation.

For many years, researchers studying phytoremediation have mainly evaluated the primary factors of plant-soil interaction in one place simultaneously under similar environmental conditions. However, the rate of the transportation of heavy metals is influenced by physiological factors like plant age, ecotype and environmental conditions (i.e., the nature of the substrate, the form and type of the available metals, climatic conditions etc.). Kumar and Maiti [5] used the concept of dynamic factors (i.e., secondary factors) to integrate the influence of site-level factors and the physiological and ecological conditions during the bioaccumulation and translocation of metals in *Oryza sativa* and *Zea mays* growing in chromite-asbestos contaminated agriculture fields of Jharkhand, India. This study confirms that dynamic factors are better for assessing heavy metals in contaminated soil and plants. To the best of our knowledge, very little research has been done on dynamic factors (BCF & TF) in relation to these plant species.

2. Methods

Many illegal leather tanning industries active from long period of time on the outskirts of Meerut, Sobhapur village (29°05″ N, 77°39′2″ E), Rhota road, Bypass Meerut, Uttar Pradesh, India, discharging millions of gallons of toxic effluent into nearby water bodies and land sites. Despite high levels of Cr contamination, *R. communis* and *C. procera* were found to be the plants that were most dominant and possessed the most biomass: growing luxuriantly without showing any toxic morphological effects, samples of these plants were collected along with soil. The soil samples (each with 5 replicates) were air dried, mixed thoroughly, passed through a <2 mm sieve, and oven dried at 105 °C. The pH (1:1; w/v) and electrical conductivity (1:1; w/v) were determined by a digital pH meter and an electrical conductivity meter, respectively. Organic carbon (OC) was determined by the rapid dichromate oxidation method [6], the available nitrogen (Avl. N) as alkaline permanganate method [7], and the available phosphorus (Avl. P) as phosphomolybdenum blue calorimetric method using a double beam UV-Visible scanning spectrophotometer [8]: the available potassium (K) was extracted by a 1N ammonium acetate solution at pH 7 (1:10; w/v) using a flame photometer (AFP-100) [1]. Accurately weighed, 1 g of soil sample was dissolved using 10 mL of nitric acid (HNO_3), followed by 0.5 mL of H_2O_2: these samples were then filtered through a Whatman#42 [5]. The samples were diluted and analyzed using an atomic absorption spectrophotometer (AAS, Hitachi Z-2000 Zeeman,).

The plant samples were washed several times to remove the adhered soil particles and oven dried at 80 °C until a constant weight was achieved. The plants were divided into root and shoot, homogenized using a mortar-pestle and passed through a < 40 BSS (British standard) mesh: 1 g was dissolved in 10 mL of HNO_3 and heated on a hot plate for complete dissolution. The samples were filtered and analyzed using an AAS.

The primary bioconcentration factor (BCF*pri*) is the ratio of metal concentration in the plant (root + shoot) to the metal concentration in the soil [1], while the primary translocation factor (TF*pri*) is the ratio of metal in the shoot to the metal in the root. The dynamic bioconcentration factor (BCF*dyn*) is the ratio of metal concentration in the plant and soil growing in TCS to the metal concentration in the plant and soil growing in CS. The dynamic translocation factor (TF*dyn*) is the ratio of metal transfer from root to shoot in plants growing in TCS to the ratio of metal transfer from root to shoot in plants growing in CS [5]. The detection limits for Cr, Pb, Cu and Mn were 0.005, 0.002, 0.01 and 0.02 mg/L, respectively. The mean, minimum, maximum, standard deviation and one way ANOVA were calculated using the SPSS 20.0 Inc. Chicago, USA and XLSTAT 2007 packages.

3. Results

The chemical, nutritional properties and heavy metal concentration in TCS and CS are presented in Table 1. The pH was found to be slightly alkaline for TCS, whereas it was neutral for CS. The EC, OC, Avl. N, Avl. P, Avl. K and heavy metals were found to be much higher in TCS compared to CS. The continuous mixing of untreated tannery effluent could be the reason for this contamination.

Table 1: The chemical and nutritional characteristics and heavy metal concentrations (mg/kg) of tannery contaminated soil and control soil, (Mean ± SD (Min–Max); n = 5).

