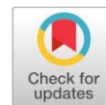


Original Research Article

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Assessment of cadmium bioremediation potential of fast-growing tree *Melia dubia* Cav.(Malabar Neem) in North Western Himalayas



Dushyant Sharma, Ankush Kumar and Kumari Shiwani*

Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, 173230, India

ABSTRACT

Cadmium (Cd) is recognized as highly toxic to humans and plants. Different methods, like chemical, physical biological, and phytoremediation have been used for the remediation of Cd contaminants from Urban and agricultural soil out of which phytoremediation is considered an eco-friendly remediation of soil, often called green remediation. On a large scale, phytoremediation using fast-growing trees may be preferred to annual/ biennial crops due to their capacity to produce large biomass, extensive deep root system, and ability to accumulate toxic metals in a form that is not biologically hazardous. The study aimed to evaluate different varieties of *Melia dubia* (Malabar Neem) for phytoremediation of applied cadmium. The study showed the significantly low effect of applied cadmium on vegetative growth parameters and biomass of trees. Further data generated through this study shows that the accumulation of Cd in plant parts in order roots > shoots > leaves in all the experimental varieties.

Novelty statement: Fast-growing tree species are one of the most important points when considering the potential tree for phytoremediation. Limited studies have been conducted to assess the cadmium bioremediation potential of *Melia dubia*. This study was done to assess the bioremediation potential of fast-growing, money-spinning trees which became popular amongst the farmers of the country due to their multipurpose uses in wood industries and suitable trees in agroforestry models wood industry. This study showed *M. dubia* can be a good candidate for heavy metals remediation for the industrial sites of the country specifically hill states due to its fast-growing nature and heavy biomass production. Further, for effective bioremediation/ bioaccumulation of heavy metals, planting of this tree species will provide a chance for rehabilitation and improvement of heavy metals polluted sites.

Keywords: Cadmium; phytoremediation; fast-growing tree; *Melia dubia*; Malabar neem

Introduction

Heavy metals are metallic chemical substance that has a relatively high density and is poisonous and toxic even at low concentrations [48]. Cadmium (Cd) is recognized as highly toxic to living organisms and humans [6] as it enters ecosystems via numerous anthropogenic activities and emissions to the environment [1]. The deposition of Cd in polluted soil poses serious problems to the health of animals and humans due to its high mobility in contaminated soils [7] which affects multiple organs of the human body mainly kidneys and causes serious damage, including pulmonary emphysema, renal tubular damage and kidney stones [27]. Apart from this, Cd affects plant growth at both the morphological and physiological levels as noticeable from a reduced leaf area, dry matter yield, and stunted growth [41;42]. Cd toxicity also includes leaf chlorosis, a delay in the growth rate and inhibition of respiration and photosynthesis [32], increased oxidative damage, and decreased nutrient uptake ability [30].

Efficient and economical remediation of Cd-contaminated urban and agricultural lands is crucial for sustainable agriculture development. Different methods, like chemical, physical, and biological; electrokinetics; and phytoremediation have been used for the remediation of heavy metal contaminants from soil.

*Corresponding Author: **Kumari Shiwani**

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Phytoremediation is considered an eco-friendly remediation of soil, often called green remediation [5]. Currently, several plants species are identified as Cd hyperaccumulating plants such as *Brassica juncea*, *Brassica rapa*, *Brassica nigra* [47], *Vigna unguiculata*, *Solanum melongena*, *Momordica charantia* [3] and *Nicotiana tabacum* [25]. However, on a large scale, phytoremediation of heavy metal from contaminated sites using trees may be preferred to annual/ biennial crops due to their capacity to produce large biomass, extensive deep root system, and ability to accumulate toxic metals in a form that is not biologically hazardous [38; 9]. The use of timber tree species for this purpose has additional benefits as these tree species can seize heavy metals in the timber for a long time. Many woody species like *Melia azedarach*, *Populus alba* [21], *Salix discolor*, *Salix eriocephala* [31], *Eucalyptus camaldulensis* [26] and *Leucaena leucocephala*, *Dalbergia sissoo* [19] have been reported to accumulate and removing significant quantities of Cd and other metals from soil.

Melia dubia which is popularly known as Malabar Neem, gaining economic importance both in domestic and global markets. It is getting popularized among farmers due to characteristics such as fast growth, stem straightness without much of branches, less shade effect, not being susceptible to pest and insect attacks, and low maintenance [12]. In different parts of India, this species is expanding rapidly due to greater demand and acceptability among farmers and industries. Because, it is used mostly in pulpwood, plywood, light timber, and medicinal industry, this species is expected to rapidly occupy large areas of fertile and marginal lands [23]. Therefore, *M. dubia* is considered an outstanding tree for the plantation as well as a good model plant among tree species for understanding

the phytoremediation potential of heavy metals in affected soils. One of the main characteristic features of a suitable tree for phytoremediation is rapid growth and high biomass [2]. The phytoremediation potential of other fast-growing trees was studied and reported by different researchers, but few scientific studies describe the phytoremediation of Cd-contaminated soils using *M. dubia*. This study includes a money-spinning fast-growing tree, *Melia dubia* which had multiple uses with high economic returns. Biomass production, metal tolerance, and uptake behavior of plants are the key factors for choosing plant species for remediation and restoration of contaminated sites. High biomass-producing plant species with metal tolerance and accumulation characteristics excellent candidates for the remediation of contaminants [35; 20]. The objective of this study includes exploring the effects of different graded levels of applied Cd on vegetative growth, biomass and mineral content of *Melia dubia* seedlings to evaluate the suitability of this tree species in phytoremediation.

