



Long-term phytoremediating abilities of *Dalbergia sissoo* Roxb. (Fabaceae)

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Abstract

The boom of tanneries in north India has converted the river Ganga into a waste dumping stream. The tanneries discharge their heavy metal-rich effluents into the river. Tissues of two-year-old tree saplings of *Dalbergia sissoo*, soil sediments and river water samples were collected from three sites along the river Ganga at Jajmau, Kanpur. Site-1 was located 1 km upstream from the point of discharge of the effluents of the tanneries, Site-2 was close to the source point, and Site-3 was about 1 km downstream from the source point. Accumulation of Cu, Cr and Ni in leaves, bark, wood growth rings, soil sediments and water samples was estimated using an atomic absorption spectrophotometer. The tissues of the two-year-old wood growth rings of *D. sissoo* accumulated large amounts of Cr, Cu and Ni. The Cr concentration in leaves, bark and wood rings had a strong, positive and linear correlation (r^2) with the Cr concentration in soil sediments. The bio-concentration factors (BCFs) of Cr, Cu and Ni were higher than 1 in the wood, bark and leaves at all three selected sites, indicating the strong phytoremediating ability of the tree. The uptake of Cr and Ni was consistent in the bark and wood growth rings of two successive years. Owing to the strong uptake and accumulating abilities of Cr, Cu and Ni as evident from high BCF values and high biomass, we propose *D. sissoo* as a suitable species for phytoremediation throughout its range of distribution in Africa, Asia and the Americas.

Keywords *Dalbergia sissoo* · Ganga river · Phytoremediation · Tannery effluents · Bio-concentration factor · Heavy metals

1 Introduction

Phytofiltration, phytoextraction, phytostabilization and phytovolatilization are being considered as effective means of phytoremediation [1–3]. The extent of uptake and accumulation of pollutants vary from plant to plant [4]. The careful selection of a phytoremediating species is of utmost importance, especially for highly polluted soil [5]. The phytoremediating species must be highly tolerant of the pollutants [6]. The preference is given to an indigenous and naturalized species of the locality, adapted to local climate conditions [7]. It is also best to select a fast growing and high biomass plant with high capacity for uptake of heavy metals [8]. *Dalbergia sissoo* (Indian

Rosewood; Fabaceae) has all these attributes (e.g., high biomass, rapid growth, climate tolerance, economic value, etc.). *Dalbergia sissoo* is the most commonly used timber tree in India, Afghanistan, Bangladesh, Iran, Iraq, Myanmar, Nepal and Pakistan, with additional advantages of easy propagation, deep root system, wide adaptability and a minimal role in the human and animal food chain [9]. *Dalbergia sissoo* shows healthy growth in metal-contaminated soil making this tree a suitable candidate for phytoremediation [10]. The heavy metals localized in the wood may get locked for a longer duration if used in furniture, doors and windows. Thus, the costs involved in harvesting and processing for recovery of metals may also be minimized.

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Dalbergia sissoo is native to the Indian sub-continent and widely distributed in Africa, Asia and Americas [11].

The river Ganga is the largest river in India and considered sacred by the majority of the Hindu community. It flows from Gangotri glaciers in upper Himalayas passing through several states of India and enters Bay of Bengal [12]. During the past several decades, large numbers of industries have come up along the Ganga river mainly in the state of Uttar Pradesh [12]. The Ganges basin is world's most heavily populated with a density of about 1000 inhabitants per square mile [13]. Inefficiently treated municipal wastes and effluents of the industries along the river are discharged in the water stream directly [12]. Kanpur (26°26'N and 80°24'E) is a major industrial city of Uttar Pradesh, India situated on the bank of river Ganga (14). Large numbers of tanneries clustered in the Jajmau area of Kanpur city (Fig. 1) discharge their effluents into the river Ganga after inadequate treatment and containing several toxic metals and organic substances [9, 14–18]. These toxins affect the aquatic organisms of almost all tropic levels [19]. All these metals are usually in soluble form and bio-available at low pH [20]. The concentration of these metals in the river consistently decreases with increasing distance from the point of discharge [21]. These heavy metals can also get absorbed and accumulated in aquatic organisms [22].

