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Accumulation and Translocation of Heavy Metals in *Hibiscus cannabinus* Grown in Tannery Sludge Amended Soil

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ABSTRACT

Digested sludge wasted by tanneries is rich in nutrients and trace elements however, the presence of toxic metals restricts their use in agriculture. The present study explores the possible application of tannery sludge amendment for the cultivation of an energy crop, *Hibiscus cannabinus*. The toxicity of various sludge amendments (25, 50, 75, and 100%, w/w) was examined during early seedling growth, followed by metal accumulation potential by performing pot experiments. Chemical characterization revealed the presence of Cr (709.6), Cu (366.43), Ni (74.6), Cd (132.71), Pb (454.8) μg.g^{-1} in tannery sludge beside N (2.1%), P 3.8 & K 316.96 (kg.hec^{-1}.) respectively. Germination of *H. cannabinus* exposed to sludge extracts ranged between 80 to 95%; Relative seed germination, 81.33 to 84.43%. Relative root growth, 0.9 to 1.16 cm; and germination index, 95 to 110%. It was found that sludge extracts have not caused adverse effects on seed germination and early seedling growth. Heavy metal accumulation was observed as follows: Ni (3.37, 2.38, 1.46 & 0.90 mg.kg^{-1}) > Pb (10.59, 10.15, 5.26, & 2.84 mg.kg^{-1}) > Cu (2.34, 2.24, 0.97 & 0.24 mg.kg^{-1}) > Cd (2.31, 1.19, 1.33 & 1.12 mg.kg^{-1}) > Cr (1458, 1136.12, 601.73 & 211.6 mg.kg^{-1}) in 100, 75, 50, & 25% sludge amended soil, respectively. The bio-concentration pattern of metals was found to be in the order of root > leaf > stem. The findings of the present study give direction for the eco-friendly and cost-effective management of tannery sludge. Further, *H. cannabinus* can be used for the restoration of metal-contaminated agricultural land, however, results need to be corroborated with field trials.

INTRODUCTION

Environmental pollution has become an ever-growing menace encompassing the world for several decades. Different industrial processes are the main causes of significant pollution in the environment. The generation of huge amounts of by-products and waste due to rapid industrialization poses a need to explore economic, eco-friendly, and aesthetically acceptable waste disposable methods (Moshood et al. 2022). Different types of industries discharge ranges of by-products/waste, from relatively simple heavy metals to complex organic compounds that affect plant life, animals, and humans in several ways (Huang et al. 2018). Environmental monitoring and epidemiological surveys have recently shown the amount of metals in the ecosystem and biological tissues, which has raised concerns about metal exposure around the world. (Sugasini & Rajagopal 2015). While certain metals, such as Mn, Cu, Zn, Mo, and Ni, are vital or beneficial micronutrients for microbes, plants, and animals, all metals have potent toxic effects at high levels and pose a threat to the environment (Małkowski et al. 2019).

In the current era, tanning industries are unavoidable entities around the world and have contributed significantly to economic development (Chiampo et al. 2023). India is the 6th top producer of finished leather in the world, housing more than 3000 tanneries (Rajamani 2023). Goat or sheep skins and cow or buffalo hide are the primary materials used in these tanneries. Tanneries use a lot of water along with significant amounts of both organic and inorganic chemicals. In the process of leather production for one ton of raw hide/skin, on average, about 50 to 100 m³ of wastewater and 45 to 150 kg of solid waste (dried sludge) are produced (Saira & Shanthakumar 2023). Of the solid waste, about 50% is utilized by the manufacturers of poultry feed, gelatine, glue, fish meal, and soap, and the remaining 50% is dumped indiscriminately (Nath et al. 2017). Chrome tanning or mineral tanning is the most common method of leather tanning worldwide. Chromium tanning is relatively quick and efficient, allowing for a faster production process compared to traditional tanning methods. Considering chromium, the hides and skins absorb roughly 70% of the available amount, with the remaining 30% going into solid waste and effluent wastewater (Ma et al. 2017). Since there are inadequate or non-existent sludge disposal systems, the disposal of solid waste (sludge) is a crucial issue. Before being removed from the drying beds, the dried sludge is carelessly dumped in the industrial area without taking environmental or ecological factors into account (Alibardi & Cossu 2016). This mode of sludge disposal is so far been considered a low-cost solution, but it is extremely harmful to the terrestrial ecosystem, including life forms occupying higher trophic levels (Sunmathi et al. 2022).