Materials and Methods

Tree species

The seeds of five commercial varieties of *Melia dubia* Cav. viz., Kshitiz, Megha, Dev, Ritu, and Kartik were procured from Forest Research Institute (FRI), Dehradun, India. Nine-month-old *Melia dubia* Cav. Plants were used in this study. This tree species of family Malvaceae was chosen for this study due to its fast-growing ability and high plant biomass. The planting material was raised in the nursery at Experimental Farm of the Department of Tree Improvement and Genetic Resources, Neri, Hamirpur, India.

Experimental site

Pot culture experiments with five commercial varieties were carried out in a polyhouse situated at the Experimental Farm of the College of Horticulture and Forestry Neri, Hamirpur, India. It is situated at an elevation of 799 meters above mean sea level at 31.68° N latitude and 76.52° E longitude. Study area map of district Hamirpur, Himachal Pradesh, India is shown in Figure 1). The study persisted from March, 2021 to April, 2022 to investigate the effect of different Cd concentrations on vegetable growth, biomass, metal uptake by a tree, and heavy metal concentration in soil before and after harvesting the plants at the end of the plantation period. The soil used in the experiment had a pH of 6.35 with low electrical conductivity and low organic carbon.

Applied cadmium concentration

Nine months seedlings of different varieties of *M. dubia* were transplanted into pots, each containing 7kg of soil mixture. After transplanting, the plants of each variety treated for five days with different concentrations of Cd in solution form i.e., control (without heavy metal treatment), 20, 40, 60, 80 and 100 mg/kg. The base of each pot was laid with plastic plates to avoid the leaching of cadmium along with water.

Harvesting and Measurements

Harvesting of plants was done after six months of heavy metal treatment and growth in pots and partitioned into leaves, stems, and roots. Before harvesting of seedlings vegetative parameters like shoot length, and leaf area were measured with the help of measuring tape, the number of leaves per plant was counted manually and collar diameter was measured with Vernier Caliper.

Each sample of tree species from each variety was washed successively with tap water and deionized water. The washed roots and shoots samples were first air-dried by keeping them in paper bags and then oven-dried at 65±5 °C. The dry weight of plant parts was recorded, these were ground and stored in paper bags for chemical analysis. Representative soil samples were taken from each pot after thoroughly mixing the soil of the pot before planting the seedlings and after harvesting the plants. These soil samples were air-dried, ground, sieved through a 2 mm sieve and stored in polythene bags for their Cd analysis. Cadmium concentration in soil before planting and after harvesting of plants, Cd concentration and uptake by different parts and total Cd uptake by the plants was determined. Soil and plant samples were digested with di-acid mixture consisting of nitric acid and perchloric acid at the ratio of 3:1 [17] followed analysis for the presence of Cd using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Analyst 200) [33]. The uptake of Cd by different plant parts was calculated from dry matter and the concentration of Cd in different plant parts was as follows:

$$\text{Cd uptake (mg/ pot)} = \text{Cd concentration } (\mu\text{g/g}) \times \text{dry matter (g)} \times 1000.$$

$$\text{Total cadmium uptake} = \text{Root uptake} + \text{Shoot uptake}.$$

Biosorption factor

The efficiency of phytoremediation can be quantified by calculating the bioconcentration factor and translocation factor, therefore Bioconcentration factor (BCF) and translocation factor (TF) were calculated as described by [29]. The bioconcentration factor indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment [24]. BCF was calculated as the ratio of Cd concentrations in plant biomass to that in soil. TF was expressed as the ratio of Cd concentration in the shoots to that in the roots. The TF% was calculated to estimate the metal ion transport efficiency from the roots to aerial plant organs [28], whereas shoots were considered equivalent to leaves and stems. The tolerance index (TI) was calculated as described by [15], $\text{TI} = \text{Root length of stressed plant} / \text{root length of control plant} \times 100$

Experimental Design

All the treatments were replicated three times and the generated data were subjected to analysis of variance (ANOVA) with factorial completely randomized design was given by [8] and [11].

Results

Effects of different cadmium concentrations on vegetative growth of *M. dubia*

The effects of various concentrations of cadmium (Cd), on different growth parameters of *M. dubia* varieties under study are shown in Table 1. The results depicted the adverse effects of Cd on all the vegetative growth parameters like shoot length, shoot fresh weight, root length, root fresh weight, leaf area, collar diameter and number of leaves per plant of all the varieties (Ritu, Kshitiz, Kartik, Megha and Dev) which was evident from the significant decreasing trend in average values of concerned traits with increase in applied Cd in pot soil. However, there was no plant lethality at any of the tested concentrations of the cadmium was observed.

The decrease in shoot length was 131.66 to 104.67 (cm) in variety Ritu, 171.00 to 118.33 (cm) in Kshitiz, 137.66-104.33 (cm) in Kartik, 114.00 to 93.33 (cm) in Megha and 185.33 to 93.33 (cm) in Dev.

