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RESEARCH ARTICLE

Studies on phytoremediation potential of *Azadirachta indica* and *Acacia nilotica*

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SUMMARY

An experiment was conducted in the pot culture house to study the phytoremediation potential of *Azadirachta indica* and *Acacia nilotica*. The tree species were raised in dune sand treated with heavy metals (Cd 10 and 20 ppm, Ni 50 and 100 ppm, Pb 50 and 100 ppm). The physiological stress indices like relative membrane injury (%) and chlorophyll content were studied to determine the tolerance of the two woody species to heavy metal stress. Keekar was found to be more tolerant in terms of relative membrane injury and chlorophyll content as compared to Neem. Among the heavy metals studied Ni was most toxic in terms of relative membrane injury. The accumulation of heavy metals in the two woody species and in their different parts varied. Cd accumulation was higher in Neem as compared to Keekar. Maximum accumulation of Cd was in the roots followed by leaves and minimum in stem. A 5-6 fold higher Ni accumulation was observed in Keekar roots as compared to Neem roots. However, in the stem and leaves Neem accumulated more Ni than Keekar. Pb accumulated mainly in leaves followed by roots and minimum in the stem. Pb accumulation was higher in Keekar. Thus it can be concluded that in terms of physiological tolerance and Pb and Ni accumulation, *Acacia nilotica* can be used for phtoremediation purposes.

Key Words : *Acacia nilotica, Azadirachta indica,* Chlorophyll, Heavy metals, Membrane injury, Phytoremediation

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Phytoremediation is an environmental clean-up strategy in which selected green plants are employed to remove, contain or render environmentally toxic contaminants harmless. This is an emerging biotechnological application and operates on the principles of biogeochemical cycling (Prasad, 2004). This remediation approach is attracting attention from various governments as a cost-effective and environment-friendly green technique to clean-up heavy metal polluted soil using hyperaccumulators. Over the past 10 years, woody plants have been shown to be excellent candidates for phytoremediation, due to rapid growth, high biomass, profuse root apparatus and low impact on the food chain and human health (Salt *et al.*, 1998; Pilon-Smits, 2005; Yadav *et al.*, 2010). Most of the hyperaccumulator plants so far identified have a small biomass for eg *Thalspi* (Prasad and Freitas, 2003). Plants with higher biomass production such as trees are of more interest in soil phytoremediation (Lanberg and Greger, 1996) and has the added advantage that it can be harvested for the production of biomass energy. Majority of such work concerns accumulation capacity and biomass production of woody plants as a response to high concentration of pollutants (Pulford and Watson, 2004). Phytoremediation using trees provides a potential opportunity to extract or stabilize metals. It involves the use of trees that readily transport targeted metals from soil to plant organs, which allows removal of metal by harvesting from the plant. This process takes longer time but helps in the greening of the land and in reducing pollution (Pulford and Dickinson, 2006).

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However, there is need to identify trees that can tolerate heavy metal stress and have the ability to uptake and translocate the metal to the aerial parts. Very little work has been done to integrate multipurpose trees into phytoremediation projects for cleaning contaminated sites. Thus, in the present investigations the ability of two woody species *Azadirachta indica* (Neem) and *Acacia nilotica* (Keekar) from heavy metal treated soil to their above-ground tissues was studied in pot culture house experiments. Nutrient culture experiments have the advantage of accessibility of the roots for observation and ease of harvesting.

MATERIALS AND METHODS

This experiment was conducted in the pot culture house. Cemented pots were filled with 8 kg dune sand. The sand was flushed thoroughly with water. The heavy metals in the following concentrations were added to the dune sand.

Cd 10 and 20 ppm

Ni 50 and 100 ppm

Pb 50 and 100 ppm

Neem and Keekar were raised in dune sand treated with heavy metals as mentioned above. The trees were supplied with equal amounts of nutrient solution every fifteen days initially and after 30 days after two months. The plants were sampled after four months and analyzed for the various parameters.