Phytoremediation is the use of plants to make soil contaminants nontoxic. Alternative methods for replacing, solidifying, or washing metal-contaminated soil, the concept of employing plants that hyper-accumulate metals to selectively collect and recycle excess soil metals has been investigated as a potentially useful and more affordable method (Chaney et al. 1997). Natural metal hyper-accumulator phenotype is much more important than high yield ability when using plants to remove metals from contaminated soils (Yaashikaa et al. 2022). Remediation of heavy metals from the soil by hyper-accumulator crops needs creative research for practical application. Various researchers have shown that some species of plants grow profusely in soil contaminated by industrial waste and can accumulate potentially toxic elements (PTE), Pb, Cr, Ni, and Cd to levels several degrees higher than in the soil (Zhang et al. 2023). However, the accumulation of PTE may vary from plant to plant and soil to soil. Properties like high biomass, rapid growth, dense root network, resistance to adverse environmental conditions, etc., are required in hyper-accumulator plant species (Ahmad et al. 2023). However, soil properties like pH, organic carbon, cation exchange capacity, stages of growth of plants, and microorganisms around the root zone also influence the process of phytoremediation (Rai et al. 2023).

Kenaf (*Hibiscus cannabinus* L.) is a dicotyledon annual crop characterized by rapid growth, large biomass, strong resistance, and wide adaptability, climates and thrives with abundant solar radiation and high rainfall, minimal fertilizers and pesticides (Coetzee et al. 2008). When the circumstances are right, kenaf can grow up to 5 m tall in 6-8 months and yield up to 30 tons of dry stem material/ha. It is considered an important and multipurpose industrial crop with fibrous stems exploited for building materials, absorbent, and in industries like textile, paper, and pulp. Further, it can be used for the preparation of bio-composites, insulation mats, animal bedding, etc (Falasca et al. 2014). The kenaf has also been explored for oil production and found to be a potential crop for energy production. The core of kenaf fiber is rich in cellulose and hemicellulose, making it suitable for the production of bio-ethanol second generation (Saba et al. 2015). Kenaf can be a good candidate to restore sites contaminated with industrial waste. Moreover, because of industrial application, the probability of transport of toxicants through the food chain is minimal.

Considering the above-mentioned facts, tannery sludge is examined for physicochemical characteristics, including analysis of potentially toxic elements that accumulate in living tissues and have detrimental effects on plants and animals. The responses of *Hibiscus cannabinus* to various combinations of industrial sludge have been studied in terms of early seedling growth. Further, bio-magnification and bio-translocation of PTE were also evaluated. The overall objective of the study was to explore the application of *Hibiscus cannabinus* in the sustainable usage of tannery sludge and the restoration of agricultural land contaminated by industrial waste.

MATERIALS AND METHODS

Sludge Collection and Sample Preparation

Tannery sludge used for the study was collected from the Common Effluent Treatment Plant (CETP) situated at Leather Technology Park, Banthar, Unnao, U.P. India. Collected sludge was air-dried at ambient room temperature and homogenized by rolling substantial aggregates to remove large slabs of sludge, plastics, etc. The homogenized sludge was brought to a fraction of less than 165 μm by grounding and sieving. Subsequently, the tannery sludge was mixed with garden soil in concentrations of 25, 50, 75, and 100% (w/w) for the pot experiment, whereas garden soil served as a control. Pot experiments were performed in five replicates, and the average were used for further analysis.