In the present study, we investigated the heavy metal extraction capacity of *D. sissoo* and its ability to compartmentalize heavy metals in tissues of three aboveground plant organs. We determined the extent of accumulation and bio-concentration factor (BCF) of Cr, Cu and Ni in wood, bark and leaves. Our objectives were to determine (a) the concentrations of these metals likely to be locked in the wood for a longer period if used in building

material and furniture making, (b) the concentrations of these metals partitioned in the bark and leaves where it can be harvested annually via natural defoliation so that the targeted toxic metals can be recovered without damaging the tree and (c) the BCF of Cr, Cu and Ni in aboveground plant organs to document the tree's capacity for phytoremediation.

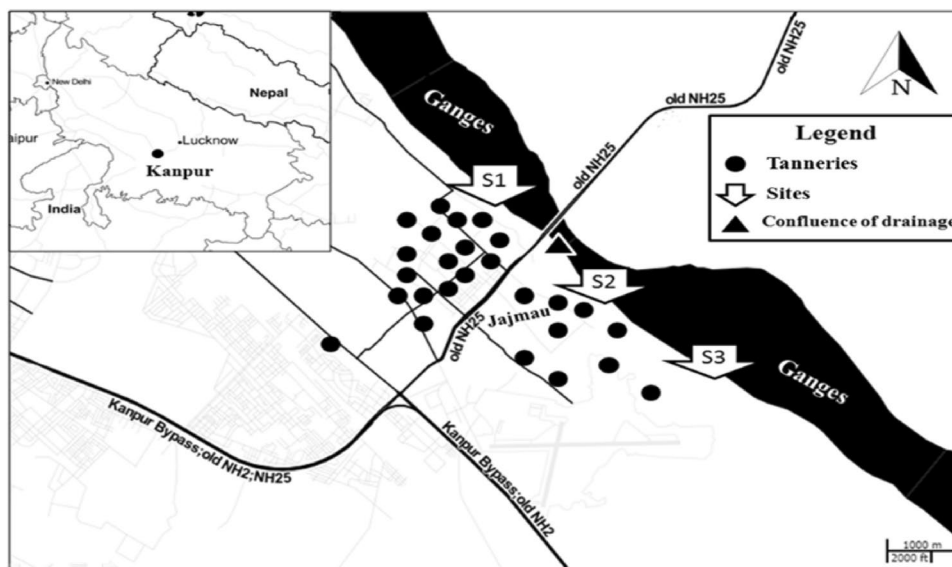
2 Methodology

2.1 Location of sites and sampling

Accumulation of Cr, Cu and Ni was estimated in varying parts of *D. sissoo* using an atomic absorption spectrophotometer (GBC Scientific Equipment, Dandenong, Australia). The plant tissue, water and soil samples were collected from three sites along the Ganga river bank. Site-1 is located on the right bank of the Ganga at Jajmau, Kanpur about 1 km before the major point of discharge of effluents of tanneries into the river. Site-2 is located at the point of the discharge of effluents of tanneries into the river and Site-3, about 1 km downstream from Site-2 (Fig. 1). Five samples of soil (over 1 kg of fresh soil sediments) were collected from 50–60 cm inland of the river stream bank, and an additional five soil samples were collected from top 20 cm layer of soil about 3–4 m inland of the river bank (close to the saplings of *D. sissoo*). Soil samples were dried in an oven at 80 °C for 72 h. The dried soil samples from each collection site were mixed to make one composite sample of each site for estimation of selected heavy metal in soil.

The trees of *D. sissoo* are widely distributed along the river banks of Ganga at Jajmau, Kanpur. Two-year-old saplings of

Fig. 1 Source World map (Harvard Education), Stamen Toner (modified) to show locations of sites. S1 = Site-1, S2 = Site-2 and S3 = Site-3 and confluence of major drainage system from tanneries in river Ganga



growing about 3–4 m away from the river bank were collected in triplicate in the first week of November (before the beginning of defoliation) for estimation of Cr, Cu and Ni in the aboveground plant organs (leaves, bark and wood). The leaves of each tree sapling were separated and dried at 80 °C for 72 h and ground to a fine powder. About 10 cm pieces of the main trunk, including bark, were separated from 30 cm above the ground and kept in the oven (80 °C) for partial drying. The bark samples were separated and chopped off with a sharp knife into thin, small pieces. The secondary xylem (wood) rings of both years in each wood block were separated using a sharp knife following guidelines of Ghose et al. [23, 24]. The wood chips of each growth ring were also chopped off into smaller pieces. The chopped bark and wood rings were re-dried at 80 °C for 48 to 72 h or until complete drying and ground to a fine powder. The powder of leaves, bark and wood rings of each tree saplings was sieved and acid digested for heavy metal analysis.

