Agroforestry Systems (2005) 64: 83–87
DOI 10.1007/s10457-005-2477-2

Phytoaccumulation of chromium by some multipurpose-tree seedlings

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Received 6 September 2003; accepted in revised form 8 March 2004

Key words: Chromium speciation, Phytoremediation, Organic acids, Vesicular arbuscular mycorrhizal fungi

Abstract

A pot culture experiment was conducted in green house to study the potential of chromium (Cr) phytoaccumulatory capabilities of four promising agroforestry tree species viz., *Albizia amara*, *Casuarina equisetifolia*, *Tectona grandis*, and *Leucaena luecocephala*. Possibility of enhancement of Cr uptake by chemical (citric acid) and biological vesicular arbuscular mycorrhizal fungi (VAM) amendments were also tried. Biologically stable speciation of Cr trivalent (Cr(III) and hexavalent Cr(VI) were used. Cr(VI) was more toxic to the tree growth in terms of collar diameter (CD) increment in all the tree species than Cr(III). In general, roots accumulated more Cr than shoots in all the tree species. There was more than 10 fold increase in root Cr content in comparison with shoot Cr content in all the trees at all the concentration of Cr and all sources of Cr. Citric acid significantly increased the Cr content in the tissues of roots in all the species under both speciation of Cr. The highest increase in Cr content brought by 20 mM citric acid addition was in *A. amara*. Unlike citric acid, VAM treatment did not bring about a significant increase in the Cr content of all the tree species studied. Results suggest that *Albizia amara* is a potential Cr accumulator with citric acid as soil amendment. The potential of this tree as a Cr phytoaccumulator may be investigated in long-term studies.

Introduction

Chromium (Cr) is the chief heavy metal contaminant found in the tannery effluent. Due to chrome leather tanning processes, large quantities of Cr compounds are discharged through liquid, solid, and gaseous wastes into the environment which can have a significant adverse biological and ecological effects. Several reports have shown that the values for Cr in tannery effluent are considerably higher than the safe limits prescribed by international standards. In India 2000–3200 tonnes of elemental Cr escapes into the environment annually (Chandra et al. 1997) and very high levels of Cr(VI) contamination (14,800 mg kg^{-1} in ground water and 25,900 mg kg^{-1} in soil) has been reported in United Chrome Products Site in Corvallis Oregon (Krishnamurthy and Wilkens 1994). Soil and water ecosystems have been contaminated to an overwhelming extent in the vicinity of leather industry and this has rendered arable land unproductive and underproductive.

Chromium is a toxic element to higher vascular plants and is detrimental to its growth, development and reproduction (Cervantes et al. 2001). The physiological impact of Cr contamination in soil and water is dependant on the speciation viz., Cr(III) and Cr(VI). Speciation of Cr in the soil differentially affects the mobilisation of the metal, subsequent uptake and resultant toxicity in the plant system. Cleaning up of the Cr contaminated sites is a challenging task. Phytoremediation by trees is an emerging technology that can be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable; at sites with low concentrations of contaminants, where treatment is required over long periods of time. Phytoextraction refers to the use of metal accumulating plants that take up and concentrate metals from the soil in roots and above ground shoots or leaves (Cunningham and Ow 1996). Tree species in association with mycorrhizae have shown promising results for rehabilitation of Cr contaminated lands in and around tannery industrial areas (Khan 2001). Organic acids have been used to enhance extraction of immobile metals from soils due their ability to complex with metals and increase their availability (Wu et al. 2003). Very little work has been done to integrate multipurpose trees into phytoremediation efforts. There is a distinct lack of literature on Cr accumulating potential of trees in general and multipurpose trees in particular. The present study was thus taken up to understand the Cr uptake pattern in potential agroforestry tree species under biological (VAM) and chemical amendment (citric acid) conditions.

Materials and methods

Tree culture and treatments

Three month old seedlings of potential agroforestry tree species *Albizia amara*, *Casuarina equisetifolia*, *Tectona grandis* and *Leucaena luecocephala* tree species of equal height and collar diameter respectively for each tree species was used in the study. Pot mixture was prepared by mixing thoroughly two parts of soil and one part each of well-decomposed farmyard manure and sand and filled in pots of size 28 × 30 cm with 10 kg of potting mixture. Fertilizer was added in the pots at the following rate: urea – 100 mg of N kg^{-1}, Di ammonium Phosphate (DAP) – 50 mg of P kg^{-1}, Potassium Chloride (KCl) – 50 mg of K kg^{-1}. The treatments consisted of control, Cr (III) as chromium sulfate ($Cr_2(SO_4)_3.2H_2O$) at 250 mg kg^{-1} of potting mixture, Cr (VI) as potassium dichromate ($K_2Cr_2O_7$) at 100 mg kg^{-1} of potting mixture, Cr (III) as $Cr_2(SO_4)_3.2H_2O$ at 250 mg kg^{-1} of potting mixture + VAM, Cr (VI) as $K_2Cr_2O_7$ at 100 mg kg^{-1} of potting mixture + VAM, Cr (III) as $Cr_2(SO_4)_3.2H_2O$ at 250 mg kg^{-1} of potting mixture + 20 mM citric acid, Cr (VI) as $K_2Cr_2O_7$ at 100 mg kg^{-1} of potting mixture + 20 mM citric acid. The potting mixture containing added chromium treatments were thoroughly mixed with chromium salts before filling into the pots. The specified amount of Cr for each treatment was added after subtracting the control value of 4.8 mg kg^{-1} of total Cr present in the potting mixture. The inoculum of *Glomus mossae* was applied at 2000 spores per pot as a band at the bottom third of the container, then covered with potting mixture and the roots of the tree seedlings were allowed to grow into the band and colonize.