Sludge Characterization

The method proposed by Walkley & Black (1934) was used to analyze the organic matter of the soil. The Kjeldahl method (1883) was used to determine total nitrogen content. Whereas the standard Analytical Method (APHA 2005) was followed to determine pH, electrical conductivity, moisture content, total solids, organic carbon, total nitrogen, available phosphorous, and potassium. For analysis of heavy metal in soil and sludge, (1g) of samples were digested with a mixture of HCl: HNO_3 in 3:1 (v/v) (McGrath & Cunliffe 1985) and analyzed by ICP-MS (Perkin Elmer Elan DRC-e).

Phytotoxicity Test

A phytotoxicity test was performed in five replicates with

25, 50, 75, and 100% (w/v) tannery sludge extract following Zucconi et al. (1985) and Visioli et al. (2014). Whereas the corresponding strength of garden soil extract has been served as a control. Tap water has been used as a solvent to obtain sludge/soil extracts. Twenty seeds of *H. cannabinus* were placed for germination in Petri dishes lined with filter paper moistened with 10 mL of sludge/soil extracts. Relative root growth, relative seed germination, and Germination index were calculated after 72 h of germination using the method given by (Hoekstra et al. 2002) as follows:

$$Relativw\ Seed\ Germination\ (\%) = \frac{number\ of\ seeds\ germinated\ in\ test}{number\ of\ seeds\ germinated\ in\ control} \times 100 \qquad \ldots(1)$$

$$Relativw\ Seed\ Growth\ (\%) = \frac{Root\ length\ of\ test\ plant\ (cm)}{Root\ length\ of\ control\ plant\ (cm)} \times 100 \qquad \ldots(2)$$

$$Germination\ index(GI) = \frac{GsLs}{Gc\ Lc} \qquad \ldots(3)$$

Where Gs and Ls denote the seed germination (%) and root elongation (cm), respectively, and Gc and Lc are the corresponding control values. To demonstrate the comparison between different tests, the GI is expressed as a percentage in comparison to the control (100 %). A 1mm emergence of radicle has been considered seed germination.

Pot Experiment

Perforated clay pots containing 5 kg of different sludge amendments were used for pot experiments in five replicates for each sludge-soil combination. Five seeds of *H. cannabinus* were soaked overnight in deionized water before sowing. Tap/groundwater was used in surface irrigation during the plant growth.

Metal Content in Plant Samples

Plant tissues of *H. cannabinus* plants were uprooted after 120 days of exposure. Plant tissues (leaves, stems, and roots) were carefully washed with deionized water to eliminate putative material surface contamination. All plant tissues were oven-dried at 70°C for 72 h. Subsequently, ground into powder using an acid-washed porcelain mortar and pestle. 0.5 g of ground tissue material was digested to determine total metal content using ICP-MS (Perkin Elmer Elan DRC-e).

Each sample underwent digestion using the usual procedure. Using a hot plate (Jeo Tech TM-14SB) at 80°C, a known quantity (0.5 g) of each plant and soil sample was digested by 14 mL concentrated acid mixture (HCL: H_2SO_4 in 3:1 (v/v)) to achieve a transparent solution. The solutions were filtered with Whatman No. 41 filter paper and, further diluted to 25 mL with distilled water and stored at ambient temperature.

Bio-concentration, translocation, and Metal Tolerance Index

The ratio of metal concentration in plant roots or aerial tissues to that in the soil or solution is defined as the bio-concentration factor (BCF). Whereas the potential of plants to move heavy metals from the roots to the shoots is determined by the translocation factor (TF). TF and BCF were calculated as follows (Yoon et al. 2006):

$$Bioconcentration = \frac{metal\ concentration\ in\ plant\ (mg/kg)}{total\ metal\ concentration\ in\ soil\ \ (mg/kg}$$

Using the following formula, the percentage of hazardous metals removed from phytoremediation tannery sludge was determined. Ghosh and Singh (2005).