The percent reduction due to Cd application in relation to control was highest in variety Dev (49.7%) followed by Kshitiz (30.80%), Kartik (24.27%), Ritu (20.50%) and Megha (18.10%). The corresponding values for shoot fresh weight of Ritu, Kshitiz, Kartik, Megha and Dev were 29.21, 18.51, 22.20, 27.88 and 30.10 percent, respectively. This indicated that the extent and magnitude of decline in shoot length and shoot fresh weight varied with varieties at different applied Cd levels. Another parameter like collar diameter (cm), the percent stem diameter reduction was observed highest in the variety Kartik (33.04%) followed by Dev (31.40%), Ritu (29.60%), Kshitiz (26.45%) and Megha (24.20%). Varieties namely, Ritu and Dev found to be affected more with increase in the Cd level as compared to other experimental varieties. The corresponding values for leaf area of these two varieties were 23.05% and 19.59% and number of leaves per plant was recorded as 20.49% and 49.80%, respectively.

The values for root parameters like root length and root fresh weight also decrease with increase in Cd levels. The percent root length reduction due to cadmium application in relation to control was highest in variety Kartik (69.95%) and Dev (69.42%), whereas variety Ritu recorded less reduction of root length i.e. 44.38%. The highest level of applied Cd (100 mg/kg) decreased fresh root weight (g) from 7.36 to 2.10 in Ritu, 7.39 to 1.99 in Kshitiz, 8.35 to 2.10 in Kartik, 6.31 to 2.02 in Megha and 6.64 to 1.88 in Dev as compared to the control. The varieties which showed a minimum decrease in root fresh weight were Megha (67.98%) and Kartik (73.10%), respectively. The different experimental varieties have variable potential to produce root biomass, thereby leading to difference in root biomass production. Different varieties showed difference in biomass production, among all the experimental varieties of *M. dubia*, Megha and Kshitiz were found to be more Cd tolerant as evidenced from less reduction of vegetative growth as compared to other investigated varieties whereas, higher Cd concentration in varieties Dev and Kartik might have leads to Cd toxicity, thus producing lower biomass than other varieties.

Effects of different cadmium concentrations on biomass of *M. dubia*

Data presented in table 2 depicted that the higher concentration of cadmium (100ppm) was the most harmful treatment that decrease the shoot dry weight of all the varieties of *M. dubia* under study. Maximum reduction in shoot dry weight was recorded by variety Kashitiz (60.5%), followed by Megha (59.0%), Dev (58.0%), Kartik (51.2%) and Ritu (47.3%). Also, root dry weight was also affected by a high concentration of cadmium almost in all the varieties. The corresponding values for root dry weight of Ritu, Megha, Kashitiz, Dev and Kartik were 75.8%, 75.4%, 74.2%, 73.6% and 73.1%. The reduction of dry matter yield of all the experimental varieties at highest cadmium concentration was mainly due to toxicity as a result of its increased accumulation in the plant. Further less reduction of dry matter was observed in Ritu and Kartik as compare with rest of the varieties.

Cadmium concentration of different varieties of *M. dubia* at different Cd levels

The Cd concentration in leaves, shoots and roots of all the five varieties was low in the control which remarkably increased with the application of soil Cd from control (0) to 100 mg/kg of soil (Figure 2a-c). The Cd concentration in leaves of different experimental varieties varies significantly.

The Cd concentration in leaves was increased from 0.18 to 5.98 (33 times), from 0.19 to 6.99 (35 times), from 1.41 to 4.05 (3 times), from 0.97 to 8.27 (9 times) and 0.71 to 8.23 (12 times) in variety Ritu, Kshitiz, Kartik, Megha and Dev, respectively, when rate of Cd increased from 0 to 100 mg/kg soil. The highest increase of metal concentration in leaves was found in variety Kshitiz and the lowest was recorded in Kartik, whereas, the maximum increase in Cd concentration in shoots was recorded in variety Dev i.e., 2.77 to 10.28 (3.7 times) and minimum increase in metal concentration was observed in variety Kshitiz i.e., 2.2 times (5.20 to 11.38). Cadmium concentration in roots of all the varieties was higher than the concentration in shoots and leaves. The concentration of Cd in roots was increase from 3.38 to 15.87 (4.5 times), 2.52 to 25.75 (10 times), 3.71 to 25.07 (6.8 times), 3.10 to 25.44 (8.3 times) and 2.37 to 40.38 (17 times) in variety Ritu, Kshitiz, Kartik, Megha and Dev upon application of Cd from 0 mg/kg to 100 mg/kg of soil. The cadmium concentration in roots of all the varieties was higher than the concentration of shoots and leaves indicating the more accumulation of cadmium in leaves and low translocation from roots to above-ground parts, particularly in leaves. But specifically, variety Ritu showed highest cadmium concentration in its leaves and lowest in roots whereas, variety Dev accumulated highest cadmium in its roots as compared to its leaves. This result is further supported by the percent reduction of root length which was observed maximum in variety Dev and minimum in Ritu. Toxic effects of Cd are evident in different metabolic and physiological plant processes, with different degrees of severity depending on how resistant the plant is to Cd [37]. Even at low dosages, Cd can inhibition of root elongation, impaired gas exchange, wilting, and it can affect uptake of macro- and micronutrients. Since root is the first contact point between the plant and Cd it often gets damaged due to oxidation of membrane proteins/thiols, inhibition of protein pumps or simply by altered membrane fluidity reviewed by [16].