Heavy metal content :

The heavy metal content of different parts of trees was estimated. The trees were extracted, washed and separated into leaves stem and roots. The plant parts were dried in oven at 70^0 C. 500 mg tissue was digested in HNO_3:$HClO_4$ mixture and the heavy metal content was analyzed by AAS.

Chlorophyll and carotenoid content :

Leaf discs (0.03g) were washed, blotted dry and then dipped in dimethyl sulphoxide overnight as described earlier by Sawhney and Singh (2002). The absorbance of the solution was recorded next day at 480, 645 and 663 nm, respectively

Relative membrane injury :

Relative membrane injury was analyzed according to the method of Zhang *et al*. (2006). Two hundred and fifty mg of fully expanded leaves were rinsed with distilled water and immersed in 10 ml de-ionised water in vials and incubated at 25^0C for 4 h. Electrical conductivity (EC) of the bathing medium was measured at 25^0C (Xi). The tissue along with leachate was then boiled at 100^0C for 30 min to completely disrupt the cell structure. The solution was brought to 25^0 C and its EC was measured again (Xt). Relative injury was calculated from the equation $[(Xi/Xt) \times 100]$.

RESULTS AND DISCUSSION

Trees have been suggested as low cost sustainable and ecologically sound solution to the remediation of metal contaminated sites. Also while a high metal content in agricultural crops is not desirable, a high metal content in trees is acceptable as long as physiological activity is not affected (Singh *et al.,* 2011). In the present investigations relative membrane injury and chlorophyll content were used as the stress markers for assessing the physiological tolerance to heavy metals. The electrolyte leakage under stress conditions has been widely accepted as selectable marker for indexing stress tolerance (Upreti and Murti, 2005). An increase in electrolyte leakage suggests a negative impact on membrane integrity and thus membrane deterioration. A higher increase in electrolyte leakage was observed in Neem leaves as compared to Keeker leaves with all the three heavy metals used (Pb, Cd and Ni) and at both the concentrations tested (Fig. 1A). In Neem Ni 50 and 100 ppm was most deleterious resulting in a 47 per cent and 52 per cent increase in electrolyte leakage followed by Cd which registered a 29 and 36 per cent increase, respectively. Pb 50 ppm treatment was least toxic in terms of electrolyte leakage in Neem. In Keekar only the higher concentrations of Pb and Ni were toxic resulting in a 31 and 26 per cent increase, respectively.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990). Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals. (Sinha *et al*., 1993; Ouzonidou, 1995). In case of Neem Cd 20 ppm was most deleterious in terms of chlorophyll content resulting in a 35 per cent decline (Fig. 1B). Pb 50 and 100 ppm resulted in a small decline of about 5 per cent. Ni 50 and 100 ppm treatments had no significant inhibitory effects on chlorophyll content. In Keekar a 10 to 15 per cent decline in chlorophyll content was observed with various heavy metals studied (Fig. 1C). Almost similar results were observed with chl. A and B content. The chlorophyll ratio, which is used as a stress indicator, increased with increasing metal treatments. This was also seen in *Empetrum nigrum*leaves near a copper and nickel smelter in the field (Monni *et al*., 2001) and in heavy metal treated *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005). Increased chlorophyll ratios due to environmental stress have been reported in spinach leaves (Delfine *et al*., 1999). A high chlorophyll ratio also indicates a change in the PSII/PSI ratio in stressed leaves (Anderson, 1986).