$$\%\ of\ remaining\ metal\ (\alpha) = \frac{metal\ concentrated\ in\ phytoremediated\ soil\ (mg/kg)}{total\ metal\ concentratiom\ in\ soil\ (mg/kg)} \times 100$$

$$Removal\ of\ metals\ (\beta)\% = 100 - \alpha$$

RESULTS AND DISCUSSION

Sludge Characterization

Standard methods were used to determine the physicochemical properties of soil and sludge samples. Each sample was evaluated in triplicate. The results are represented as average ± standard deviation (Table 1). Sludge application increased the total metal concentration in soils. The primary

Table 1: Physico-chemical characteristics of tannery sludge and garden soil.

Parameters	Tannery sludge	Garden soil
pH	8.36±0.96	8.6±0.92
Electrical conductivity [µS cm^{-1}]	0.21±0.02	0.18±0.02
Organic carbon [%]	1.26±0.8	1.8±0.26
Total organic matter [$mg.kg^{-1}$]	1.36±0.24	1.53±0.18
Total nitrogen [%]	2.1±0.12	1.54±0.4
Available phosphorus [$kg.hec^{-1}$]	3.8±0.42	13.5±2.21
Available Potassium [$kg.hec^{-1}$]	316.96±52.74	480.7±65.18
Available zinc [ppm]	4.66 ± 0.62	5.81±0.72
Manganese [ppm]	4.15 ± 0.62	1.39±0.18
Sulfur [ppm]	15.78 ± 2.46	14.6±2.24
Boron [ppm]	3.03 ± 0.46	1.9±0.20
Chromium [$\mu g.g^{-1}$]	709.6±15.23	0.9 ±0.35
Copper [$\mu g.g^{-1}$]	366.43 ±12.76	0.48 ±0.01
Nickel [$\mu g.g^{-1}$]	74.6±10.2	0.71 ±0.01
Cadmium [$\mu g.g^{-1}$]	132.71 ±20.1	1.1 ±0.026
Lead [$\mu g.g^{-1}$]	454.8 ±54.2	6.23 ±0.001

determinants of metal availability and uptake in soil are its physico-chemical properties, such as pH, organic matter, organic carbon, CEC, etc. the effect of organic compounds on heavy metal solubility. In addition, considerably hinges on how much of their biologically bound form of organic matter has been humified and how it influences the pH of the soil. The physicochemical characteristics of soil are known to control the fate of the metals through rhizospheric activities and to have a major effect on how plants and soil interact.

Phytotoxicity Test

Seed germination (72 h), along with other early seedling growth parameters, were analyzed at different concentrations of tannery sludge extracts (Table 2). GI less than 50% indicates high phytotoxicity, between 50 to 80% indicates moderate, and more than 80% indicates no phytotoxicity (Zucconi et al. 1985). Research shows that root length is a better indicator compared to while calculating (Bożym et al. 2021). The germination range of *H. cannabinus* was not significantly affected by sludge extract. The average root length of *H. cannabinus* was 0.99 ± 0.005 cm in control. Tap water was taken as control. The highest root length was found in 50% sludge extracts, i.e., 1.166 ± 0.05 cm, whereas the minimum was recorded in 75% concentration 0.9 ± 0.01 cm. Further, 100% concentration has a root length of 0.9133 ± 0.005 cm (Fig. 1), while 25% concentration showed 1.033 ± 0.032 cm. The germination index was found to be more than 80%, which indicates no phytotoxicity. Similar results were reported by Gonçalves et al. (2020) in *Lactuca sativa* exposed to sewage sludge. It might be explained by the increase in mineralization and availability of organic components by sludge extract (Tangredi et al. 2023).

