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Natural Accumulation of Copper and Distribution of Metals in Plants Growing in Copper Mining Area, Rajasthan, India

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ABSTRACT

Phytoremediation helps not only to stabilize erosion but also to extract metals from mine waste. The present study aimed to identify naturally-growing hyperaccumulator plants in mining areas. Plants growing naturally in copper mining areas of Kolihan and different locations of Khetri, a town in Jhunjunu district of Rajasthan, India were selected for the study. Different samples collected from mining sites were analyzed by atomic absorption spectroscopy. More copper accumulation was observed in the leaves of *Prosopis juliflora* (Sw.) DC. and *Ailanthus excelsa* Roxb. than in the twigs. Leaves and twigs of *P. juliflora* showed a 42- and 8-fold increase in the amount of copper, 775.29 μg g^{-1} and 146.8 μg g^{-1}, respectively than plants growing on metal contamination-free soil (controls). The present results indicate that these plants could be hyperaccumulators and possibly be used for the removal of Cu from contaminated sites. The distribution of other metals such as Mn, Zn and Fe was also studied. A high Transfer Coefficient of 6 was observed in *P. juliflora* leaves of Khetri site "A" (near temple Mata mandir) while it was 4.65 in *A. excelsa* for Zn.

Keywords: *Ailanthus excelsa*, metal accumulation, *Prosopis juliflora*, transfer coefficient

INTRODUCTION

Heavy metals are elements having their density above 5 g cm^{-3} (Weast 1984). Heavy metals released into environment at increasing rates through mining industries and agriculture, cause serious problems for environment and health (Ross 1994). Contamination of soils, sediments and water with toxic chemicals is one of the major problems facing the industrialized world, today (Gratão *et al.* 2005). In India, mining, in particular the metal ore extraction, is the second source of heavy metal contamination in soil, after sewage sludge (Singh *et al.* 2005).

Phytoremediation is a sustainable, inexpensive technology for the removal of metals from soil by using plants (Salt *et al.* 1995, 1998). Phytoremediation strategies mainly depend upon stabilization and accumulation of metals (Salt *et al*. 1998). Phytostabilization of metals may employ plants to reduce leaching, runoff and erosion via either stabilization of soil by plant roots or by transferring of metals to less toxic forms (Berti and Cunningham 2000). Accumulation of metal in the shoot tissue followed by harvesting of shoot biomass is called phytoextraction (Blaylock and Huang 2000). The harvested plant tissue can be used for non-food purposes; they can be converted to ash and be used for recycling the metals or for disposing in landfills (Chaney *et al.* 2000).

The effects induced by metal stress on plant growth and development involve biochemical changes mainly through oxidative damage. An exaggerated increase in the production of reactive oxygen species (ROS), such as the superoxide radical (O^{-2}) and hydrogen peroxide (H_2O_2), triggers a complex detoxification mechanism which may involve non-enzymatic and enzymatic systems, capable of preventing the cascades of uncontrolled oxidation (Gratão *et al*. 2005).

There are many reports on evaluation of plant species for phytoremediation and phytomining potential. The distribution and mobility of heavy metals (Fe, Mn, Cu, Zn and Cd) in the surrounding soils of a mining valley in NW Madrid (Spain), and their transfer to wild flora of twenty-five vascular plant species (3 ferns and 22 flowering plants) were analyzed by Moreno-Jimenez *et al.* (2009). The study of dispersal of arsenic in soils around a former mining area, measuring arsenic levels in the wild flora and evaluation of the transfer and availability of arsenic in the soil–plant system was studied by Moreno-Jimenez *et al.* (2010).

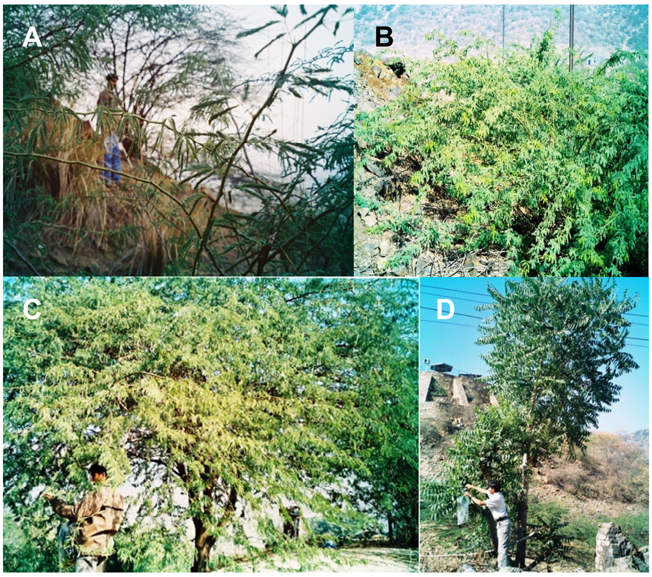

Fig. 1 Plants growing at Kolihan and different locations of Khetri Copper mines. (**A**) *Prosopis juliflora* growing at Kolihan mine, (**B**) *P. juliflora* growing at Khetri site "A", (**C**) *P. juliflora* growing at Khetri site "B", (**D**) *Ailanthus excelsa* growing at Khetri site "C".