The Cr status of the soil at midway stage (6 months) is given in Table 1. Replications were seven and the design was completely randomized design, periodic repositioning of pots was done once a month. The experiment was repeated two times between 1999–2001. Plant samples were drawn 1 year after the initiation of the experiment from each treatment for recording growth and chromium uptake pattern.

Chromium content and growth

Growth in terms of collar diameter (CD) was recorded using a measuring tape and expressed in cm. Measurement of chromium content ($\mu g\ g^{-1}$)

Table 1. DTPA extractable Cr in the treatmental pots at midway stage of the experiment (6 months).

Treatment	Total Cr (mg kg^{-1} of potting mixture)	DTPA extractable Cr (mg kg^{-1} of potting mixture)
Control	4.8	1.3
Cr (III)	250	52.6
Cr (VI)	100	31.3
Cr (III) + VAM	250	54.8
Cr (VI) + VAM	100	38.2
Cr (III) + citric acid	250	61.8
Cr (VI) + citric acid	100	48.8

was made according to Shanker et al. (2004). The chromium accumulation factor (ACF) of the tree species was estimated according to Baker et al. (1994).

$$\mathrm{ACF} = \frac{\text{Cr content in tissue}}{\text{Total Cr in soil}}$$

Statistics

Seven replications were taken for every estimation and the experiment was repeated twice. The mean of both the experiments (n = 14) was analysed statistically using a general linear model (Wilkinson et al. 1996) for analysis of variance in completely randomised design. Least Significant Difference (LSD) was used to compare treatment differences.

Results

Among the tree species highest CD increase was seen in *C. equisetifolia*, followed by *L. leucocephala* and *A. amara* (Table 2). The least growth was observed in *T. grandis*. Among the treatments, Cr(VI) affected the CD increment significantly in comparison to control in all the tree species except *A. amara*. The decrease in CD caused by Cr (III) was not significantly different from Cr (VI), although both were significantly different from control. The general pattern of Cr accumulation (Table 3) was highest in *A. amara* followed by *T. grandis*, *C. equisetifolia* and the least accumulation was seen in *L. leucocephala*. Among the Cr treatments the Cr (III) treated trees accumulated significantly more Cr in tissue in comparison to Cr (VI). Cr (III) treated trees exhibited a Cr content of 425, 464, 476 and 600 μg g^{-1} in the roots of *L. leucocephala*, *C. equisetifolia*, *T. grandis* and *A. amara*, respectively. In general roots accumulated more Cr than shoots in all the tree species. There was more than 10-fold increase in root Cr content in comparison with shoot Cr content in all the trees at all concentration of Cr and all sources of Cr. Citric acid significantly increased the Cr

Table 2. Effect of chromium speciation, VAM and citric amendments on increase in collar diameter of different tree seedlings.

Treatments	Collar Diameter (cm)			
	L. luecocephala	*C. equisetifolia*	*T. grandis*	*A. amara*
Control	1.65	2.15	1.09	1.29
Cr (III)	1.38	1.85	0.64	1.24
Cr (VI)	1.45	2.00	0.76	1.25
Cr (III) + VAM	1.40	1.90	0.68	1.25
Cr (VI) + VAM	1.54	1.92	0.75	1.23
Cr (III) + citric acid	1.40	1.90	0.69	1.27
Cr (VI) + citric acid	1.47	2.00	0.65	1.28
CD at 0.05	0.072	0.091	0.093	0.092

Note: Data represent mean of two experiments. Observations were taken 1 year after treatment imposition.

Table 3. Effect of chromium speciation. VAM and citric acid amendments on chromium content of different tree seedlings.

Treatments	Chromium content (μg g^{-1} dry matter)							
	L. luecocephala		*C. equisetifolia*		*T. grandis*		*A. amara*	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
Control	1.2	nd	1.9	nd	1.5	nd	1.8	nd
Cr (III)	425.2	47.1	464.1	53.0	475.9	67.6	599.6	57.2
Cr (VI)	344.3	35.2	379.7	42.5	384.4	56.4	451.9	47.3
Cr (III) + VAM	450.8	49.2	495.0	56.6	507.5	71.9	603.2	62.6
Cr (VI) + VAM	358.1	36.1	397.9	47.2	399.7	59.6	470.8	49.3
Cr (III) + citric acid	463.5	51.3	504.9	57.6	516.8	73.4	639.1	63.3
Cr (VI) + citric acid	375.0	37.5	412.4	43.0	417.5	57.8	489.1	50.0
CD at 0.05	30.13	5.32	33.18	5.76	32.76	6.41	35.48	6.97

Note: nd - indicates not detected. Data represent mean of two experiments. Observations were taken 1 year after treatment imposition.