Several studies have demonstrated that the metal concentrations in plant tissue are a function of heavy metal content in the growing environment (Cui *et al*., 2004; Lorestoni *et al.,* 2011). All plants take up metals to varying degrees from the substrates in which they are rooted (Baker *et al*., 2000). Moreover, the level of tolerance developed can often be related

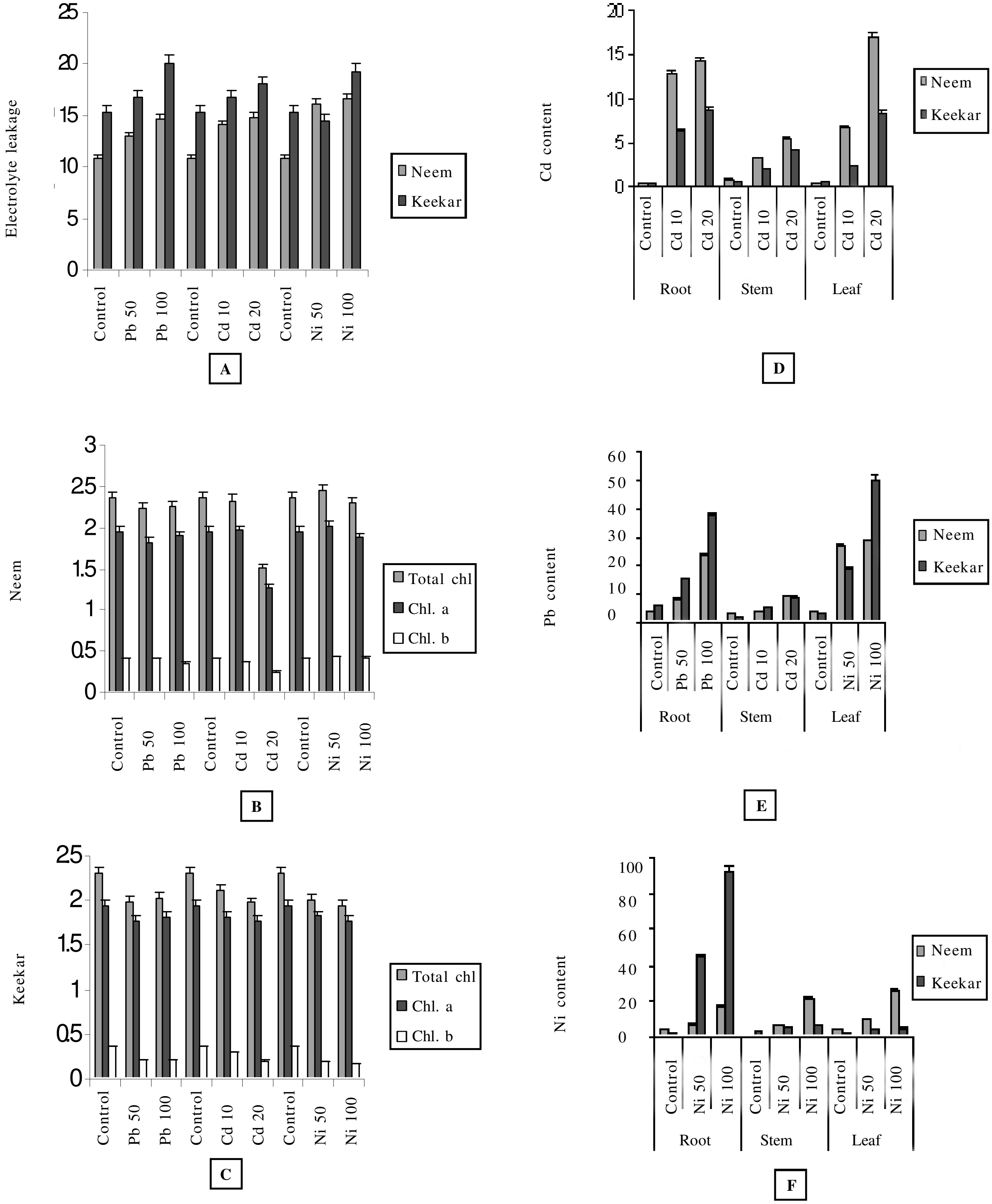

Fig 1: **Effect of heavy metal treatments on A-Electrolyte leakage (%) in Neem and Keekar, B- Total chlorophyll, cholrophyll A and chlorophyll B (mg/g F.Wt) in Neem, C- Total chlorophyll, cholrophyll A and chlorophyll B (mg/g F.Wt) in Keekar, D- Cd content (µg/g D.Wt) in Neem and Keekar, E- Pb (µg/g D.Wt) content in Neem and Keekar, F- Ni content (µg/g D.Wt) in Neem and Keekar**