2.2 Acid digestion

The powdered plant samples were digested following the method of Parkinson and Allen [25]. 0.3 g of the powdered plant samples and soil sediment samples were weighed and transferred to conical flasks. Nitric acid (69%) of the analytical grade was added and kept on a hot plate for digestion until the brown fume evolving from the conical flask turned white. The digest was allowed to cool, diluted with DDW and filtered with Whatman's filter paper 42, leaving a whitish residue. The volume of the filtrate was made to 25 ml with double distilled water and analyzed for heavy metal using the AAS (GBC Scientific Equipment, Dandenong, Australia). The AAS results were converted to actual concentration of metal in the sample using the following equation:

$$\text{Concentration } \mu\text{g g}^{-1} = \frac{\text{Calibration reading} \times \text{Extract volume}}{\text{Sample weight}},$$

where calibration reading is obtained from the AAS with pre-installed Avanta Software Package, version 2.02. Extract volume is the final volume of the digest used for spectrometric analysis.

2.3 Bio-concentration factor (BCF)

Bio-concentration factor (BCF) or transfer factor (TF) is defined as the ratio of total metal concentration in the harvested tissues (barks, leaves and wood rings in the present study) and metal concentration in the soil [26]. In previous studies, BCF has been used to estimate the ability of plants to accumulate certain heavy metals absorbed from soil [27, 28]. BCF was calculated using the following equation:

$$\text{BCF} = \frac{C_{\text{harvested tissues}}}{C_{\text{soil}}},$$

where $C_{\text{harvested tissue}}$ = concentration of heavy metal in harvested tissues and C_{soil} = concentration of heavy metal in soil.

The data were analyzed using SPSS Version 17.0 for Windows [29].

3 Results and discussion

3.1 Soil and water

The concentrations of Cr, Cu and Ni were higher in the soil sediments at the Site-2 close to source point than either at Site-1 (1 km upstream) or Site-3 (1 km downstream). The concentration of all three heavy metals in the soil sediments were in the order as Site-2 > Site-3 > Site-1. The presence of Cr, Cu and Ni at Site-1 may largely be due to the addition of these heavy metals through the effluents of several other sources of Kanpur city rather than tanneries at Jajmau area alone (Table 1). This result is in agreement with the findings of Bhatnagar et al. [30]. The Cr concentration in water samples was relatively higher at source point (Site-2) than in the water samples of Site-1 (1 km upstream) and Site-3 (1 km downstream). The presence of high quantities of Cr in the water and soil sediments at Site-2 as compared to other sites indicates that the tannery effluents consist of higher quantities of Cr and it is readily retained at the site of confluence of the major drainage system of the effluents of tanneries and the river Ganga. In a similar study, it was reported that during the tanning process, large amounts of un-reacted Cr remain in water [31]. But, Cu and Ni concentrations in water samples of Site-2 (at source point of the tannery effluents) were comparatively less than those in the water samples of Site-1 and Site-3 (Table 1). The concentration of Cu was very high in water samples of Site-1 followed by Site-3 and Site-2, respectively (Table 1). The sedimentation of Cr, Cu and Ni was reduced at Site-3 (1 km distance from source point). The sedimentation of Cr and Ni was relatively higher at 1 km downstream from the source point (Site-3) than that of Cu (Table 1). There are no specific studies to our knowledge investigating the rate of sedimentation of these metals; therefore, we are unable to compare our results to other published studies. In our findings (Table 1), relatively larger quantities of Cr were recorded in the soil sediments at Site-2 (source point) than at 1 km downstream (Site-3). The Ni sedimented in larger quantities at 1 km (Site-3) away from the source point (Site-2). This indicates that the Cr sedimented quickly near the source point, while Ni