To evaluate the capability of *M. dubia* varieties to extract accumulated cadmium in the plant, bioconcentration factor was calculated. In Figure 3 (a) the BCF of *M. dubia* varieties referring to the root system and the aerial part of the plant, is reported. The BCF of roots of all the varieties under study were the most efficacious in bioaccumulating Cd in the roots. The value of BCF for roots was found to be more than 1 which showed a remarkable ability to bio-concentrate cadmium in the root system, could be efficiently used in the remediation of polluted water or contaminated sites to limit metal percolating to the water layer (phytostabilisation). The capability of *M. dubia* varieties to accumulate cadmium in the roots system was further confirmed by calculating the translocation factor (TF), which indicated the percentage of the absorbed metal that reached the aerial part of the plant respect to that present in the roots. The value of TF is found to be less than 1 in all the varieties under study showed minimum translocation of Cd from roots to shoots. Evaluation of the BCF and TF in *M. dubia* in the present experiment confirms that this has a considerable potentiality to remove cadmium from a contaminated medium and bind the metal to its root system and low rates of heavy metal accumulation into aboveground biomass, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation. The tolerance index (TI) based on root elongation, for different levels of applied Cd levels are presented in Figure 3 (b). TI was decreased with increasing applied cadmium level in soil.

Varieties Kshitiz (68.23 %) and Dev (67.77 %) recorded maximum reduction in tolerance index as concentration of applied cadmium was increased from 20 to 100 mg/kg soil whereas, Ritu showed minimum reduction in TI i.e. 43.43 %, indicating the high cadmium tolerance of this variety.

Cadmium uptake by different varieties of *M. dubia* at different Cd levels

Cadmium uptake by shoots and roots as well as total uptake was comparatively low in the control which increases significantly with Cd application. (Table 1 and Figure 4a-c). The total Cd removal by variety Kartik was greatest at a concentration of 60 ppm whereas other varieties namely, Ritu, Megha and Dev showed maximum uptake at 40ppm concentration of Cd. The increase in uptake with increasing levels of Cd was due to increase in concentration of Cd in tissues with increasing levels of Cd in the present study. The cadmium removal by all the varieties of *M. dubia* was higher by roots than by shoots. The uptake pattern of Cd from soil depends on the tree species and within tree species it varies between different parts of the tree. In case of variety Kartik the total uptake started decreasing at Cd concentration of 20 ppm which was due to a reduction in root dry matter caused by cadmium toxicity at this level.

Discussion

Present study revealed that all the varieties showed the lowest average value for all the vegetative traits at highest Cd levels (100 mg/kg of soil). The reduction of vegetative growth parameters (including shoots and roots parameters) was parallel to increasing heavy metal in the soil. Variety Dev found to be affected more as compare to other studied experimental varieties, as evident from the lowest values for most of the vegetative growth whereas, Megha, Ritu and Kshitiz were not much affected by various applied cadmium levels. Cd is recognized as water soluble pollutant that can easily be taken up by plants and can affects plant growth at both the morphological and physiological level as noticeable from a reduced leaf area, dry matter yield and stunted growth [41; 42]. Cd toxicity in plants includes leaf chlorosis, a delay in the growth rate, and inhibition of respiration and photosynthesis [32], increased oxidative damage, and decreased nutrient uptake ability [30]. The stunted growth of plants under Cd stress may be attributed to reduced nutrient and water uptake, respiration, photosynthesis, assimilation of nitrogen and carbon, and antioxidant activity [36]. In the context of Cd effects on plant growth, most prominent effect is reduction of root length and dry mass which is related to decrease of mitotic activity in root meristems under Cd stress [44; 43], which leads to reduced root length and dry biomass, and enhanced the root diameter [14].

Khamis et al. (2014) while working on *Melia azedarach*, noticed the adverse effects of high doses of Cd on stem height increment, stem diameter, number of branches, leaf area and length of longest root in the soil was evident from the significant decrease in values of both these parameters. Our results are further supported by [13] who observed the growth reduction in different varieties of *M. dubia* under different cadmium levels. Here variety Megha showed tolerance to high concentrations of applied cadmium as compared to other varieties. Similarly, toxic effect of Cd on various growth indices of other species of genus *Melia* like *M. azedarach* was observed by [18] and [19] in different studies. Experiments done in relation to effect of heavy metals on other tree species belongs to family Meliaceae were also addressed by [45] and [46].

Decrease in dry biomass both for shoots and roots with increasing applied cadmium was observed in our study. Less reduction of dry matter was observed in Ritu and Kartik as compared with rest of varieties. The results showed a significant decrease in plant biomass with the increase of metal concentration probably due decrease in photosynthesis, carbohydrate metabolism as well as production of reactive oxygen. Also, the exposure of plant to heavy metals can stimulate the production of reactive oxygen species that causes the oxidation of proteins, lipids and nucleic acid, member damage, mutagenesis and the inactivation of the enzymes [40; 21]. Our results are in the lane of [19] where all the tree species (*Eucalyptus tereticornis*, *Leucaena leucocephala*, *Melia azedarach* and *Dalbergia sissoo*) under the study had the lowest stem, leaf and root dry matter at the highest Cd level i.e. 120 mg/kg of soil. The highest Cd level resulted in 49, 41, 26 and 43% reduction in total dry matter of *Eucalyptus tereticornis*, *Leucaena leucocephala*, *Melia azedarach* and *Dalbergia sissoo*, respectively over control.

The cadmium content in the different plant parts (leaves, shoots and roots) gradually increased with increased levels of the corresponding metal in the soil. The results indicated that the highest significant metal concentrations in the plant parts were recorded for the highest concentrations of heavy metal used. The highest increase of cadmium concentration in leaves was found in the variety Kshitiz, whereas Dev showed maximum increase of cadmium in shoots and roots. Similarly, the cadmium concentrations increased in various plant parts with increased soil Cd levels were reported by [19].