to the amount of metal in the soil (Foy *et al*., 1978). There is evidence from natural establishment of trees on contaminated sites that some types of trees can survive under such adverse conditions, e.g. *Salix* (willow), *Betula* (Birch), *Populus* (Poplar), *Alnus* (Alder) and *Acer* (Sycamore) (Roselli *et al*., 2003). The main characteristics of trees that make them suitable for phytoremediation is their large biomass, both above and below ground level. Physical phytostabilization can be readily achieved, and is often the main benefit of using trees on such sites. Vegetation of tree species helps in decreasing the risk of soil, water and wind erosion. Phytoremediation and especially the use of trees is an emerging and developing technology, and this has grown rapidly in recent years (Van der Lelie *et al*., 2001). However, the accumulation of heavy metals in different tree species and in their different parts varies. This could be due to differences in the mechanisms of uptake and sequestration of this metal in different plant systems (Clemens, 2001). In the present studies heavy metal uptake in two tree species from different families were studied. Cd accumulation was twofold higher in Neem as compared to Keekar (Fig. 1D). The metal accumulated mainly in the roots but at the higher Cd concentration substantial accumulation was observed in the leaves in both Neem and Keekar. Minimum accumulation was observed in the stem. A 5-6 fold increase in Ni accumulation was observed in Keekar roots as compared to Neem roots, however, in the stem Neem accumulated more Ni than Keekar and a threefold increase in Ni accumulation was observed with Ni 100 ppm treatment (Fig. 1F). In the leaves also a 2 to 5 fold increase in Ni accumulation was observed in Neem leaves as compared to Keekar leaves. Pb accumulated mainly in the leaves followed by roots and minimum in the stem (Fig. 1E). A significantly higher accumulation of Pb was observed in Keekar leaves as compared to Neem with Pb 100 ppm treatment. In roots also accumulation was higher in Keekar as compared to Neem and a two fold increase in Pb accumulation was observed with the Pb 50 ppm treatment.

Thus, it can be concluded that in terms of physiological tolerance Pb and Ni uptake *Acacia nilotica* can used successfully for phytoremediation purposes.

REFERENCES

Anderson, J.M. (1986). Photoregulation of the composition, function and structure of the thylakoid membranes. *Ann. Rev. Plant Physiol.,* **37**:3–136.

Baker, A.J.M., McGrath, S.P., Reeves, R.D. and Smith, J.A.C. (2000). Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metalpolluted soils. *In: Phytoremediation of contaminated soil and water* (Ed.) Terry, N. and Banuelos, G., Lewis Publishers, FLORIDA, (U.S.A.) pp. 85–107.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, **212**: 475–486.

Cui, Y., Wang,Q. and P. (2004). Christie: Effect of elemental sulphur on uptake of cadmium, zinc and sulphur by oilseed rape growing in soil contaminated with zinc and cadmium. *Comm. Soil Sci. Plant Anal*., **35**: 2905-2916.

Delfine, S.A., Alvino, M.C., Villiani and Loreta, F.(1999). Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiol.,* **119**: 1101–1106.

Foy, C.D., Chaney, R. L. and White, M.C. (1978). The physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol*., **29**: 511–566.

Grifferty, A. and Barrington, S. (2000). Zinc uptake by young wheat plants under two transpiration regimes. *J. Environ. Qual*., **29:** 443-446.

Landberg, T. and Greger, M. (1996). Differences in uptake and tolerance to heavy metals in salix from unpolluted and polluted areas. *Appl. Geochem*., **11**: 175-180.