Table 1 Accumulation of Cr, Cu and Ni in water, sediments and aboveground parts of *D. sissoo* collected from three sites around the confluence of the effluents from major tanneries on the bank of river Ganga at Jajmau, Kanpur

Parameters	Heavy metals	Site-1	Site-2	Site-3	LSD
Water ($\mu\text{g ml}^{-1}$)	Cr	0.5 ± 0.1a	0.9 ± 0.2a	0.5 ± 0.2a	0.57
	Cu	1 ± 0.2a	0.7 ± 0.2a	0.9 ± 0.2a	0.66
	Ni	0.4 ± 0.1a	0.3 ± 0.1a	0.9 ± 0.2a	0.58
Sediment ($\mu\text{g g}^{-1}$)	Cr	25.4 ± 2.8c	68.6 ± 2.3a	40.8 ± 2.3b	7.21
	Cu	10.7 ± 2a	16.2 ± 2.8a	13.1 ± 2.3a	7.02
	Ni	45.1 ± 2.9c	56.2 ± 2.9b	78.2 ± 2.3a	7.79
Leaf ($\mu\text{g g}^{-1}$)	Cr	69.1 ± 2.3b	80.9 ± 2.6a	68.8 ± 1.9b	6.58
	Cu	42.1 ± 2.3a	20.8 ± 2.6a	72 ± 1.7a	9.54
	Ni	126 ± 3.5a	42.1 ± 1.1b	136 ± 3.4a	8.33
Bark ($\mu\text{g g}^{-1}$)	Cr	70 ± 1.7c	92 ± 1.1a	84.2 ± 2.3b	5.19
	Cu	25.2 ± 2.9b	35.2 ± 1.7a	30.2 ± 0.7ab	5.72
	Ni	90.6 ± 3.1b	105.6 ± 3.8a	96 ± 3.4ab	10.02
Ring 1 ($\mu\text{g g}^{-1}$)	Cr	70.1 ± 1.8b	84.9 ± 2.6a	89.7 ± 2a	6.29
	Cu	26.8 ± 2c	62.6 ± 1.4a	33.5 ± 1.8b	5.12
	Ni	102.9 ± 1.7b	119.5 ± 2.5a	109.8 ± 2.8b	7.04
Ring 2 ($\mu\text{g g}^{-1}$)	Cr	75 ± 2.8b	90.7 ± 3.1a	87 ± 2.8a	8.57
	Cu	20.3 ± 2.9b	39 ± 2.5a	30.6 ± 2a	7.19
	Ni	90.7 ± 2b	123.6 ± 2a	96.5 ± 3.5b	7.49

Mean ± Standard Error

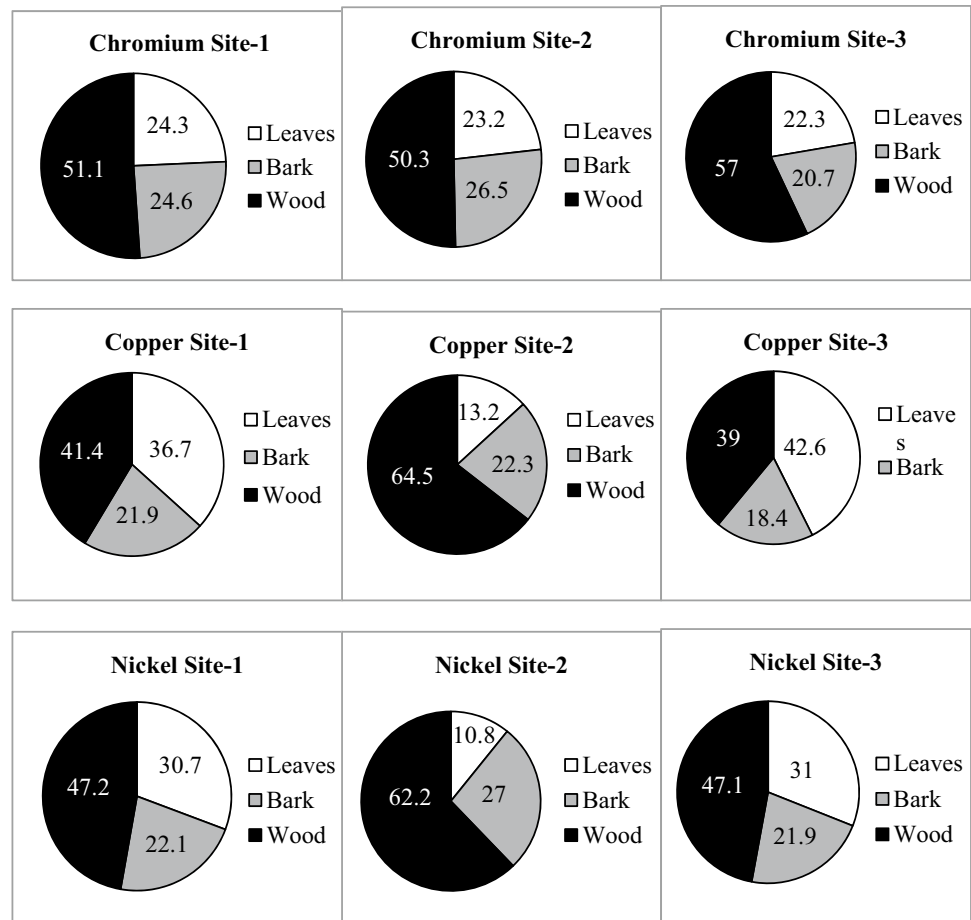
sedimentation was highest at 1 km from the point of discharge. Further studies based on sampling from the point of discharge (Site-2) to downstream at regular intervals are needed to examine various factors that may contribute to the patterns of sedimentation of various metals (Table 1).

