**Adarsh Kumar*, Poonam Rani**,**
**Tripti***, Ramesh Chandra Arya******

# Phytostabilization of Tannery Contaminated Soil Using Naturally Colonized Plant Species *Ricinus communis* and *Calotropis procera*

The present study involves assessment of four metals (Cr, Pb, Cu and Mn) and its mobility in *Ricinus communis* and *Calotropis procera* growing on tannery contaminated soil (TCS). The area is moderately to strongly contaminated with Cr. Except for Cr, all analyzed metals were found within critical range in TCS and in both the plants. Translocation and bioconcentration factor assessment showed TF< 1 and BCF > 1 for both the plants,which justifies major transfer and accumulation of Cr from soil to root. As these plants are not grazed by grazing animals, ecological metal transfer risks from these plants are quite low. High commercial importance such as biofuel production with medicinal values further enhances its utilization for phytostabilisation of moderately Cr contaminated sites.

Population explosion and rapid urbanization resulted in establishment of different industries and induced heavy metal pollution problem which raised critical concern over human health and ecosystem. Among all the industries, chrome tanning industry is one of the most potent, carcinogenic and toxic industry. It is remunerative and used in many part of the world for good quality products (leather). But direct discharge of their untreated, heavy metals loaded effluent (specially Cr VI) into the environment is matter of concern [1], its concentration as low as 0,5 mg $kg^{-1}$ in solution and 5 mg $kg^{-1}$ in soil can be toxic to plants [2; 3]. Heavy metals are toxic, non-degradable and persist in the environment for a long period of time which causes adverse effect on human health and other living biota.

Plants growing in and around tannery contaminated soil (TCS) accumulates significant concentration of heavy metals such as chromium (Cr), lead (Pb), copper (Cu) and manganese (Mn) in their tissues [4; 5]. Cr (VI) is highly toxic for plants and causes DNA and membrane damage, inhibition of seed germination, root tip cell division and photosynthesis [6; 7]. Prolonged intake of Cr via plants, vegetables and crops has long been considered as the predominant pathway for human exposure which leads to the contamination of the environment and food chain and causes respiratory, gastrointestinal, dermatological diseases, neurotoxic disorders and cancer [1; 8].

Phytoremediation is a eco-friendly, cost effective and resource generating technology, gaining attention throughout the world to use the tannery contaminated fallow and agricultural lands for resource generation [1]. R*icinus communis* and *Calotropis procera* are two potentially important plant species found suitable for bioenergy/ biofuel production with medicinal and commercial values. Present research primarily investigates status of heavy metal contamination of TCS. Secondly, the metals mobility and uptake by two plants species (*R. communis* and *C. procera*) using translocation factor and bioaccumulation factor was also evaluated to check the potentiality of phytoremediation.

Many illegal leather tanning industries are running continuously from long period of time in out-skirts of Meerut, Sobhapur village (29°0'5"N77°39'2"E), Rhota road, By-

* **Adarsh Kumar,** Ural Federal University (Ekaterinburg, Russia).
** **Poonam Rani,** Chaudhary Charan Singh University, Meerut Institute of Engineering and Technology (Meerut, India).
*** **Tripti,** Ural Federal University (Ekaterinburg, Russia).
**** **Ramesh Chandra Arya,** Chaudhary Charan Singh University (Meerut, India).
E-mail: adarsh.biorem@gmail.com

pass Meerut, Uttar Pradesh,India, discharging million gallons of toxic effluent into the nearby water bodies and land sites. In spite of high Cr contamination, *R. communis* and *C. procera* were found to be the most dominant and high biomass plants, growing luxuriantly without showing any toxic morphological effects were collected along with soil. Soil samples (each with 5 replicates) were air dried, mixed thoroughly, passed through < 2 mm sieve, and oven dried at 105 °C. The pH (1:1; w/v) and electrical conductivity (1:1; w/v) were determined by digital pH meter and electrical conductivity meter, respectively. Organic carbon (OC) was determined by rapid dichromate oxidation method [9], available nitrogen (Avl. N) by alkaline permanganate method [10], available phosphorus (Avl. P) by phosphomolybdenum blue calorimetric method using double beam UV-Visible scanning spectrophotometer [11], and available potassium (K) was extracted by 1N ammonium acetate solution at pH 7 (1:10; w/v) using flame photometer (AFP–100) [11]. Accurately weighed, 1 g of soil sample was digested using 10 mL of nitric acid ($HNO_3$) followed by 0.5 mL of $H_2O_2$ and filtered through Whatman#42 [12]. Samples were diluted and analyzed using atomic absorption spectrophotometer (AAS, Hitachi Z-2000 Zeeman).