Parameters	Tannery Contaminated Soil (TCS)		Control Soil (CS)	
	R. communis	C. procera	R. communis	C. procera
Nutritional parameters (mg/kg)				
Avl. N	147.16 ± 28.97a (104.5 – 170.0)	141.3 ± 14.06a (120.00 – 159.00)	72.80 ± 2.37b (70.90 – 76.40)	71.00 ± 4.53b (66.00 – 76.00)
Avl. P	46.70 ± 6.62a (38.00 – 53.00)	45.00 ± 9.21a (32.00 – 58.00)	15.18 ± 4.50b (11.00 – 22.45)	12.04 ± 1.41b (10.00 – 14.00)
Avl. K	133.03 ± 3.33a (128.28 – 137.50)	130.22 ± 2.35ab (127.68 – 134.00)	130.00 ± 3.80ab (125.00 – 135.00)	127.00 ± 5.43b (120.00 – 135.00)
Heavy metals (mg/kg)				
Cr	163.41 ± 37.31a (103.89 – 199.35)	159.01 ± 26.76a (115.69 – 186.23)	43.43 ± 4.11b (38.27 – 49.25)	42.41 ± 3.71b (38.95 – 48.26)
Pb	21.00 ± 1.59a (19.00 – 23.00)	20.01 ± 2.71a (16.00 – 23.00)	15.01 ± 2.80b (11.12 – 18.06)	14.89 ± 3.42b (10.56 – 19.62)
Cu	41.86 ± 8.27a (28.32 – 49.00)	39.70 ± 2.19a (36.95 – 42.56)	20.08 ± 4.85b (14.53 – 26.12)	19.69 ± 6.41b (11.12 – 26.55)
Mn	200.02 ± 27.45a (165.12 – 239.26)	199.04 ± 24.06a (166.88 – 231.56)	170.29 ± 23.64a (140.00 – 199.56)	169.45 ± 30.33a (120.12 – 198.13)
Source: Authors' own work.				
Note: Avl. N: available nitrogen; Avl. P: available phosphorus; Avl. K: available potassium.				

The heavy metal concentrations in TCSs ranged between 104–200 mg Cr/kg, 16–23 mg Pb/kg, 28–49 mg Cu/kg, and 165–239 mg Mn/kg. In addition, Avl. NPK (104–170, 32–58, 125–137 mg/kg) and OC (11–12%) were sufficient to enhance plant growth [9]. In TCS, only the Cr concentration was found to be above the critical total metal concentration in the soil. Metal accumulation in both plants was found in the order Mn > Cr > Cu > Pb (Table 2). The concentration of Cr in *R. communis* (303.83 mg/kg) and *C. procera* (258.89 mg/kg) growing in TCS was above the critical limits [10]. For all the metals, the average concentration in the whole plants was much higher in *R. communis* than in *C. procera*. Similar patterns were followed by both plants growing in CS, with low metal concentrations [11, 12].

Table 2: Heavy metal concentrations (mg/kg) in the shoots and roots of *R. communis* and *C. procera* growing in tannery contaminated soil (TCS) and control soil (CS) (Mean ± SD (Min – Max); *n* = 5).

Soil	Metal	Plant Part	R. communis		C. procera	
			Mean ± SD	Min – Max	Mean ± SD	Min – Max
TCS	Cr	Shoot Root	108.99 ± 2.95 194.84 ± 2.70	105.59 – 112.90 190.49 – 197.72	85.93 ± 1.46 172.96 ± 0.87	83.91 – 87.85 171.73 – 174.20
	Pb	Shoot Root	11.92 ± 0.90 13.49 ± 1.27	10.65 – 13.19 11.56 – 14.76	11.56 ± 1.08 12.13 ± 1.05	9.99 – 12.90 10.84 – 13.18
	Cu	Shoot Root	31.59 ± 1.80 25.36 ± 2.69	29.31 – 34.12 21.22 – 28.28	29.02 ± 1.57 23.00 ± 1.86	27.64 – 31.69 20.83 – 25.68
	Mn	Shoot Root	144.81 ± 8.80 115.16 ± 6.15	133.66 – 156.69 109.69 – 122.66	142.66 ± 7.89 113.25 ± 1.73	130.66 – 150.66 111.19 – 115.59
CS	Cr	Shoot Root	21.16 ± 2.94 36.77 ± 1.29	18.65 – 25.69 35.36 – 38.65	19.31 ± 2.95 32.66 ± 1.96	15.63 – 21.66 31.09 – 36.12
	Pb	Shoot Root	7.89 ± 1.08 10.96 ± 0.87	6.23 – 9.12 9.98 – 12.06	5.50 ± 0.57 12.60 ± 0.63	4.87 – 6.10 11.95 – 13.44
	Cu	Shoot Root	14.97 ± 2.17 9.75 ± 0.96	12.36 – 17.86 8.62 – 11.00	14.80 ± 0.49 9.44 ± 0.83	13.95 – 15.23 8.42 – 10.65
	Mn	Shoot Root	120.05 ± 2.04 61.96 ± 2.24	117.99 – 122.91 59.11 – 64.49	119.64 ± 1.70 59.63 ± 1.53	117.86 – 121.95 57.78 – 61.56

Source: Authors' own work.

In both plants, significantly higher concentrations of Cr and Pb were observed in the roots than in the shoots. For Cr, this might be due to the fact that the complexation of metals with the sulphydryl group (-SH) of soil constituents resulted in less translocation of heavy metals to the upper parts of the plants [13], since they are immobilized in the root vacuoles [1]. Similarly, Pb binds to the carboxylic acid group of mucilage uronic acids on the root's surface and remains stored in the root [14]. Higher accumulations of Cu and Mn were observed in the shoots than in the roots for both plants, which may be because of the different metal transporters present in plants, which can easily translocate Cu and Mn from the roots to the aerial parts via the plasma membrane and tonoplast [15].