Rashed (2010) carried out a study on wild plants *Acia raddiena* and *Aerva javanica*, growing on mine tailings of Southeast Egypt on Hg, Cd, Pb, As and associated heavy metals (Cr, Ag, Ni, Au, Mo, Zn, Mn and Cu). The study showed distribution and mobility of these metals from

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tailing to the surrounding soils and wild flora. Similarly a study was conducted on 10 plant species growing naturally on the largest lead–zinc mines in Northern Vietnam (Ha *et al.* 2011). They determined the concentrations of multiple heavy metals and As in plant species growing on a contaminated site and assessed the feasibility of using these plants for phytoremediation and phytomining. Recently metalloid such as arsenic and heavy metal (Cd, Cr, Cu, Ni, Pb, and Zn) accumulation and their translocation in ten native plant species growing in soils polluted by mining activities at the Mónica mine (NW Madrid, Spain) were studied (Garcia-Salgado *et al.,* 2012). High element concentrations were found in the aboveground parts of *Corrigiola telephiifolia* (As and Pb), *Jasione montana* (Cd and Zn), and *Digitalis thapsi* (As, Cd, Cu, Pb and Zn).

Copper (Cu) is a major contaminant which is released into environment by different anthropogenic activities, e.g. from bactericides, fungicides and industrial waste (Ducic and Polle 2005). Cu is both, a micronutrient for plants as well as a heavy metal capable of stress induction (Thomas *et al*. 1998). This trace element plays important role in CO_2 assimilation, ATP synthesis and is a component of various proteins; particularly those involved in both, the photosynthetic (plastocyanin) and the respiratory (cytochromeoxidase) electron transport chain (Demirevska-Kepova *et al*. 2004). The uptake of Cu from soil by plant depends upon the ability of the plants to transfer the metal across the soil-root interface and the total amount of Cu present in the soil (Fargasova and Beinrohr 1998). Excessive amount of Cu in soil plays cytotoxic role, induces stress and can cause damage and symptoms in plants, including growth retardation and leaf chlorosis (Baker and Proctor 1990; Waldermar *et al*. 1994; Lewis *et al*. 2001).

There are evidences that plants such as *Silene armeria* (Dinelli and Lombini 1996), *Typha latifolia* (Ye *et al*. 2001), *Salix viminalis* (Rosseli *et al*. 2003), *Juncus conglomerates* (Freitas *et al*. 2004) grown on metal-enriched soil can accumulate high amount of heavy metals in their tissues. In Rakha mining area, Jharkhand, India there are several abandoned Cu-tailing ponds causing severe metal pollution in the nearby areas. The concentration of Cu, Ni, Mn, Zn, Pb and Cd was examined in the above and underground tissues of five naturally growing plant species (Das and Maiti 2007). The study on differential accumulation of Mn in three mature tree species (holoptelia, cassia and neem) growing on mine tailing of Gumgaon, Nagpur, India showed that holoptelia accumulates high amount of Mn in leaves from dumpsite (Raju *et al*. 2008). It has been shown that *Prosopis* plants accumulated higher amounts of Fe, Mn, Cu, Zn and Cr in various fly ash amendments than in garden soil (Rai *et al*. 2004). This shows the potential of *Prosopis juliflora* (Sw.) DC. to grow in plantations on fly ash landfills and to reduce the metal contents of fly ash by bioaccumulation in its tissues. The use of *P. juliflora* for the biorecovery of aluminium from urban industrial sites of Coimbatore was reported by Thangaval *et al.* (2000). Senthilkumar *et al*. (2005) suggested the use of *P. juliflora* to decontaminate heavy metal-polluted soil in view of its ability to accumulate various heavy metals such as Cd, Cu, Ni, Cr and Al. Recently, Varun *et al*. (2011) have also shown that *P. juliflora* has a good phytoextraction potential demonstrated by the accumulation of Cd and Pb under natural conditions.

The present study was carried out on *P. juliflora* (Sw.) DC. plants growing in Kolihan and different locations of Khetri mining area, as well as on *Ailanthus excelsa* Roxb. growing at Khetri. Thus, a study was conducted to generate information on Cu accumulation and distribution of other metals like Mn, Zn and Fe, in aerial parts of both these plants.