3.2 Plant tissues

The concentration of Cr in the leaves of *D. sissoo* varied from 21 to 23% and in the bark from 24.7 to 26.3% at all three sites (Fig. 2). In a similar study, high quantity of Cr, Cu and Ni, besides some other heavy metals, accumulated in the bark and the leaves of the *Juglans regia* growing in contaminated soil [32]. The concentration of Cr in the wood (rings 1 and 2 together) varied from 50 to 52% at all three sites (Fig. 2). In our study, the Cu concentration is higher in the leaf samples rather than in the bark samples at all sites (Table 1). The defoliated and green leaves may differ in accumulated quantities of these metals. Rafati et al. [33] reported that the accumulation of Cd and Cr was relatively higher in defoliated leaves than in the green leaves of *Populus alba*. High concentration of Cu has also been reported in leaves of an oak species growing at a contaminated site [34]. In the present study, the Cu concentration in the wood rings 1 and 2 varied from 38 to 41% (Fig. 2) at all three sites. The partitioning of Cu and Ni was relatively higher in the leaves than in the bark at Site-1 and Site-3 than at the point of discharge of tannery effluents (Table 1). The quantity of Ni has also been reported higher in the defoliated leaves of *Morus alba*

[33]. The overall trend of accumulation of Cr was wood rings (1 + 2) > bark > leaves and that of Cu and Ni was wood > leaves > bark (Fig. 2 and 3). Further, there are differences in the levels of metal accumulation in different tissues. For examples, leaves and stem of *Salix* sp. growing in metal-contaminated soil accumulated high quantities of Cd and Zn compared to other parts of the plant [35]. Zhao et al. [36] found high phytoextracting potentials for Cd, Cu, Pb and Zn in 18 woody species of China. Among these trees, *Broussonetia papyrifera* had the highest multi-metal phytoremediating ability. In contrast to our results, the partitioning of metal from trunks to leaves consistently increased in the order of trunks < branches < leaves [36]. Many *D. sissoo* germinate under metal-contaminated soil and grow to maturity along the Ganga river banks. Plants that are fast growing, able to tolerate heavy metals, accumulate and translocate high quantities of metals to aboveground tissues and easy to harvest make ideal candidates for phytoremediation [37]. *Dalbergia sissoo* satisfies all these criteria and therefore makes an ideal candidate for the remediation and recovery of metals. In our study, accumulation of Cr, Cu and Ni in the wood of *D. sissoo* was relatively higher than in the other plant parts (Fig. 3). The partitioning of these toxic heavy metals in the dead wood may possibly be a defensive strategy to avoid toxicities to more physiologically active organs and tissues.