The primary and dynamic translocation (TF) and bioconcentration (BCF) factors for Cr, Pb, Cu and Mn are presented in Table 3. In both plants, the TF*pri* for Cr and Pb was found to be low (< 1), which indicates a reduction in the translocation to the shoot parts. This may be due to a lack of carriers for the transportation of Cr and Pb in the plants [5]. However, the TF*pri* was found to be > 1 for Mn and Cu because of its high mobility toward aerial parts, which support metabolic activities and are beneficial for plant growth. When dynamic factors are used for evaluation, the TF*dyn* was < 1 for Cr, Cu and Mn, whereas values were higher in *R. communis* than in *C. procera.* The $TF_{(dyn)}$for Pb was > 1 in both plants: this result was the opposite for the other metals (*C. procera* > *R. communis*).

Table 3: The primary *(pri)* and dynamic *(dyn)* translocation (TF) and bioconcentration factors (BCF) of heavy metals in *R. communis* and *C. procera* growing in tannery contaminated soil (TCS) and control soil (CS).

	TCS	CS	TCS	CS
TF(*pri*) > 1	$Mn_{1.25}$ > $Cu_{1.24}$	$Mn_{1.93}$ > $Cu_{1.53}$	$Cu_{1.26}$ > $Mn_{1.25}$	$Mn_{2.00}$ > $Cu_{1.56}$
TF *(pri)* < 1	$Pb_{0.88}$ > $Cr_{0.55}$	$Pb_{0.71}$ > $Cr_{0.60}$	$Pb_{0.95}$ > $Cr_{0.49}$	$Cr_{0.59}$ > $Pb_{0.43}$
TF *(dyn)* > 1	$Pb_{1.22}$	–	$Pb_{2.18}$	–
TF *(dyn)* < 1	$Cr_{0.92}$ > $Cu_{0.81}$ > $Mn_{0.64}$	–	$Cr_{0.84}$ > $Cu_{0.80}$ > $Mn_{0.62}$	–
BCF*(pri)* > 1	$Cr_{1.85}$ > $Cu_{1.36}$ > $Mn_{1.29}$ > $Pb_{1.21}$	$Cr_{1.35}$ > $Pb_{1.25}$ > $Cu_{1.23}$ > $Mn_{1.06}$	$Cr_{1.62}$ > $Cu_{1.31}$ > $Mn_{1.28}$ > $Pb_{1.18}$	$Cu_{1.23}$ > $Cr_{1.22}$ > $Pb_{1.21}$ > $Mn_{1.05}$
BCF *(pri)* < 1	–	__	–	
BCF *(dyn)* > 1	$Cr_{1.37}$ > $Mn_{1.21}$ > $Cu_{1.10}$	–	$Cr_{1.32}$ > $Mn_{1.21}$ > $Cu_{1.06}$	–
BCF *(dyn)* < 1	$Pb_{0.96}$		$Pb_{0.97}$	

Source: Authors' own work.

The primary BCF was found to be > 1 for all the metals, which shows the metal-accumulating ability of both plants. However, the $BCF_{(dyn)}$value (which shows the overall metal transfer from TCS to CS and the plant) were high ($BCF_{(dyn)}$> 1) for all metals except for Pb. Their order was: Cr > Mn > Cu. This suggests heavy metal contamination of the soil and its subsequent accumulation in both naturally growing plant species. However, a low $BCF_{(dyn)}$(< 1) for Pb indicates its lower bioavailability than the other metals in both plants. Comparison between the primary and dynamic factors revealed that primary factors can easily be influenced by environmental changes (contaminated and reference soil) and may show diversity in metal uptake and mobility. So, it was insufficient to determine the exact metal concentration in soil and its bioaccumulation and transfer pattern in plants [5]. While dynamic factors were insensitive to environmental changes because they incorporate the influence of environment on metal uptake (i.e., external factors), they demonstrate the translocation process in plants growing in the contaminated and control sites (i.e., internal factors), which eliminates systematic errors of analysis and improves the precision of the result. It can be preliminarily stated that high metal contamination of soil may adversely affect the protective barrier functions in plants with changes in the metal accumulation pattern, resulting in a high uptake of heavy metals in the studied plants. Similar findings regarding heavy metal accumulation and translocation were reported by Nagaraju and Guru [16] in *C. procera* and by Ananthi et.al. in *R. communis* [17].

4. Conclusion

The current study concludes that TCS was strongly contaminated with Cr. The accumulation of metals in the whole plant was observed in the order of Mn > Cr > Cu > Pb, which was higher in *R. communis* than in *C. procera* growing naturally in TCS. Assessment of the TF*dyn* and BCF*dyn* factors proved that the translocation of Cr from root to shoot was low (< 1), whereas its accumulation in both plants was higher (> 1) than for the other associated metals. The dynamic factors used for the evaluation of heavy metal toxicity in TCS and selected plant species (*R. communis* and *C. procera*) further confirm and justify the primary factor results. As these plants are not grazed upon by grazing animals, the ecological metal transfer risks from these plants are quite low. The high commercial importance of these plants for biofuel production and their medicinal value further enhances the probability that they can be used for the phytostabilization of moderately Cr contaminated sites. In addition, the present study provides a better assessment of metal toxicity in the soil and plants: dynamic factors can be implemented for any metal contaminated sites.

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