Plant samples were washed several times to remove the adhered soil particle and oven dried at 80 °C until the constant weight was achieved. Plants were divided into root and shoot, homogenized using a mortar-pestle and passed through < 40 BSS (British standard) mesh and 1 g was dissolved in 10 mL of $HNO_3$ and heated on a hot plate for complete dissolution. The samples were filtered and analyzed using AAS.

Contamination factor (CF) is the ratio of metal contamination in TCS with respect to reference soil [13]. Bioconcentration factor (BCF) is the ratio of metal concentration in plant (root + shoot) to the metal concentration in soil [1] and translocation factor (TF) is ratio of metal in shoot to root was calculated. Detection limit for Cr, Pb, Cu, and Mn were 0,005, 0,002, 0,01 and 0,02 mg $L^{-1}$, respectively. The mean, minimum, maximum, standard deviation and one way analysis of variance were performed using SPSS 20.0 Inc. Chicago, USA and XLSTAT 2007 package.

The pH was found slightly alkaline for both the soils. The OC, avl. N, Avl. P, Avl. K were found high whereas concentration of Pb, Zn and Cu were found within the critical soil limits. However, the concentration of Cr was found very high for both soil samples. Heavy metals concentration in TCSs was ranged between 104–200 mg Cr $kg^{-1}$, 16–23 mg Pb $kg^{-1}$, 28–49 mg Cu $kg^{-1}$, and 165–239 mg Mn $kg^{-1}$for *R. communis*(Table 1). In addition, Avl. NPK (104–170, 32–58, 125–137 mg $kg^{-1}$) and OC (11–12 %) were sufficient to enhance plant growth [14, 15]. Continuous mixing of untreated tannery effluent would be the reason of this contamination [16; 17].

Table 1

**Chemical, nutritional characteristics and heavy metal concentrations of tannery contaminated soil (Mean ± SD) (Min–Max) n = 5**

| Parameters | *R. communis* | *C. procera* |
|---|---|---|
| $pH_{H2O}$ (1:1) | 8,20 ± 0,47 (7,68–8,65) | 8,15 ± 0,23 (7,86–8,40) |
| $E.C_{H2O}$ (ms $cm^{-1}$) (1:1) | 0,73 ± 0,02 (0,71–0,77) | 0,60 ± 0,10 (0,50–0,70) |
| Organic Carbon (%) | 11,50 ± 0,35 (11,00–12,00) | 11,23 ± 0,43 (11,00–12,00) |
| Available Nitrogen (mg $kg^{-1}$) | 147,16 ± 28,97 (104,5–170,0) | 141,33±14,06 (120,0–159,0) |
| Available Phosphorus (mg $kg^{-1}$) | 46,70 ± 6,62 (38,00–53,00) | 45,00±9,21 (32,00–58,00) |
| Available Potassium (mg $kg^{-1}$) | 133,03 ± 3,33 (128,28–137,50) | 130,22 ± 2,35 (127,68–134,0) |
| Cr (mg $kg^{-1}$) | 163,41 ± 37,31 (103,89–199,35) | 159,01 ± 26,76 (115,69–186,23) |
| Pb (mg $kg^{-1}$) | 21,00 ± 1,59 (19,00–23,00) | 20,01 ± 2,71 (16,00–23,00) |

| Cu (mg kg$^{-1}$) | 41,86 ± 8,27 (28,32–49,00) | 39,70 ± 2,19 (36,95–42,56) |
|---|---|---|
| Mn (mg kg$^{-1}$) | 200,02 ± 27,45 (165,12–239,26) | 199,04 ± 24,06 (166,88–231,56) |

Critical soil total concentration [18, 19]: Cr: 75-100; Pb: 100-400; Cu: 60-125; Mn: 1500–3000 mg kg$^{-1}$.