The content of cadmium in plant parts was in the order of roots > shoots > leaves. The variability of metals in various plant tissues may be due to compartmentalization and translocation in the vascular system [22]. The Cd concentration in shoots of all the varieties was higher than the concentration in stems and leaves indicating its less translocation from roots to above ground parts particularly leaves. Accumulation of cadmium by roots of different trees species were reports by [21], [34], [4], [10], [13] and [46]. Therefore, *M. dubia* roots accumulated much more Cd than the shoots and leaves; in this case, the risk of contamination of the wider environment through leaf fall can be considered minimal.

We found that all the commercial varieties of *M. dubia* (Ritu, Kshitiz, Kartik, Megha and Dev) can effectively absorb cadmium. Further, Bioconcentration Factor (BCF) was greater than one for all the Cd treatments, whereas the translocation Factor (TF) was found to be less than 1. The results are supported by [13] in which some of the same varieties of *M. dubia* were used to study the bioconcentration factor, tolerance index and remediation factor, where the values of all the factors found to be more than 1 except translocation factor which was recorded less than 1, indicated that more bioaccumulation of heavy metals in root system as compare to shoot system and minimum translocation of metals from roots to shoots. Tolerance Index (TI) values were significantly reduced with increasing Cd concentrations in the soil.

The highest uptake was of Cd was recorded for roots as compared to leaves for all the varieties which may be due to the fact that plants roots can release into the rhizosphere chelating agents with binding ability for metals [39]. These metal Chelators or other molecules within plant cells that have an affinity for metals can help in the metal sequestering. Consequently, most of the Cd uptake occurs in the epidermis of the root tips.

Root tips lack the casparian band, and Cd is therefore transported apoplastically through cell wall directly to the xylem. Cation in the xylem moves towards in the negative walls of the xylem, but most (70-90%) remain in the root tissue. The reason for this may that Cd is absorbed to negative charges on cell walls and macromolecules in cells, or is taken up by the root cells and accumulates in the cytoplasm and vacuoles. the risk of contamination of the wider environment through leaf fall can be considered minimal. [13] reported more Cd levels in roots as compared to leaves or shoots. Therefore, these data suggest that *M. dubia* roots accumulated much more Cd than the shoots and leaves in this case the risk of contamination of the wider environment through leaf fall can be considered minimum. Therefore data suggests that it is a suitable choice for phytostabilization of heavy metals as compare to other deciduous trees.

Conclusion

Melia dubia is a fast-growing tree and commercially important short-duration tree species with higher productivity, it is used mostly in pulpwood, plywood, light timber, and medicinal industry, this species is expected to rapidly occupy large areas of fertile and marginal lands of the country. In this study we found *M. dubia* as a good option for phytostabilizer of heavy metal, cadmium. Apart from its economic importance, this tree species could be extremely useful tools for rehabilitation of heavy metal affected soils near industrial areas. Fully or partially rehabilitation of contaminated/ problematic soils through developing agroforestry models with this tree can not only lead to profitable use of contaminated and degraded lands like production wood raw material, but they can also have environmental repercussions in terms of conservation and enhancement for long-term ecological security. In addition to this, planting this tree species in problematic areas near industries will also help in creating the green belts near industries.

Table 1 Response of vegetative growth parameters of different varieties of *Melia dubia* at different levels of applied Cadmium

Varieties	Cd concentration (mg/kg soil)						Mean
	Control	20	40	60	80	100	
Shoot length (cm)							
Ritu	131.66	125.00	119.67	115.67	111.00	104.67	117.94
Kshitiz	171.00	158.34	144.00	128.33	135.00	118.33	142.50
Kartik	137.66	131.33	130.33	122.00	114.68	104.33	123.38
Megha	114.00	107.33	103.34	100.00	99.67	93.33	102.94
Dev	185.33	176.33	162.00	146.00	101.66	93.33	144.11
Mean	147.93	139.67	131.86	122.40	112.40	102.80	
Shoot fresh weight (gm)							
Ritu	8.46	8.16	7.23	7.08	6.63	5.98	7.26
Kshitiz	12.42	11.99	11.65	11.34	10.85	10.12	11.39
Kartik	10.00	9.70	9.21	8.78	8.33	7.78	8.96
Megha	10.58	10.35	9.78	9.06	8.16	7.63	9.26
Dev	9.40	8.90	8.13	7.95	6.96	6.57	7.98
Mean	10.17	9.82	9.20	8.84	8.19	7.61	
Root length (cm)							
Ritu	14.87	14.62	13.32	11.23	8.67	8.27	11.83
Kshitiz	10.35	9.79	8.76	5.98	4.72	3.11	7.12
Kartik	8.10	7.99	7.32	5.64	4.07	3.17	6.05
Megha	12.37	11.88	10.73	8.92	6.86	5.13	9.31
Dev	10.89	10.33	9.54	7.16	5.48	3.33	7.79
Mean	11.31	10.92	9.93	7.79	5.96	4.60	
Root fresh weight (gm)							
Ritu	7.36	7.08	6.07	3.15	2.16	2.10	4.65

As per the Ministry of Environment, Forests and Climate Change (MoEFCC) of GOI guidelines for greenbelt development, an industry should generate greenbelt of 1/3rd (about 33%) of the plant area. Therefore, a greenbelt technology can be developed using this forestry plants species along with phytoremediation of heavy metals which will help the industries in fulfilling the regulations/norms of regulatory authorities.