Fig. 2 The proportion of the selected heavy metals accumulating in three organs (leaves, bark and both the rings of wood) of *D. sissoo* collected from three sites (Site-1 located at 1 km before source point, Site-2 close to source point and Site-3 1 km away from the source point in the direction of flow) located on the bank of Ganga river near Jajmau, Kanpur



3.3 Bio-concentration factor (BCF)

Bio-concentration factors of Cr, Cu and Ni in *D. sissoo* are shown in Table 2. The values of BCF were determined in the leaves, bark and wood (Ring 1 and Ring 2) (Table 2). The bio-concentration factors of Cr, Cu and Ni in all the harvested tissues of *D. sissoo* were above 1 at all three selected sites. Maximum BCF of Cr (2.9) was recorded in the wood at Site-1. In a similar study, Algreen et al. [38] found that BCF values of Zn > Cu > Cd > Ni were highest in the wood of willow and poplar trees. In our study, the highest BCF values of Cu (5.5) and Ni (11.8) were found in leaves at Site-3 and Site-1, respectively. Native plant species are the best option for phytoremediation in any region of the world even if they have a lower ability for metal accumulation, instead of non-native plant species with higher ability for heavy metal uptake and remediation [39].

3.4 Correlation analysis

The correlation coefficients (r^2) were determined between the concentration of Cr, Cu and Ni in leaves, bark and wood rings of *D. sissoo* and water as well as sediments (Table 3).

There were weak correlations of heavy metals concentration between water samples and Cr in the leaves, bark and wood rings (Table 3). The Cr concentration in leaves, bark and wood and Cu concentrations in the bark was strongly correlated with their respective concentrations in the soil sediments (Table 3). The Ni accumulation in plant tissues had weak correlations with Ni concentrations in water or sediments (Table 3). The strong correlation of Cr in plant tissues with respect to sediments (Table 3) indicates concentration-dependent uptake. The weak correlation between Cu and Ni concentration in tissues and their corresponding concentration in water and sediments appears concentration independent.

4 Conclusion

We observed compartmentalization of Cr, Cu and Ni in leaves, bark and growth rings of wood in *D. sissoo*. A significant amount of absorbed heavy metals is locked into the wood growth rings, where the metals can be retained for long without reentering the soil through decomposition. The heavy metals may also be recovered annually