Table 4. Effect of chromium speciation.

Treatments	Accumulation factor (ACF)			
	L. luecocephala	*C. equisetifolia*	*T. grandis*	*A. amara*
Control	0.25	0.39	0.31	0.37
Cr (III)	1.88	2.06	2.17	2.62
Cr (VI)	3.79	4.22	4.40	4.99
Cr (III) + VAM	2.00	2.20	2.31	2.66
Cr (VI) + VAM	3.94	4.45	4.59	5.20
Cr (III) + citric acid	2.05	2.25	2.36	2.80
Cr (VI) + citric acid	4.12	4.55	4.75	5.39

Note: VAM and citric acid amendments on ACF of different tree seedlings. Data represent mean of two experiments. Observations were taken 1 year after treatment imposition.

content in the tissues of roots in all the species under both speciation of Cr. The highest increase in Cr content brought by 20 mM citric acid addition was in the case of *A. amara* followed by *C. equisetifolia*, *T. grandis* and the least was seen in *L. leucocephala* in the same treatments in roots of the respective tree species. Unlike citric acid, VAM treatment did not bring about a significant increase in the Cr contents of all the tree species studied. Although the total content of Cr was more in Cr (III) treatment the ACF values were more in Cr (VI) treatment irrespective of the tree species studied. *L. leucocephala* had ACF of 1.88 in Cr (III) treatment as against 3.79 in Cr (VI) treatment (Table 4). VAM and citric increased the ACF values for both the Cr speciation in all the tree species. The increase brought about by citric acid was higher than that by VAM. The highest accumulation factor was observed in *A. amara* followed by *T. grandis*, *C. equisetifolia* and the least ACF was seen in *L. leucocephala* irrespective of the speciation of Cr added to the soil.

Discussion

Potential toxicity of Cr speciation added to soils may be affected by the interactions between oxidation–reduction and organic complexation. Plants cannot usually access the total pool of a metal present in the growth substrate. Instead, the fraction of the metal, which plants can absorb, is known as the available or bio available fraction. The deleterious effect of Cr on the growth of all the tree species was evident from the reduction in collar diameter of the seedlings. The reduction in collar diameter could have been due to Cr induced toxicity to the outer cells of the tree barks affecting the secondary thickening processes of the trees.

The possibility of modification of the Cr form after it is supplied in either Cr (III) or Cr (VI) cannot be ruled out in the experiment. The reason for the high accumulation in roots of the trees could be because Cr is immobilised in the vacuoles of the root cells to render it non-toxic, which may be a natural toxicity response of the plant. Since both Cr (VI) and Cr (III) must cross the endodermis via symplast, the Cr (VI) in cells is probably readily reduced to Cr (III) and retained in the root cortex cells (Zayed et al. 1998). Another important reason for the lack of transport of Cr from roots to shoots could be because the plants lack any specific mechanism of transport of Cr, as it is a toxic and nonessential element to plant growth. The accumulation factor for both Cr speciation derived in the present study in tree species revealed the differing capacity of the four tree species studied. The results are in confirmation with the report of Khan (2001). This could be because the uptake pattern of Cr from soil depends on the tree species and that within the tree species the concentration largely differs between different parts of the trees (Pulford et al. 2001). In general, it is possible that the restriction of Cr to roots is more in non-accumulator species. There was a pronounced enhancement of Cr uptake in tree species treated with citric acid. Similar results have been reported by Shahandeh and Hossner (2000) and Srivastava et al. (1999). The reason for this could be that the organic acids modified soil properties, increased the labile fraction of Cr and mobilised Cr for easy uptake by roots (Wu et al. 2003).

The poor transfer of Cr from root to shoot means that the prospects for using trees as phytoremediators on chromium contaminated sites are poor, but it should be noted that Cr is very poorly translocated in all the higher vascular plants (Zayed et al. 1998). Hence the use of these trees as potential phytoremediators can only be proven over very long period of time. Our results suggests that *A. amara* is a potential Cr accumulator with citric acid as soil amendment. Although fodder is one of the economic component of this tree in an agroforestry system, the tree if only used for firewood and timber and not for fodder would ensure no reentry of the Cr accumulated by the tree into biological systems. This tree can be taken up for future long term on site trials in Cr contaminated lands to establish its usefulness as Cr phytoaccumulator.

Acknowledgements

Thanks are due to Dr. K.R. Solanki Deputy Director General (Agroforestry), Indian Council of Agricultural Research, New Delhi, for having permitted study leave to the first author to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The first author also acknowledges the Dr. P. Rai, Director, National Research Centre for Agroforestry, Jhansi, for having supportive and encouraging during the documentation of the manuscript. ICAR funded the study in terms of salary to the first author during the course of the broad study on "Physiological biochemical and molecular aspects of chromium toxicity and tolerance in crops and tree species" a part of which forms this paper.

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