CF (metal concentration in contaminated soil to the metal in control reference soil) was found between 2,51–4,38 for Cr, 1,50–3,37 for Cu, 1,10–2,17 for Pb, and 0,84–1,74 for Mn which showed TCS soil is moderately to strongly polluted with Cr, none to strongly for Cu, moderately for Pb, and medium for MnTable 2.

Table 2

**Contamination factor (CF) of tannery contaminated soils. (n = 5; Mean ± SD)**

| Metals | *R. communis* growing site | | *C. procera* growing site | |
|---|---|---|---|---|
| | Mean ± SD | Min–Max | Mean ± SD | Min–Max |
| Cr | 3,74 ± 0,73 | 2,51–4,38 | 3,73 ± 0,43 | 2,97–3,91 |
| Pb | 1,44 ± 0,37 | 1,10–1,97 | 1,40 ± 0,43 | 1,16–2,17 |
| Cu | 2,13 ± 0,76 | 1,50–3,37 | 2,18 ± 0,82 | 1,56–3,43 |
| Mn | 1,20 ± 0,30 | 0,88–1,70 | 1,21 ± 0,32 | 0,84–1,74 |

CF = 0: none; CF = 1: none to medium; CF = 2: moderately; CF = 3: moderately to strong; CF = 4: strongly polluted; CF = 5: very strongly polluted soil

Partitioning of heavy metals in *R. communis* and *C. procera* is presented in Table 3. Metal accumulation in both plants were found in the order Mn > Cr > Cu > Pb. The average concentration of Cr in *R. communis* (303.83 mg kg$^{-1}$) and *C. procera* (258.89 mg kg$^{-1}$) growing on TCS was found higher than critical limits [20]. For all detected metals average metal concentrations in whole plants were much higher in *R. communis*than in *C. procera.*The results were in consistence with those of [21–23] in these plants. In both plants, significantly higher concentration of Cr and Pb were observed in roots than in shoots. For Cr, this might be due to complexation of metals with sulphydryl group (-SH) of soil constituents resulted into less translocation of heavy metals to the upper parts of plants and get immobilized in the root vacuoles [1; 24; 25]. Similarly, Pb get binds to carboxylic acid group of mucilage uronic acids on root surface and remains stored in root [26; 27]. Higher accumulation of Cu and Mn were observed in shoots than in roots for both plants that may be because of different metal transporter present in plants which can easily translocate Cu and Mn from root to aerial parts *via* plasma membrane and tonoplast [28; 29].

Table 3

**Heavy metal concentrations (mg kg$^{-1}$) in shoot and root of *R. communis* and *C. procera* growing on tannery contaminated soil. (Mean ± SD; n = 5)**

| Metal | Plant part | *R. communis* | | *C. procera* | |
|---|---|---|---|---|---|
| | | Mean ± SD | Min–Max | Mean ± SD | Min–Max |
| Cr | Shoot | 108,99 ± 2,95* | 105,59–112,90 | 85,93 ± 1,46 | 83,91–87,85 |
| | Root | 194,84 ± 2,70* | 190,49–197,72 | 172,96 ± 0,87 | 171,73–174,20 |
| Pb | Shoot | 11,92 ± 0,90 | 10,65–13,19 | 11,56 ± 1,08 | 9,99–12,90 |
| | Root | 13,49 ± 1,27 | 11,56–14,76 | 12,13 ± 1,05 | 10,84–13,18 |
| Cu | Shoot | 31,59 ± 1,80 | 29,31–34,12 | 29,02 ± 1,57 | 27,64–31,69 |
| | Root | 25,36 ± 2,69 | 21,22–28,28 | 23,00 ± 1,86 | 20,83–25,68 |
| Mn | Shoot | 144,81 ± 8,80 | 133,66–156,69 | 142,66 ± 7,89 | 130,66–150,66 |
| | Root | 115,16 ± 6,15 | 109,69–122,66 | 113,25 ± 1,73 | 111,19–115,59 |

Critical plant total concentration [20]: Cr : 5–30; Pb: 30–300; Cu: 20–100; Mn: < 400 mg kg$^{-1}$. * represents significant difference at $p < 0,05$ level of significance.