Future Scope

M. dubia is a good candidate for phytoremediation of heavy metal contaminated soils because of its tolerance, rapid growth rate and low rates of herbivory. Results obtained from experiments done on *M. dubia* showed the excellent growth and survival under various doses of heavy metals. In addition, *Melia* efficiently Phytoremediate the toxic metals from the soil and accumulate more in roots as compare to shoots which further showed that planting of this would contribute significantly in rehabilitation of waste and problematic soils of industrial areas of the hilly states of the country thus contributing towards clean up-of soil and water pollution.

Conflict of interest

No potential conflict of interest was reported by the authors. The authors hereby confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. All the authors have seen and approved the manuscript for submission.

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ORCID

Dushyant Sharma <https://orcid.org/0000-0002-4767-5123>,
Kumari Shiwani <https://orcid.org/0000-0002-9906-7293>

Kshitiz	7.39	7.17	6.14	4.15	2.29	1.99	4.85
Kartik	8.35	8.16	7.06	4.07	2.26	2.10	5.33
Megha	6.31	5.98	5.06	3.80	2.17	2.02	4.22
Dev	6.64	6.22	5.32	3.27	1.88	1.88	4.20
Mean	7.21	6.92	5.93	3.69	2.15	2.01	
Collar diameter (mm)							
Ritu	6.62	6.36	5.73	5.08	4.85	4.66	5.55
Kshitiz	7.41	7.18	6.90	6.31	5.93	5.45	6.53
Kartik	7.01	6.20	5.55	5.50	5.11	4.69	5.68
Megha	6.61	6.24	6.01	5.65	5.38	5.01	5.82
Dev	7.45	7.05	6.53	5.98	5.51	5.11	6.27
Mean	7.02	6.61	6.14	5.70	5.36	4.98	
Leaf area (cm²)							
Ritu	82.11	78.39	76.15	71.80	66.04	63.18	72.95
Kshitiz	75.84	71.75	69.45	68.08	65.79	61.37	68.71
Kartik	108.74	105.12	97.18	94.48	90.45	87.81	97.29
Megha	80.04	76.87	74.29	70.56	68.29	64.61	72.44
Dev	81.86	79.25	76.60	73.00	68.97	65.82	74.25
Mean	85.72	82.27	78.74	75.58	71.90	68.55	
Number of leaves/ plant							
Ritu	131.66	125.00	119.67	115.67	111.00	104.67	117.94
Kshitiz	171.00	158.34	144.00	128.33	135.00	118.33	142.50
Kartik	137.66	131.33	130.33	122.00	114.68	104.33	123.38
Megha	114.00	107.33	103.34	100.00	99.67	93.33	102.94
Dev	185.33	176.33	162.00	146.00	101.66	93.33	144.11
Mean	147.93	139.67	131.86	122.40	112.40	102.80	

Table 2 Response of biomass of different varieties of *Melia dubia* at different levels of applied cadmium

Varieties	Cd concentration (mg/kg soil)					Mean	
	Control	20	40	60	80		100
Shoot dry weight (gm)							
Ritu	3.93	3.89	3.49	2.96	2.27	2.07	3.10
Kshitiz	5.77	5.55	5.24	4.04	2.99	2.28	4.31
Kartik	4.57	4.67	4.19	3.32	3.04	2.23	3.67
Megha	4.93	4.73	4.35	3.03	2.36	2.03	3.57
Dev	4.40	4.29	3.99	3.08	2.19	1.86	3.30
Mean	4.72	4.63	4.25	3.28	2.57	2.09	
Root dry weight (gm)							
Ritu	4.34	4.36	3.59	2.34	1.38	1.05	2.84
Kshitiz	4.34	4.17	3.17	2.54	1.17	1.12	2.75
Kartik	4.76	4.49	3.46	2.67	1.46	1.28	3.02
Megha	3.94	3.69	3.11	2.19	1.61	0.97	2.58
Dev	3.49	3.32	2.59	2.06	1.36	0.92	2.29
Mean	4.17	4.00	3.18	2.36	1.39	1.07	

Table 3 Mean cadmium content and uptake of cadmium in shoots, roots and total uptake of different varieties of *M dubia* at different levels of applied cadmium

Variety Treatment	Cadmium concentration (mg/ kg)		Uptake (mg)		Total uptake (mg/plant)
	Shoots	Roots	Shoots	Roots	
Ritu					
Control	2.24	3.38	0.009	0.014	0.023
20	2.66	5.84	0.010	0.025	0.035
40	4.71	7.81	0.016	0.028	0.044
60	5.81	10.79	0.017	0.025	0.042
80	8.35	12.47	0.018	0.017	0.035
100	9.00	15.87	0.018	0.016	0.034
Kshitiz					
Control	2.22	2.52	0.012	0.010	0.022
20	5.20	18.60	0.028	0.077	0.105
40	6.48	21.03	0.033	0.066	0.099
60	7.86	22.66	0.031	0.057	0.088
80	9.68	23.02	0.028	0.026	0.054
100	11.38	25.75	0.025	0.028	0.053
Kartik					
Control	1.14	3.71	0.005	0.017	0.022
20	4.24	8.17	0.019	0.036	0.036
40	6.06	13.16	0.020	0.045	0.065
60	6.75	17.75	0.022	0.047	0.069
80	8.71	22.68	0.026	0.033	0.059
100	10.73	25.07	0.023	0.032	0.055
Megha					
Control	0.87	3.10	0.004	0.012	0.016
20	3.97	9.50	0.018	0.035	0.053
40	5.35	15.58	0.023	0.048	0.072
60	5.95	22.31	0.018	0.049	0.067
80	8.44	23.47	0.019	0.037	0.056
100	11.25	25.14	0.022	0.024	0.046
Dev					
Control	1.84	2.37	0.008	0.008	0.016
20	2.77	23.12	0.011	0.076	0.087
40	4.79	26.50	0.019	0.068	0.087
60	5.50	29.53	0.016	0.060	0.076
80	7.76	36.07	0.017	0.049	0.066
100	10.28	40.38	0.019	0.037	0.056