Lorestoni, B., Cheraghi, M. and Yousefi, N. (2011). Phytoremediation potential of native plants growing on a heavy metal contaminated soil of copper mine in Iran. *World Acad. Sci. Tech*., **77** :377-382.

Monni, S., Uhlig, C., Junttila, O., Hansen, E. and Hynynen, J. (2001). Chemical composition and ecophysiological responses of *Empetrum nigrum* to above ground element application. *Environ. Poll.,* **112**: 417–426.

Ouzounidou, G. (1995). Cu-ions mediated changes in growth, chlorophyll and other ion contents in a Cu-tolerant *Koeleria splendens. Biol. Plant*., **37** :71-78.

Parekh, D., Puranik, R.M. and Srivastava, H.S. (1990). Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie Physiologie der Pflanzen.,* **186**: 239–242.

Pilon-Smits, E. (2005). Phytoremediation. *Annu. Rev. Plant Biol*., **56**: 15–39.

Prasad, M.N.V. and Freitas, H. (2003). Metal hyperaccumulation in plants-biodiversity prospecting for phytoremediation technology. *Electr. J. Biotech.,* **6** :285-321.

Prasad, M.N.V.(2004). Phytoremediation of metals in the environment for sustainable development. *Proc. Indian Natl. Sci. Acad. Part B*., **70**, 71–98.

Pulford, I.D. and Watson, C. (2003). Phytoremediation of heavy metal contaminated land by trees – A review. *Environ. Internat*., **29**: 529–540.

Pulford, I.D. and Dickinson, N.M.(2006). Phytoremediation technologies using trees. In: *Trace elements in the environment: Biogeochemistry, biotechnology and bioremediation* (Ed. Prasad, M. N. V., Sajwan, K. S. and Naidu, R.), C.R.C. Press, Boca Raton, pp. 375–395.

Rosselli, W., Keller, C. and Boschi, K. (2003). Phytoextraction capacity of trees growing on a metal-contaminated soil. *Plant Soil*, **256**: 265–272.

Salt, D.E., Smith, R.D. and Raskin, I. (1998). Phytoremediation. *Annu.Rev. Plant Physiol. Plant Mol. Biol*., **49**: 643–668.

Sawhney, V. and Singh, D. P. (2002). Effect of chemical dessication at the post anthesis tage on some physiological and biochemical changes in flag leaf of contrasting wheat genotypes. *Field Crops Res.*, **77**:1-6.

Singh, D., Gupta, R. and Tiwari, A. (2011). Phytoremediation of lead from wastewater using aquatic plants. *Internat. J. Biomed. Res.*, **2** (7).

Sinha, S. K., Srivastava, H.S. and Tripathi, R.D. (1993). Influence of some growth regulators and cations on the inhibition of chlorophyll biosynthesis by lead in maize. *Bull. Environ Contam. Toxic*., **51**: 241-246.

Upreti, K.K. and Murti, G.S.R. (2005). Water stress induced changes in common polyamines and abscisic acid in French bean. *Indian J. Plant Physiol.,* **10** :145-150.

Van der Lelie, D., Schwitzguébel, J. P., Glass, D. J.,Vangronsveld, J. and Baker, A. (2001). Assessing phytoremediation's progress in the United States and Europe. *Environ. Sci. Technol*., **35**(22): 446-452.

Yadav, R., Arora, P. Kumar, S. and Chaudhary, A. (2010). Perspectives for genetic engineering of Poplars for enhanced phytoremediation abilities. *Exotoxicol*., **19**: 1574-1588.

Zengin, F.K. and Munzuroglu,O. (2005). Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biologica Cracoviensia*., **47/2**: 157-164.

Zhang, Y., Wang,L.,Liu, Y., Zhang, Q., Wei, Q. and Zhang, W. (2006). Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na^+/H^+ antiport in the tonoplast. *Planta*, **224**:545-555.

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