Bio-concentration Factor

A bio-concentration factor (BCF) was calculated to determine the efficiency of *H. cannabinus* to accumulate a heavy metal from the soil. The finding of the bio-concentration factor for metals is presented in Fig. 1. The concentration of Cr was found to be the highest among all the heavy metals, followed by Ni and Cd, whereas the lowest concentration was observed of Pb, followed by Cu**.** The order of heavy metal uptake was observed as Cr > Ni > Cd > Pb > Cu in the tannery and garden soil. Further, the bio-concentration factor was found to be highest in plants grown in 50% sludge-amended soil. The results were observed as Cr (76%), Ni (59%), Cd (42%) & Pb (23%). The lowest bio-concentration factor was observed in 100% sludge: Cr (43%), Ni (39%), Cd (28%), Pb (14%) & Cu (12%) (Table 3)**.** The plants' elevated concentration factor highlighted their role in the treatment of tannery sludge wastes with high metal concentrations. The ability of metal accumulation in proportion to plant biomass is referred to as the "bio-concentration factor," and it has been observed for an extensive range of plants (Zhao et al. 2020). A high metal concentration factor led to a reduction in the sludge's metal content and an improvement in the physico-chemical properties of the treated sludge (Ahmad et al. 2016).

Translocation Factor Analysis

Heavy metal distribution in different parts of cultivated plants is observed by multiplying metal concentration with dry matter. The translocation factor in plants cultivated in tannery sludge and garden soil is represented in Fig. 1. Results revealed that accumulation is higher in roots than in aerial parts of plants. Similar findings were reported by Shabani and Sayadi (2012). TF was observed higher in plants cultivated in tannery sludge than those of control soil, which was as Cd > Cr > Cu > Pb > Ni and Ni > Cr > Pb > Cu > Cd in tannery and control, whereas the trend of distribution of heavy metals in plant parts is root > leaf > stem in almost all the concentration of amendment of soil.

Cr was more accumulated than other studied metals. Results revealed that roots have maximum accumulation with values 128.85, 106.66, 77.44 & 32.7 ($\mu g.g^{-1}$) further, minimum Cr was translocated to stem, i.e., 72.24, 55.33, 62.12 & 21.34 ($\mu g.g^{-1}$) in 100, 75, 50 & 25% respectively. However, accumulation of Cr was found to be higher in leaves than in stem, and the values were recorded as 57.3, 74.13, 33.21 & 14.5 ($\mu g.g^{-1}$) in 100, 75, 50, & 25%, correspondingly. Toxicity of Cr affects yield and root growth, further causing a mutagenic impact. Reported toxicity ranges from 10-100 $mg.kg^{-1}$ (Kabata-Pendis & Pendias 2011). Results revealed that 100, 75, and 50%

Table 2: Effect of tannery sludge extract on Relative seed germination (RSG), relative root growth (RRG), and germination index (GI) of *H. cannabinus*.

Concentration	Germination [%]	RRG [cm]	RSG [%]	GI [%]
Control	80	100 ± 0.0	100 ± 0.0	100 ± 0.0
100	95±1.13	0.91 ± 0.01	84.43±0.51	87.07 ± 0.94
75	80±1.23	0.9 ± 0.01	82.36 ± 0.55	84.02 ± 1.15
50	90±0.41	1.16 ±0.05	84 ± 1.52	90.38 ± 1.27
25	80±0.15	1.03 ± 0.03	81.33 ± 1.15	76.21 ± 0.94

Table 3: Total heavy metal accumulation in *H. cannabinus* grown in garden and tannery sludge amended soil (μg.mg^{-1}).