MATERIALS AND METHODS

Sample collection

Khetri and Kolihan underground mines of Khetri Copper Complex (KCC), which comes under Hindustan Copper Ltd., are located in Rajasthan, India. KCC is in the cradle of Aravelly hills in Jhunjhunu district, Rajasthan which is about 190 km southwest of Delhi and 180 km north of Jaipur (27° 59′ N 75° 48′ E). KCC has two well-developed townships at Khetri-Nagar and Kolihan. Plants, including *P. juliflora* and *A. excelsa* growing naturally at Khetri mine and *P. juliflora* at Kolihan mine, Rajasthan, India were identified (**Fig. 1**). A single *A. excelsa* tree was seen growing at Khetri mining area, whereas several plants of *P. juliflora* could be seen growing naturally at both the mine areas. Leaf and twig samples of *P. juliflora* were collected from different locations of Khetri mine. The area near temple Mata mandir was named as “A” and the place where the explosive waste material was dumped was named as “B”. At site “C”, another area near Mata mandir, *A. excelsa* was growing near a trolley used for carrying crude ore for smelting and refining purpose. These samples were compared with the samples obtained from *P. juliflora* and *A. excelsa* growing in soil, free from metal contamination (control), near National Chemical Laboratory (NCL), Pune. Soil samples were collected from different sites from a depth of 10 cm where plants were growing. Assessment of metal content in natural vegetation in contamination-free soil was done. Sampling was carried out in a randomized design. *A. excelsa* at Khetri site “C” was seen growing on heaps of waste dumps left after the metal extraction from ore. Due to difficulty in obtaining soil sample from there, the data of soil from the nearest site “A” was taken for further studies. Copper tailings were disposed in slurry impoundments. No habitat was observed at this place.

Soil and plant analysis

Soil samples were collected from both the mines, and from different places. Soil from NCL was taken as control. The soil samples were dried at 100°C for two days and ground to a fine powder with mortar and pestle. Dust that was accumulated on the surface of the leaves and twigs was removed by washing thoroughly with tap water. This was followed by thorough washing of the samples with deionized water. The samples were then dried on a filter paper to eliminate any moisture from the surface. The same process was repeated for the control leaves and twigs of all plants. Fresh leaves and twigs of *P. juliflora* and *A. excelsa* were taken (1–3 g) in pre-weighed 50 ml glass beakers. Fresh weights of the tissues were determined from the difference in the weights. Samples were dried in an oven at 100°C and weighed intermittently until constant weights were observed. Dried plant materials were ground in mortar and pestle to a fine powder for metal analysis.

An amount of 150 mg of finely powdered plant tissues or soil was taken in Borosil vials. This was digested with 3 ml of nitric acid and 1 ml of 70% perchloric acid on a hot plate under the hood. The volume of digested sample solution was made up to 10 ml with deionized water. Metal content in analytes was determined using Atomic Absorption Spectroscopy (Perkin Elmer 1100B). Metal content was calculated and expressed in $\mu g\ g^{-1}$ of dry tissue (Kashem *et al*. 2007). The values were expressed as mean ± standard deviation (SD) of three replicates. The data was subjected to ANOVA (Analysis of Variance) at a significance level of $P < 0.05$. Individual comparisons of treatments were made with controls and significantly different values were determined using Student’s *t*-test at $P < 0.05$.

Metal accumulation by plants was evaluated using a simple index, termed as Transfer Coefficient (TC). The TC has been calculated by dividing the concentration of a metal in the aerial portion of the plant by the total metal concentration in the soil (Kabata-Pendias and Pendias 1984; Kloke *et al*. 1984; Yan *et al*. 2007). The root samples of the plant species studied in this report could not be collected due to the deep root system of the tree species growing in the mine area.