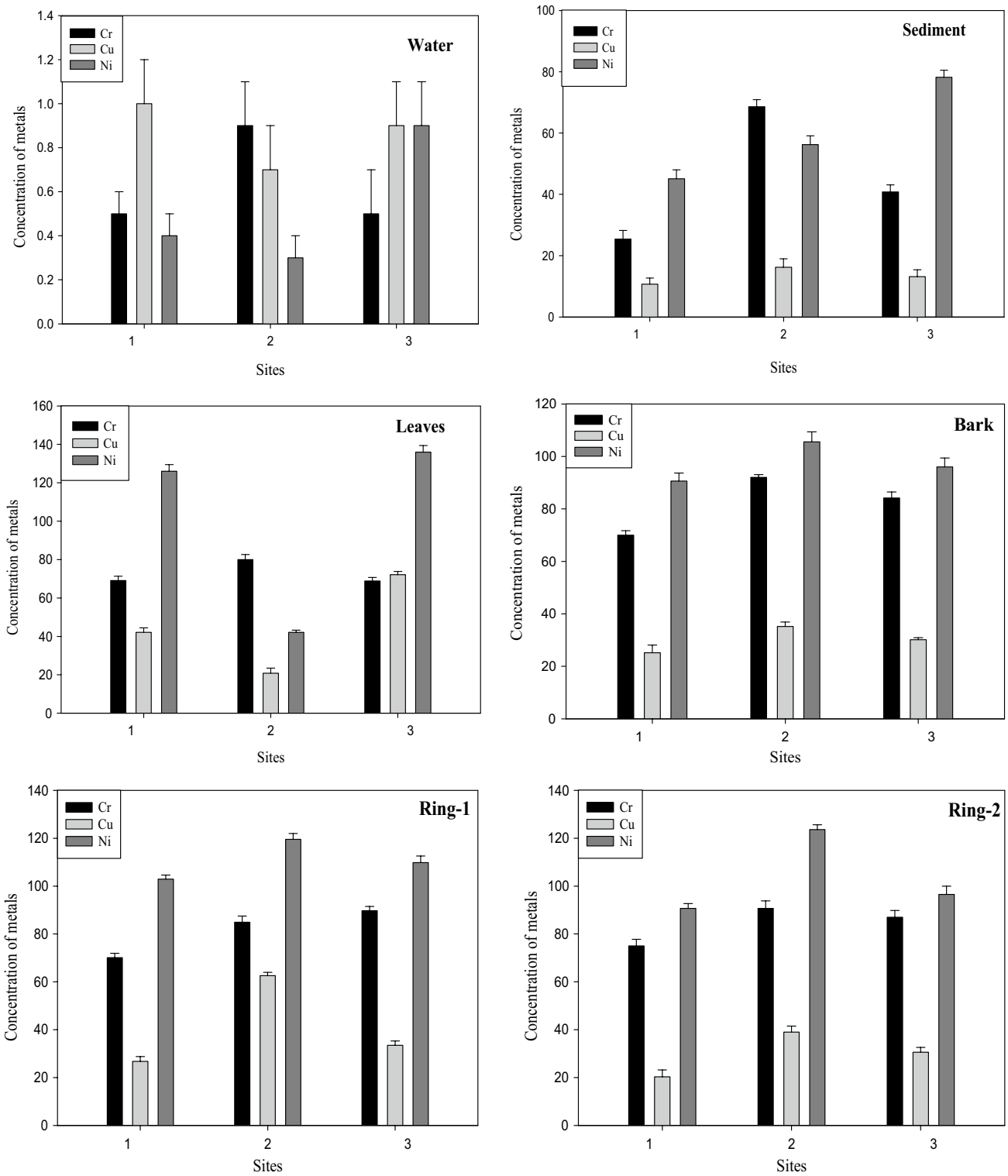


Fig. 3 Accumulation of the Cr, Cu and Ni in water, sediments, leaves, bark and in first and second year growth rings of *D. sissoo* collected from three sites located on the bank of river Ganga at Jajmau Kanpur. The Site-1 is located 1 km before the confluence

of the drainage carrying tannery effluents to river Ganga. Site-2 is located near the confluence point and Site-3 about 1 km downstream of river Ganga

Table 2 Bio-concentration factor (BCF) of Cr, Cu and Ni in varying organs of *Dalbergia sissoo* at Site-1, Site-2 and Site-3

BCF/TF	Site-1			Site-2			Site-3		
	Cr	Cu	Ni	Cr	Cu	Ni	Cr	Cu	Ni
Leaves/soil	2.7	3.9	11.8	1.1	1.3	2.6	1.9	5.5	10.4
Bark/soil	2.7	2.3	8.5	1.3	2.2	6.5	2	2.3	7.3
Wood/soil	2.8	2.2	8.9	1.5	3.5	7.3	2.2	2.4	7.9

Table 3 Correlation coefficient (r^2) between the metal concentrations in different tissues and water as well as sediments

Correlation coefficient (r^2)	Chromium	Copper	Nickel
Leaves versus water	0.27	0.19	0.28
Bark versus water	0.31	0.09	0.03
Wood versus water	0.22	0.08	0.07
Leaves versus sediment	0.61	0.10	0.06
Bark versus sediment	0.83	0.61	00
Wood versus sediment	0.43	0.34	00

(via phytomining) by collecting and processing the defoliated leaves and barks. In India, the timber of this tree is very commonly used in making furniture, doors and other building material, and thus, the significant amounts of Cr, Cu, and Ni localized in the annual wood rings can become locked in for a long period of time. These features, in addition to the wide distribution and high biomass, make *D. sissoo* an ideal candidate for the phytoremediation of Cr and Ni.

Acknowledgements The first author is grateful to the Aligarh Muslim University for the award of university fellowship and chairperson, Department of Botany for providing necessary laboratory facilities. NR was funded by a South and Central Asia Regional Travel Grant, The United States-India Educational Foundation.

Funding Financial support from the University Grants commission of India and Department of Botany, AMU for required laboratory supplies and facilities is gratefully acknowledged.

Compliance with ethical standards

Conflict of interest There is no conflict of interest associated with this publication

Declaration On behalf of all authors, the corresponding author confirms that that manuscript has been approved for submission.

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