Heavy metals		Cr	Metal removal [%]	Cd	Metal removal [%]	Ni	Metal removal [%]	Pb	Metal removal [%]	Cu	Metal removal [%]
	Control	0.9 ±0.35	38	0.31 ±0.026	39	0.71±0.01	71	1.1 ±0.02	29	6.23±0.01	17
	Uptake in plant	0.56±0.032		0.096±0.01		0.50±0.01		0.31±0.01		1.04±0.34	
25%	Amended soil	120.21±23.2	57	41.21±9.56	42	19.87±2.12	57	128.32±32.1	19	84.2±12.1	29
	Uptake in plant	68.5±7.21		17.30±4.32		11.32±3.21		24.3±7.2		23.58±7.81	
50%	Amended soil	228.22±42.11	76	88.43±23.11	58	42.4±8.5	59	254.87±32.21	46	168.84±21.1	28
	Uptake in plant	173.28±32.91		51.28±7.12		25.01±4.87		58.62±8.42		47.27±9.12	
75%	Amended soil	582±88.11	41	103.22±21.1	32	62.11±13.4	45	381.32±43.2	16	284.11±25.1	12
	Uptake in plant	236.12±54.7		33.03±6.78		27.9±11.12		61.01±12.11		42.61±12.11	
100%	Amended soil	709.6±15.2	37	132.71 ±20.1	28	74.6±10.2	39	454.8±54.2	14	366.43±12.76	12
	Uptake in plant	258.03±20.62		37.15±11.12		29.09±6.5		63.67±12.1		43.97±13.9	

concentrations of Cr accumulation were above the toxicity range.

In tannery sludge amended soil, heavy metal Ni was maximum accumulated after Cr. Translocation observed in root were 14.05, 13.97, 11.62 & 5.031(μg.g^{-1}) in 100, 75, 50, & 25%, respectively. Further minimum translocation was observed in leaves of plants grown in 100 and 75% concentration whereas, in 50 and 25%, it was kept in the stem part with values 9.4 & 4.31, respectively.

Cd was found to be less accumulated than Cr & Ni among the studied metals. Maximum accumulation was observed in the root, i.e., 15.2., 13.3, 27.69 & 7.81 in the concentration of 100, 75, 50 & 25%, respectively. Whereas minimum accumulation was observed in stem 7.60, 14.42 & 5.22 in 100, 50 & 25% sludge amendment. While leaf metal accumulated more than stem at 100 & 25 % with values 6.3 & 5.22 (μg.g^{-1}), whereas accumulation values were equal in 50% concentration, i.e., 9.32 mg.kg^{-1}, and at 75% sludge strength. Stem accumulated more metal compared to leaf. Cd is a highly toxic element that impacts germination, metabolism, and water status in plants and also causes a reduction in plant biomass. The toxicity range of Cd was reported to be 5-30 mg.kg^{-1} (Kabata-Pendis & Pendias 2011, Eid et al. 2016).

Lead was found lesser accumulated than Cr, Ni & Cd. Results revealed that in 100% concentration, metal accumulated maximum in the root (6.5 mg.kg^{-1}) and minimum in the stem area (14.0 μg.g^{-1})) whereas leaves exhibited accumulation more than the stem, i.e., 18.23 μg.g^{-1}. In 75% sludge amended soil, the values are found to be 25.66 (root), 18.2 (leaf), and 17.2 (stem); similarly, in 50%, it was found to be 26.9 (leaf), 18.1(root), and 13.2 (stem), further, in 25% it was observed as 11.7(root), 6.32(leaf) and 5.01 (stem). Plants absorb Pb, but it is not an essential element for any significant biological function, rather a toxic element for plants if found in a range of 30-300 mg.kg^{-1} (Nawab et al. 2015, Kabata-Pendis & Pendias 2011).

Cu was accumulated in a relatively lower amount than other observed metals. Results showed values accumulated in root were 20.3, 19.2, 20.03 & 23.5 μg.g^{-1} in concentrations of 100, 75, 50 & 25%, respectively, whereas 09.23, 10.58 & 11.19 μg.g^{-1} was accumulated in leaf and 0.43, 0.53 & 0.25 μg.g^{-1} accumulated in stem area of plant grown in 100, 75, 50% sludge respectively.