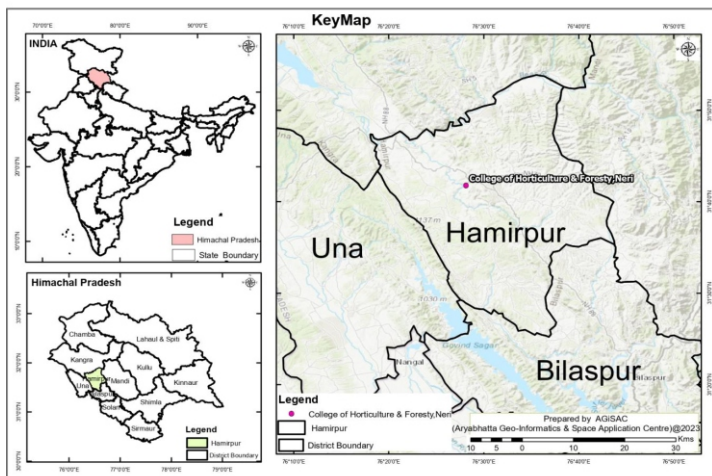
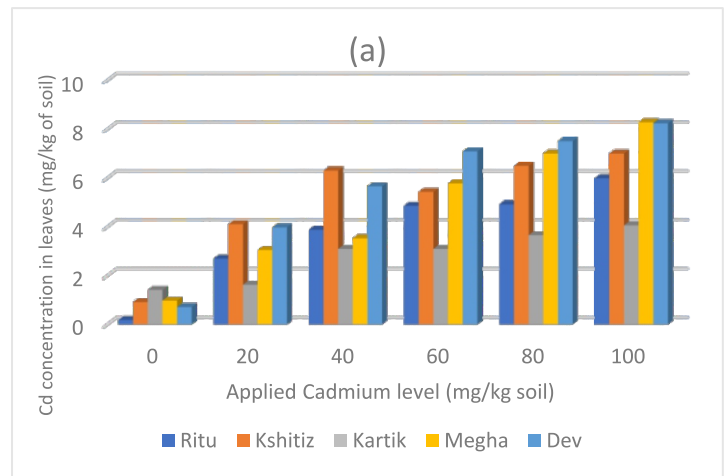


Figure 1: Map of the study area (Source-AGISAC, Kasumpti, Shimla, Himachal Pradesh, India)



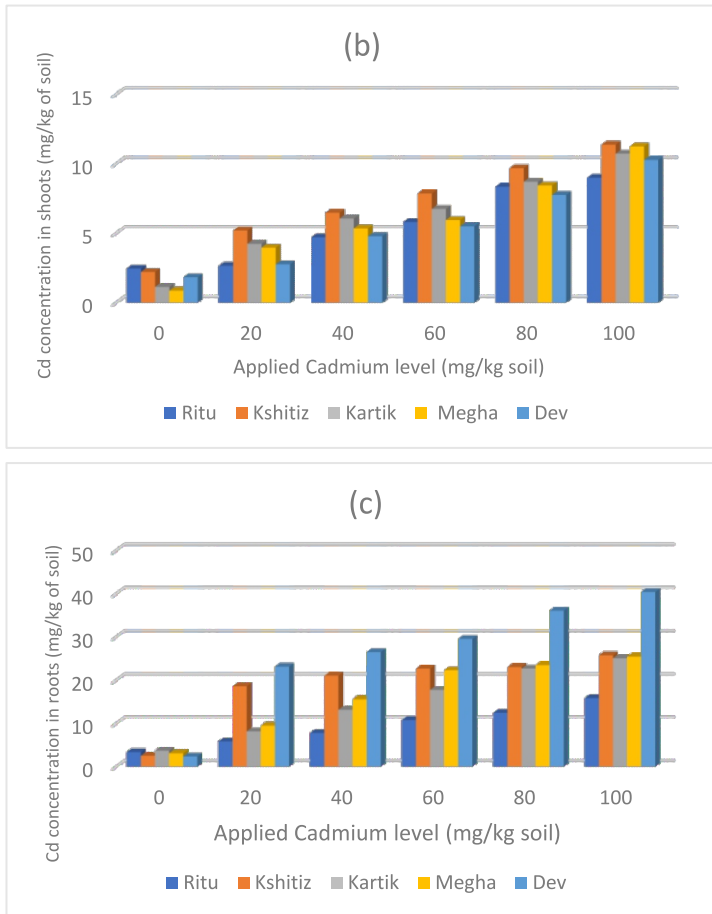


Figure 2: Cd concentration in different parts (leaves, shoots and roots) of different varieties of *M. dubia* at various levels of applied cadmium, a-c