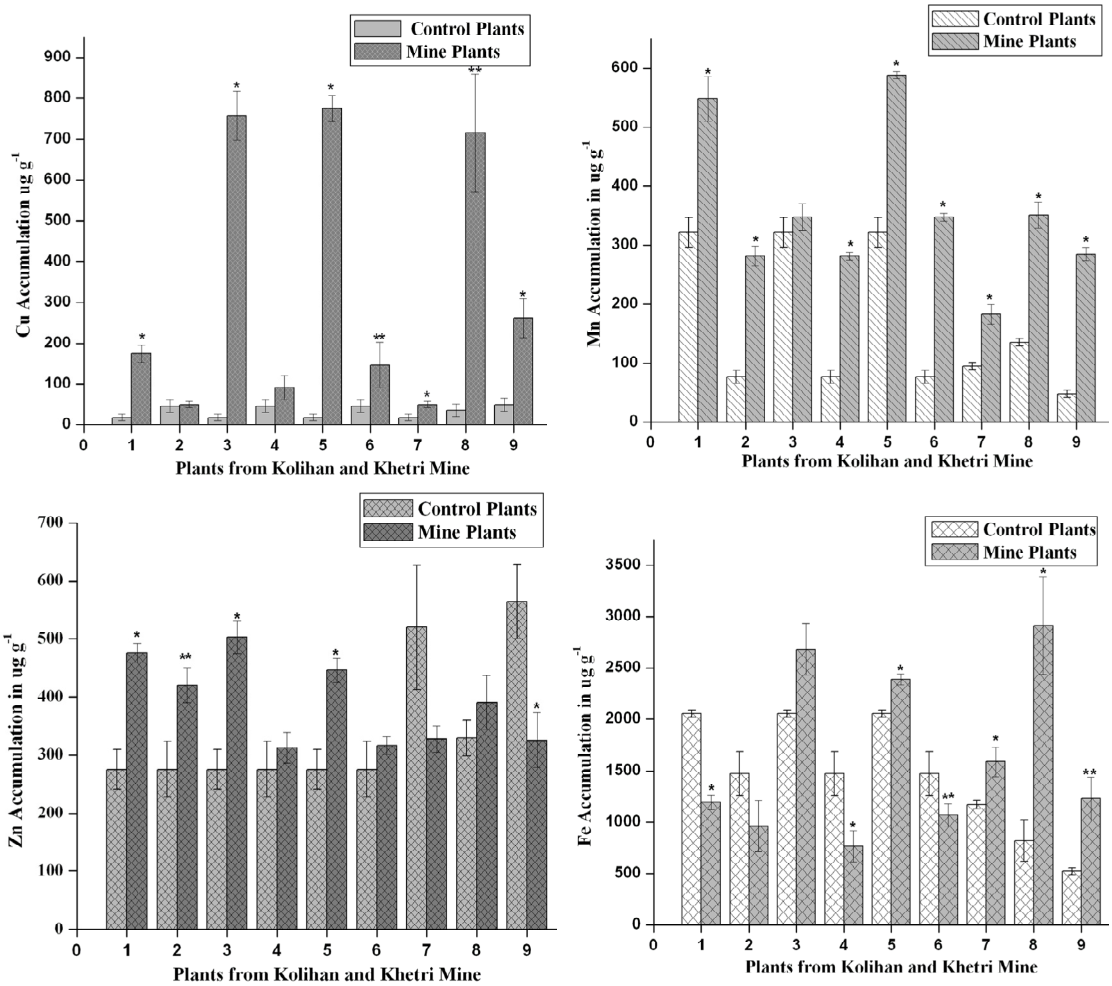

Fig. 2 Accumulation of Cu and distribution of other metals (Mn, Zn, Fe) in plants growing at Kolihan, Khetri mine and its controls. (**1**) Kolihan *Prosopis juliflora* leaf, (**2**) Kolihan *P. juliflora* twigs, (**3**) Khetri site "A" *P. juliflora* leaf, (**4**) Khetri site "A" *P. juliflora* twigs, (**5**) Khetri site "B" *P. juliflora* leaf, (**6**) Khetri Site "B" *P. juliflora* twigs, (**7**) Khetri "B" site *P. juliflora* green pods, (**8**) Khetri site "C" *Ailanthus excelsa* leaf, (**9**) Khetri site "C" *A. excelsa* twigs.

Table 1 Analysis of metals in soils of different mine sites as compared to control.

Metal	NCL (control) ($\mu g\ g^{-1}$) (Mean ± SD)	KolihanMine ($\mu g\ g^{-1}$) (Mean ± SD)	Khetri Tailing Dam ($\mu g\ g^{-1}$) (Mean ± SD)	Khetri Site "A" ($\mu g\ g^{-1}$) (Mean ± SD)	Khetri Site "B" ($\mu g\ g^{-1}$) (Mean ± SD)
Cu	216.95 ± 83.67	594.31 ± 39.70*	2059.05 ± 169.75*	1840.95 ± 120.45*	715.27 ± 40.48*
Mn	66.59 ± 10.47	172.63 ± 22.6*	404.45 ± 45.2*	133.17 ± 14.8**	611.58 ± 47.59*
Zn	160.99 ± 27.8	164.92 ± 7.86†	445.03 ± 32.7*	83.77 ± 4.53†	212.04 ± 28.3†
Fe	14721.57 ± 233.6	23855.23 ± 586.12**	26980.66 ± 236.5†	31420.22 ± 228.3†	17207.09 ± 609.7†

ANOVA carried out using 3 replicates of samples.
SD Standard Deviation
* Significant at P<0.01
** Significant at P<0.05
† Non-significant

RESULTS AND DISCUSSION

Cu accumulation study

Among the soil samples collected, high amount of Cu was found at Khetri tailing dam (**Table 1**) where the waste product after recovery of Cu from the ore was dumped. Absence of vegetation was noted at this site. The next high amount of Cu was found at site "A" followed by "B" where *P. juliflora* plants were growing. When compared with the control soil, the ratio of Cu was 1:9 in tailing dam, 1:8 at site "A" and 1:3 at site "B" of Khetri mine. In the soil of Kolihan mine the ratio of Cu content was 1:3. The plants at Kolihan and Khetri mine were growing in soil containing high Cu content. The permissible limit of Cu in India is 135 to 270 mg kg^{-1} (Awashthi 2000). The Cu content in mine area soil was found to be above the permissible limit (**Table 1**).