Further, the maximum accumulation of metal in plants cultivated in garden soil was observed in Cd, and a translocation trend of metal was found in the root (0.02) = leaf (0.02) > stem (0.015 μg.g^{-1}). Cu showed less accumulation than Cd, and the trend of translocation is as follows: root (0.44) > leaf (0.33) > root (0.26) mg.kg^{-1}. Whereas Pb was

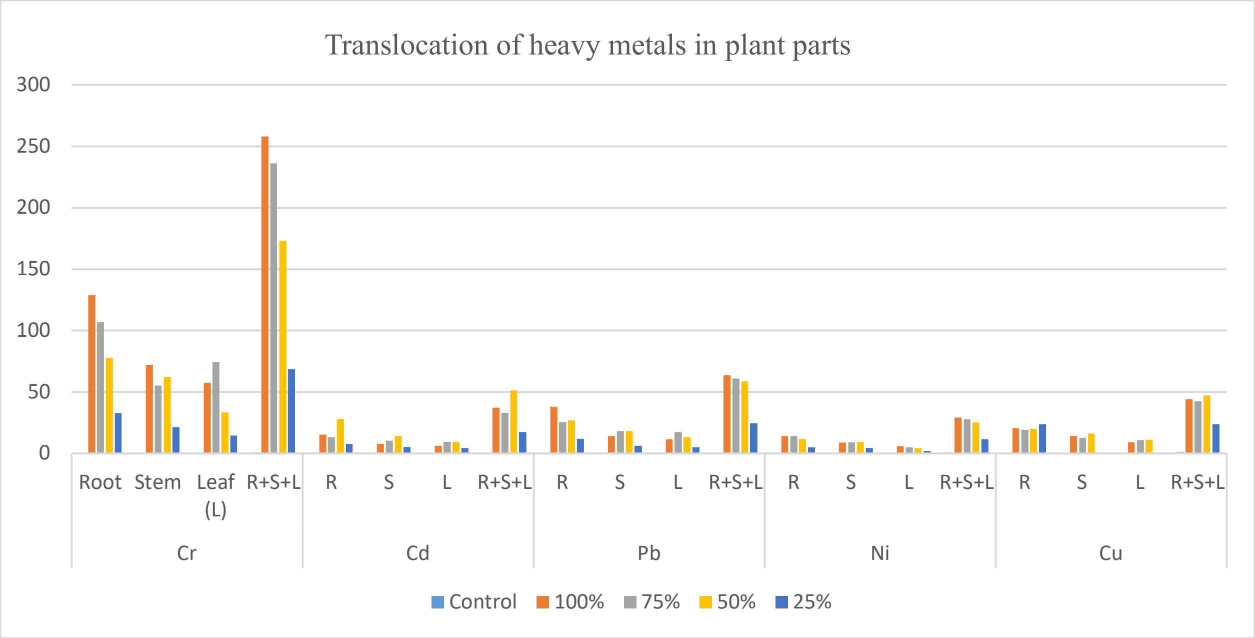

Fig.1: Heavy metal concentration in different parts of plant cultivated in garden and tannery sludge amended soil (µg.kg^{-1}).

found to be less accumulated than Cd and Cu, and it was found to be 0.19 > 0.17 > 0.12 µg.g^{-1} in root, leaf & stem, respectively. Further, Cr and Ni were found to be minimum accumulated, and values are as follows: 0.13 & 0.19 (root) > 0.09 & 0.15 (leaf) > 0.14 & 0.09 µg.g^{-1} (steam), respectively. Heavy metals are highly leachable; they are more likely to enter the environment and become available for uptake by plants (Kumar et al. 2013).

CONCLUSION

Utilization of tannery sludge in the agricultural field owing to the availability of essential nutrients can be an attractive alternative. However, the study revealed the presence of high levels of Cr (709 mg.kg^{-1}) and other metals (e.g., Cu, Ni, Pb, and Cd), which can be a hindrance. Noticeably, some plants have adapted over time to the locally elevated metal levels. Results exhibited that *H. cannabinus* is tolerant towards tannery sludge leachate during germination and early seedling growth and, consequently, can be grown in tannery sludge-amended soil. Efficient bioaccumulation and translocation of toxic metals from sludge-amended soil to roots and subsequently to aerial parts countered in this study gives direction for the application of *H. cannabinus* for reclamation of land contaminated with industrial waste/heavy metals.

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