In both the plants, TF for Cr and Pb was found low (< 1) which indicates reduction in translocation to shoot parts. This may be due to lack of carriers for the transportation of Cr and Pb in plants [24]. However, TF was found >1 for Mn and Cu because of its high mobility towards aerial parts within the plant which support in metabolic activities and beneficiary for plant growth. The BCF was found > 1 for all the metals which shows metal accumulating ability of both plants. It can be preliminarily stated that, high metal contamination of soil could adversely affect the protective barriers functions with change in metal accumulation pattern which resulted in high uptake of heavy metals in studied plants [30]. Findings, similar to present study regarding heavy metals accumulation and translocation were reported by Nagaraju and Guru [31] in *C. procera* and for *R. communis.*

The current study concludes that TCS was moderate to strongly contaminated with Cr. Accumulation of metals in whole plant was observed in order: Mn > Cr > Cu > Pb which was higher in *R. communis* than *C. procera* growing naturally in TCS. Assessment of TFas well as BCFfactor proved that translocation of Cr from root to shoot was low (< 1) in both *R. communis* and *C. procera.*As these plants are not grazed by grazing animals, ecological metal transfer risks from these plants are quite low. High commercial importance such as biofuel production with medicinal values further enhances its probability to be/can be used for phytostabilisation of moderately Cr contaminated sites.

**Acknowledgments:** Authors are grateful to Upadhyay NC, Principal Scientist, Soil Science Department, Central Potato research Institute, Modipuram, India for providing laboratory facility for soil and plant analysis. Thanks are due to Meerut College and Ural Federal University for e-research facility supported by the Act 211 Government of the Russian Federation, agreement No. 02.A03.21.0006.

## References

1. Rani P., Kumar A., Arya R. C. Stabilization of tannery sludge amended soil using *Ricinus communis*, *Calotropis procera* and *Nerium oleander* // Journal of Soil Sediment. – 2017. – V. 17. – P. 1449–58.

2. Turner M. A., Rust R. H. Effects of chromium on growth and mineral nutrition of soybeans. Soil Science Society of America Journal. – 1971. – V. 35. – P. 755–758.

3. Kumar A., Maiti S. K. Effect of organic manures on the growth of *Cymbopogon citrates* and *Chrysopogon zizanioides* for the phytoremediation of chromite-asbestos mine waste: A pot scale experiment // International Journal of Phytoremediation. – 2015. – V. 17. – P. 437–447.

4. Barman S. C., Sahu R. K., Bhargava S. K., Chatterjee C. Distribution of heavy metals in wheat, mustard, and plant grown in fields irrigated with industrial effluents // Bulletin of Environmental Contamination and Toxicology. – 2000. – V. 64. – P. 489–496.

5. Bareen F., Tahir S. A. Metal accumulation potential of wild plants in tannery effluent contaminated soil of Kasur, Pakistan: Field trails for toxic metal clean up using Suaeda fruticose // Journal of Hazardous Material. – 2011. – V. 186. – P. 443–450.

6. Barcelo J., Poschenrieder C. Chromium in plants, In: S. Canali, F. Tittarelli and P. Sequi, Chromium Environmental Issues, Pub. – Milano : Franco Agnelli, 1997. – P. 101–129.

7. Rodríguez E., Azevedo R., Fernandes P., Santos C. Cr (VI) induces DNA damage, cell cycle arrest and polyploidization: a flow cytometric and Comet assay study in *Pisum sativum* // Chemical Research and Toxicology. – 2011. – V. 24. – P. 1040–1047.

8. Beyersmann D., Hartwig A. Carcinogenic metal compounds; recent insight into molecular and cellular mechanism // Archives of Toxicology. – 2008. – V. 82. – P. 493–512.

9. Walkely A. E., Black J. A. An examination of the Degtjareff Method for determining soil organic matter and a proposed modification of the chromic acid titration method // Soil Science. – 1934. – V. 37. – P. 29–38.

10. Subbiah B. V., Asija G. L. A rapid procedure for the determination of available nitrogen in soil // Current Science. – 1956. – V. 25. – P. 259–260.