In the present study, Cu content in leaves collected from all the mine sites was higher than that collected from the control sites (**Fig. 2**). Leaf samples collected from Khetri mines particularly showed maximum Cu accumulation, among which, leaf samples collected from location "A" and "B" accumulated significantly higher amount of Cu. However, in Kolihan mines, the plants displayed low Cu accumulation in leaf tissues, probably because the plants were growing in less Cu-containing soil. The Cu content was less in the soil of Kolihan mine area than Khetri, hence the metal uptake by plant was also low.

Twigs also showed a similar pattern of higher Cu accumulation in samples collected from the mine dumps when compared with controls, although the capacity for Cu accumulation was lower than the respective leaf samples. Variable amounts of Cu have been noticed among the twigs with respect to mine locations. However, in *A. excelsa* tree growing in Khetri mine site "C", the twigs contained significantly higher amount of Cu when compared with those from control and *P. juliflora* growing at different mine sites. The maximum accumulation of Cu (775.29 µg g^{-1}) with respect to leaf tissue was observed at "B" site. The least accumulation of Cu was in green pod of *P. juliflora* growing at site "B" (50.46 µg g^{-1}). When compared with controls, the leaves of *P. juliflora* accumulated more Cu (1: 42.2 times) at site "B", while 1: 41.2 times at site "A". Leaves of *A. excelsa* growing at site "C" accumulated 1: 19.5 times more Cu. In leaves of *P. juliflora* of Kolihan mine, the ratio of Cu content was 9.5 times while green pods of plants growing at site "B" accumulated 1: 2.75 times higher Cu content than control.

In case of twigs, highest amount of Cu was noticed in *A. excelsa*. Leaves of *P. juliflora* were found to be highly efficient in Cu accumulation, being (8 and 5 times, respectively at site "A" and "B") higher than twigs. Similarly, it was 3.45 times higher in leaves than twigs of the plant growing at Kolihan mines. Leaves of *A. excelsa* accumulated 2.73 times higher amount of Cu than twigs. Cu accumulation was found to be higher in leaf tissues when compared to that in twigs at all the mining sites studied. In Kolihan mine the accumulation rate was less than that Khetri mine.

In the literature, the highest concentration of Cu was found in the leaves of *Rumex acetosa* ranging from 340 to 1102 mg kg^{-1} and averaging 601 mg kg^{-1}. Kelepertsis and Andrulakis (1983) reported a maximum of 800 mg kg^{-1} Cu in *Rumex acetosella*. Most of these samples had Cu concentrations ranging from 230 to 400 mg kg^{-1} in the species. Although Cu, a component of enzymes, is an essential element for plant growth, it can cause toxic effects when shoots or leaves accumulate more than 20 mg kg^{-1} (Borkert *et al*. 1998). The plant samples collected in the present study, from different mines and locations, accumulated more than 20 mg kg^{-1}. The Cu concentration ranged from 50.46 to 775.29 µg g^{-1} in samples from mine areas, whereas in control it was 18.35 to 50.46 µg g^{-1}. The highest amount of Cu was 775.29 µg g^{-1}. The high amount of Cu found in leaf samples of mining sites suggest, the uptake of metal by plant through roots and transportation to leaves. The twig samples from mining sites showed less amount of Cu. It was not more than 3.2 times than its control. In case of control sample of all the plants, Cu content was higher in twigs than in leaves. Cu is mostly utilized in leaves as it is used by plastocyanin in photosynthesis and Cytochrome *c* oxidase in respiration (Rodriguez *et al*. 1999). This could be the possible reason for less amount of Cu in leaves than twigs in control plants.

Distribution of other metals (Mn, Zn and Fe)

The distribution of other metals (Mn, Zn and Fe) was also studied in the soil of Cu mining areas. Manganese (Mn) is an essential micronutrient and an activator for enzymes involved in tricarboxylic acid cycle (Pittman 2005). Mn content in the soil of Khetri at different sites and Kolihan mine is shown in **Table 1**. In control soil, Mn content was 66.59 µg g^{-1}. When compared with control soil the ratio was 1: 6 at tailing dam, 1: 2 in Khetri site "A", and 1:9 at site "B". In Kolihan mine the ratio of Mn content was 1: 2. The permissible limit of Mn was not available, but, the range in uncontaminated soil of India is between 100 to 4000 mg kg^{-1} (Bowen 1966). Content of Mn in Khetri Cu mining area soil showed that it is within limits.

The amount of Mn present in leaves and twigs of *P. juliflora* growing in the surrounding areas of NCL is shown in **Fig. 2**. Control leaves accumulated more amount of Mn than the twig samples of both the plant species studied. Significant content of Mn was observed in leaves of *P. juliflora* plants growing at both mines, highest being at Khetri site "B" and Kolihan mine, followed by Khetri site "A" but it was less than the Cu content. The amount of Mn in twigs was less than that in leaves. Green pods of *P. juliflora* at site "B" accumulated 182.68 µg g^{-1}, whereas in control it was 94.99 µg g^{-1}, which shows that Mn also moves to other parts of the plant.