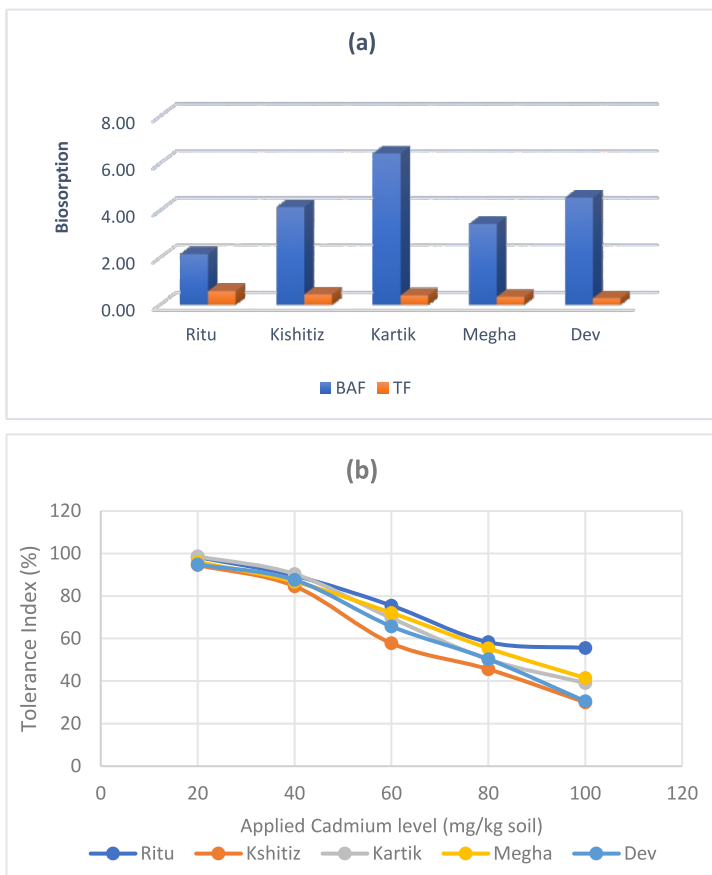


Figure 3: Biosorption of cadmium for different varieties of *M. dubia*, a-b

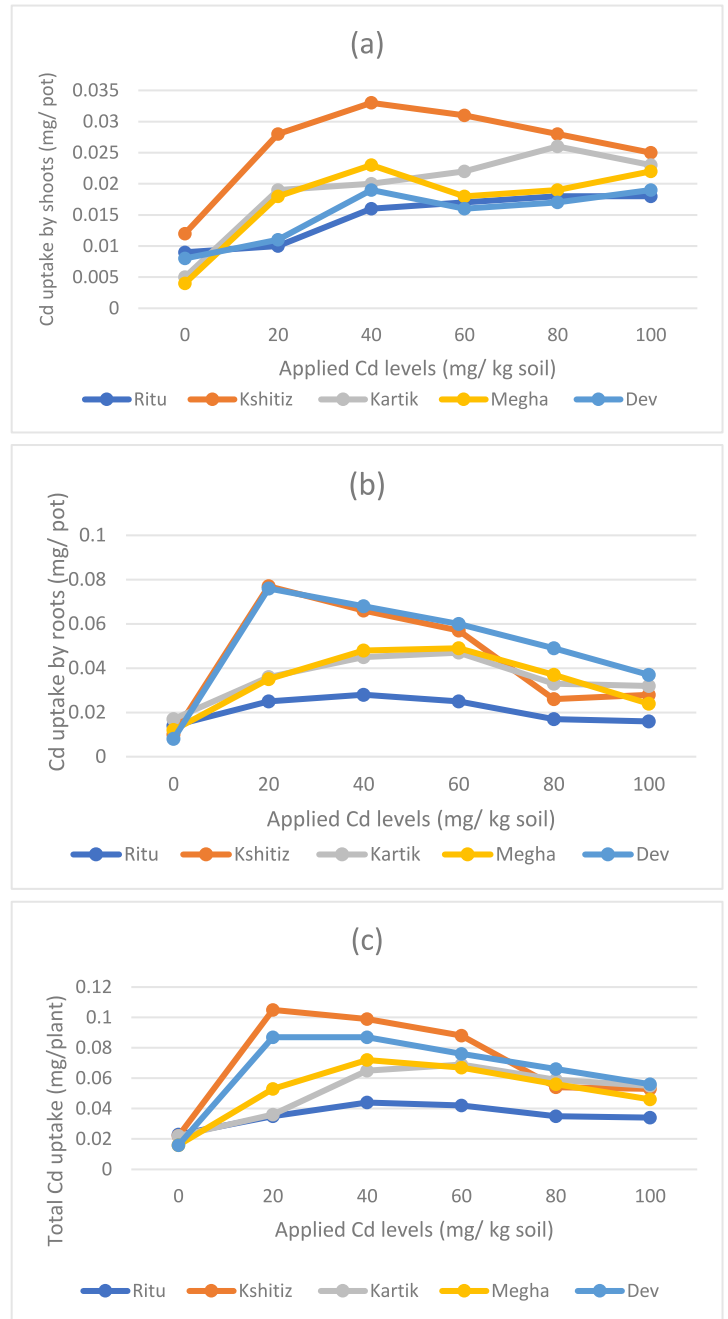


Figure 4: Cadmium uptake by shoots, roots and total uptake by different varieties of *M. dubia* at different levels of applied cadmium

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