11. Jackson M. L. Soil chemical analysis. – Prentice Hall of India : Ltd. New Delhi, 1958.

12. USEPA, Method 3050B.: Acid digestion of sediments, sludge and soils, Revision 2, 1996.

13. Kumar A., Maiti S. K., Translocation and bioaccumulation of metals in *Oryza sativa* and *Zea mays* growing in Chromite-Asbestos contaminated agricultural fields, Jharkhand, India // Bulletin of Environmental Contamination and Toxicology. – 2014. – V. 93. – P. 434–441.

14. Chhonkar P. K., Datta S. P., Joshi H. C., Pathak H. Impact of industrial effluents on soil health and agriculture – Indian Experience: Part II Tannery and Textile Industrial Effluents // Journal of Science and Industrial Ressearch. – 2000. – V. 59. – P. 446–454.

15. Gupta A. K., Sinha S. Phytoextraction capacity of the plants growing on tannery sludge dumping sites // Bioresource Technology. – 2007. – V. 98. – P. 1788–1794.

16. Revathi K. T., Haribabu E., Sudha P. N. Phytoremediation of Chromium contaminated soil using Sorghum plants // International Journal of Environmental Research. – 2011. – V. 2. – P. 417–428.

17. Sangeetha R., Kamalahasan B., Karthi N. Use of tannery effluent for irrigation: An evaluative study on the response of antioxidant defense in maize (Zea mays) // International Food Ressearch Journal. – 2012. – V. 19. – P. 607–610.

18. Alloway B. J. Heavy metals in soils. – Wiley : New York, 1990.

19. Alloway B. J. Heavy metals in soils. Environmental Pollution Springer. – Netherland. 2013.

20. Kabata – Pendias A. Trace elements in soils and plants. – London : CRC Press, 2011.

21. Annapurna D., Rajkumar M., Prasad M.N.V. Potential of caster beans (*Ricinus communes* L.) for phytoremediation of metalliferous waste assisted by plant growth promoting bacteria: possible cogeneration of economic products. – Elsevier, USA, 2016.

22. Nakbanpote P. C, Meesungnoep O., Prasad M. N. V. Potential of ornamental plants for phytoremediation of heavy metals and in coming generation. In: M.N.V. Prasad, Bioremediation and Bioeconomy. – Elsevier, USA, 2016. – P. 179–217.

23. Al-Yamni M., Sher H., El- Sheikh M., Eid E. Bioaccumulation of nutrient and heavy metals by *Calotropis procera* and *Citrullus colocynths* and their potential use as contamination indicators // Science Research Essays. – 2011. – V. 6. – P. 966–976.

24. Shanker A. K., Cervantes C., Loza-Tavera H., Avudainayagam S. Chromium toxicity in plants // Environment International. – 2005. – V. 31. – P. 739–753.

25. Morel J. L., Mench M., Guckert A. Measurement of Pb+2, Cu+2 and Cd+2 binding with mucilage exudates from maize (*Zea mays* L.) roots // Biology and Fertility of Soils. – 1986. – V. 2. – P. 29–34.

26. Sharma P., Dubey R. S. Lead toxicity in plants // Brazilian Journal of Plant Physiology. – 2005. – V. 17. – P. 35–52.

27. Kramer U., Cotter-Howells J. D., Charnock J. M., Baker A. J. M., Smith A. C. Free Histidine as a metal chelator in plants that accumulate nickel // Nature. – 1996. – V. 379. – P. 635–638.

28. Kramer U., Talke I. N., Hanikerne M. Transition metals transport // FEBS Letters. – 2007. – V. 581. – P. 2263–2272.

29. Ramamurthy N., Kannan S. Analysis of soil and plant (*Calotropis gigantea* Linn) collected from an industrial village, Cuddalore DT, Tamil Nadu, India // Romanian Journal of Biophysics. – 2009. – V. 19. – P. 219–226.

30. Baltrenaite E., Lietuvninkas A., Baltrenas P. Use of dynamic factors to assess metal uptake and transfer in plants-example of trees // Water Air and Soil Pollution. – 2012. – V. 223. – P. 4297–4306.

31. Nagaraju, Guru K. R. Environmental impact of Barytes Deposit: A case study from Mangampeta Area, Cuddapah Basin, Andhra Pradesh. – 2003. – P. 1–6. – URL: www.academia.edu.