Twigs accumulated more Mn than leaves when compared with their respective controls. Twigs of *A. excelsa* growing in Khetri site "C" accumulated 6 times higher Mn than the controls followed by the twigs of *P. juliflora* growing in Khetri site "B" (4.52 times) and site "A" (3.66 times) and also to those of the plants growing in Kolihan mine (3.6 times). The content of Mn was more in leaves, but when compared with control, the twigs had more amount of Mn. Higher amount of Mn was found in *A. excelsa* twigs than *P. juliflora*, when compared to their respective controls.

Mn concentration in the range of 20-300 mg kg^{-1} in plant is considered to be normal by Kabata-Pendias and Pendias (1992). The Mn concentration ranged from 182.68 to 588.25 µg g^{-1} in plant samples from mining areas, whereas in control it was 47.49 to 321.53 µg g^{-1}. Mn content was found to be more in leaves than twigs. Leaves of both the plants of Cu mines showed higher accumulation of Mn than the normal range. In other parts it is in normal range except in twigs of *P. juliflora* of Khetri site "B" and control leaves, where it is slightly higher than the normal limits.

More amount of Zinc (Zn) was found in the soil of tailing dam (**Table 1**) followed by that at Khetri site "B" and Kolihan mine. Site "A" of Khetri mine had least amount of Zn content. The ratio of Zn content when compared with control soil was 1: 2.8 in tailing dam, 1: 0.5 at site "A" and 1:1.3 at site "B" of Khetri mine. In Kolihan mine, Zn content was almost same as that of control. The permissible limit of Zn in India is 300 to 600 mg kg^{-1} (Awashthi 2000). The concentration of Zn in soils of both the mines, fall in the normal limit.

The amount of Zn present in *P. juliflora* growing in the area around NCL and plants growing at Cu dumpsite were compared (**Fig. 2**). Amount of Zn was more in leaves than twigs. In green pods of *P. juliflora*, Zn content was less than its control. The amount of metal in *Prosopis* twigs of dumpsite was more than its control. It was more in Kolihan mine followed by almost similar amount in twigs of plants growing at site "A" and "B". In green pods of *P. juliflora*, Zn content was less in dumpsite than control while it was similar to that in case of *A. excelsa* twigs. When compared with control, the ratios of Zn content in *P. juliflora* leaves was 1.8 and 1.6 times more at site "A" and "B", respectively. The Zn content in twigs of *P. juliflora* growing at Khetri site "A" and "B" was similar to that of control. In *A. excelsa* leaves of Khetri site "C", Zn accumulation was 1.18 times, while twigs did not accumulate more Zn than the control. In Kolihan mine, Zn content was 1.7 times more in *P. juliflora* leaves than in control, while in twigs it was 1.52 times higher. In the dump samples, Zn content of leaves was more than twigs in both the plant species studied, while in control samples, *A. excelsa* twigs had more Zn content than its leaves.

Zinc is an essential element as divalent cation, which acts as co-factor to enzymes (Alloway 1990). The normal level of Zn is 20-400 mg kg^{-1} as reported by Reeves and Baker (2000). Highest content of Zn in plants growing at Cu mine site was 312.61 to 475.40 µg g^{-1}. In all the plants, content of Zn was within normal range except leaf samples of *P. juliflora* of Kolihan and Site "A" of Khetri mine, which were slightly higher. In control samples, Zn content in plants was in normal limits except the pods of *P. juliflora* and twigs of *A. excelsa*.

High amount of Iron (Fe) was present in soil of Khetri site "A" (31420.22 µg g^{-1}), followed by Khetri tailing dam, Kolihan mine and Khetri site "B" (**Table 1**). When compared with control soil, the ratio of Fe content was 1: 2.1 in Khetri site "A", 1: 1.83 in tailing dam, 1:1.6 in Kolihan mine and 1:1.1 in Khetri site "B". Regarding Fe, there is lack of specific criteria for its toxicity. The form of Fe, the growth stage, the nutrient status and environmental factors, all affect the toxicity level (IRRI, 2009). However, safe limits of Fe are not available in Indian standard (Islam *et al*.

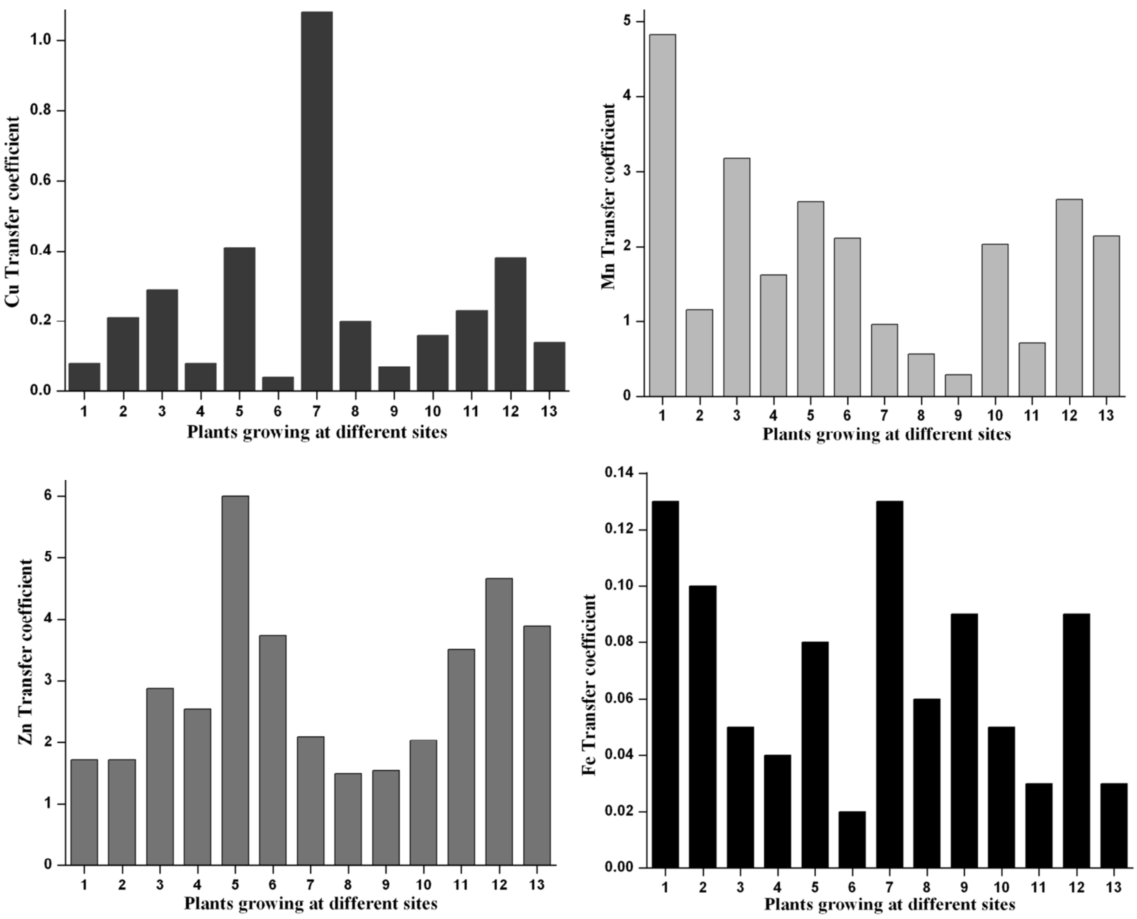

Fig. 3 Transfer Coefficient of different metals (Cu, Mn, Zn, Fe) in leaf and twigs of plants growing on both mines and its controls. (**1**) Control *Prosopis juliflora* leaf, (**2**) Control *P. juliflora* twigs, (**3**) Kolihan *P. juliflora* leaf, (**4**) Kolihan *P. juliflora* twigs, (**5**) Khetri site "A" *P. juliflora* Leaf, (**6**) Khetri site "A" *P. juliflora* twigs, (**7**) Khetri site "B" *P. juliflora* leaf, (**8**) Khetri site "B" *P. juliflora* twigs, (**9**) Khetri site "B" *P. juliflora* green pods, (**10**) Control *Ailanthus excelsa* leaf, (**11**) Control *A. excelsa* twigs, (**12**) Khetri site "C" *A. excelsa* leaf, (**13**) Khetri site "C" *A. excelsa* twigs.

2009).

The amount of Fe present in leaves and twigs collected from both the mines and control plants are shown in **Fig. 2**. Irrespective of plant species and sites, all the leaves had more Fe content as compared to twigs. Leaves of the plants at all other locations were accumulating high amount of Fe, except in Kolihan mine which had less amount of Fe when compared with control. All the twigs of *P. juliflora* plants growing in mining area accumulated less amount of Fe than the controls. In case of *A. excelsa* tree, the twigs had high amount of Fe than its control. Leaves of *A. excelsa* at Khetri site "C" had 3.3 times more Fe when compared with control. *P. juliflora* leaves from Khetri site "A" and "B" showed 1.3 and 1.16 times more Fe, respectively. Green pods showed 1.3 times more Fe content than their controls. The accumulation of Fe in *Prosopis* twigs was not higher than its control, while the twigs of *Ailanthus* growing at Khetri site "C" had 2.3 times more Fe than control.

Fe is an essential nutrient for plants. It functions to accept and donate electrons and plays important role in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot 2002). Leaf tissues of all Cu mining sites accumulated high amount of Fe except in Kolihan mine which had less amount of Fe when compared with control. All the twigs of *P. juliflora* plants growing in mining area accumulated less amount of Fe than the controls. This could possibly be the effect of Cu on these plants. Further study needs to be conducted to know the exact mechanism of metal uptake.

Transfer coefficient

The TC quantifies the relative differences in the bioavailability of metals to plants and is a function of both soil and plant properties. In the present study, coefficient is calculated by dividing the concentration of a metal in the plant tissue dry weight (DW) by the total metal concentration in the soil. Madejón *et al.* (2004) calculated TC in leaves and stems of white poplar trees from spill-affected and non-affected sites. The TC in the vegetables of metal contaminated sites in Huangshi, China was studied in Cu, Cd, and Pb. The values ranged from 0.009-0.065, 0.057-0.146, 0.031-0.132, respectively (Yan *et al.* 2007). Ayari *et al.* (2010) reported TC in plants growing on soil amended by municipal solid waste composts (MSWC) for metals such as Cr, Ni, Cu and Zn. The TC values were studied for four consecutive years at three levels, 0 (control), 40 t MSWC/ha/y and 80 t MSWC/ha/y. TC values for Cr ranged from 0.01-0.02 for control, 0.03-0.13 for 40 t MSWC/ha/y and 0.07-0.26 for 80 t MSWC/ha/y level. For Ni, TC was 0.01-0.03, 0.04-0.15 and 0.09-0.33; for Cu, TC values were 0.03-0.04, 0.08-0.23 and 0.13-0.44; whereas for Zn, it was 0.19-0.21, 0.41-0.53 and 0.47-0.71, respectively. The root samples of the plant species studied in the present report could not be collected, hence, only the data on translocation of the metal from soil to stem and leaves have been calculated.

TC for Cu of control leaves and twigs was 0.08 and 0.21, respectively. TC of all the samples ranged from a minimum of 0.05 in *P. juliflora* twigs of Khetri site "A" to a maximum of 1.08 in Khetri site "B" leaves (**Fig. 3**). It was noted that the uptake of metal from soil and accumulation in leaves was more than that in twigs. The TC of Cu was different in same plant species from different sites. In *P. juliflora* growing at Khetri site "A" it was 0.41, but in site "B" it was 1.08. In control plants of *A. excelsa*, the TC of leaves and twigs was 0.16 and 0.23, respectively, it was 0.38 and 0.14 in leaves and twigs of plants growing at

Khetri site "C", indicating metal transport from soil and its increased accumulation in leaves than in twigs. Similarly Ayari *et al*. (2010) reported TC value of 0.03-0.04 for control, 0.08-0.23 for 40 t MSWC/ha/y and 0.13-0.44 80 t MSWC/ha/y for Cu in wheat.

In control plants of *P. juliflora*, the TC for Mn was 4.28 in leaves and 1.15 in twigs. TC factor of Mn ranged from 3.17 (Kolihan mine *Prosopis* leaves) to 0.29 (Khetri site "B" *Prosopis* green pods). Maximum transport of Mn from soil to leaves of *P. juliflora* was observed at Kolihan mine and minimum transport in green pods of plants from Khetri mine. In control leaves and twigs the TC of Zn was 1.72, whereas in plants growing in the mining area, it ranged from 6 (Khetri site "A" *Prosopis* leaves) to 1.49 (Khetri site "B" *Prosopis* twigs). The TC of Zn was more than the reported values by Ayari *et al*. (2010) for wheat plants. In case of Fe in the present study, it was 0.13 and 0.02 at site "A" leaves and site "B" twigs, respectively. Among the TC of Mn, Zn and Fe in *A. excelsa*, Zn was highest, followed by Mn and then Fe.

Maximum transport of Zn was noted from soil to *P. juliflora* leaves of Khetri site "A". Among all the metals studied, TC of Fe was the least. The soil had high concentration of Fe, but it was not absorbed by the plants growing there.

This study reveals that the plants studied here, can tolerate excess amount of Cu. They accumulate higher amount of Cu in aerial parts of plants. Higher accumulation of the metal than the toxic level proves that these plants have mechanism for stress tolerance, defense and detoxification of Cu, which has to be elucidated.

The present results show that Cu accumulation was higher in leaves than twigs of both the plants of Khetri mine. High amount of metal accumulated in leaves of both the plant species, of which *P. juliflora* accumulates at a higher-rate than *A. excelsa*, when compared to their respective controls. This suggests that these plants could be hyperaccumulators. Further studies need to be carried out to determine whether they can accumulate higher amount of metal. This field study shows that these plants can be used for phytoextraction of Cu from contaminated sites.

Among the distribution of other metals in plant samples, Mn content was found to be more. The content of Mn in the soil of Cu mining area was higher than the control soil, which may have led to higher uptake of Mn by the plants growing there. Less amount of Zn in green pods of *P. juliflora* and twigs of *A. excelsa* in the sample from dumpsite than control could be the effect of Cu on these tissues. In case of Fe, all the leaves had more amount of metal than its control except those from Kolihan mines. Dumpsite leaf and twig samples of *A. excelsa* had higher Fe content than the respective *Prosopis* tissues. This indicates that distribution of metals differ from one part to other part and also between the two different plant species. Highest TC was observed in *P. juliflora* leaves of Khetri site "A" for Zn.

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