Reclamation of Tannery Polluted Soil through Phytoremediation

V. Sakthivel¹ and M. Vivekanandan²

¹Department of Biotechnology, Bharathidasan University College, Perambalur – 621 212, Tamil Nadu, India

²Department of Biological Sciences, Vivekananda arts and Science College for women, Elayampalayam, Thiruchengodu - 637205, Tamil Nadu, India

ABSTRACT

The huge volume of sludge emanating from the tannery effluent treatment plants poses a serious environmental problem. Phytoremediation is an emerging technology in which the plants are employed to reclaim the contaminated soil strewn with heavy metals (metalloids) and toxic compounds. This work focuses the impact of application of tannery sludge on biochemical properties of 6 months old tree saplings of *Azadirachta indica* A. Juss. (Neem), *Melia azedarach* Linn. (Wild Neem) and *Leucaena leucocephala* (Lam) de Wit (Subabool) raised over the tannery sludge in an attempt to use these plants for phytoremediation. The plants raised over the garden soil served as the control. The porosity and water holding capacity of the tannery sludge were higher. The plant growth supporting elements such as Ca, total N₂, NO₃ and Mg were higher in the sludge. The plants raised over the sludge were found to be dark green with increased morphometric parameters. Electrophoretic profile revealed amplification of a few polypeptides (100, 105, 49 and 55 KDa). The levels of biomolecules and the CO₂ absorption increased in 6 months old plants. There was a significant uptake and transport of chromium in all the three tree species suggesting that these plants could be employed in phytoremediation of soils contaminated with heavy metals. [Physiol. Mol. Biol. Plants 2009; 15(2) : 175-180] E-mail : tosakthivel@scientist.com, sakthiii@yahoo.com

Key words : *Azadirachta indica* (Neem), CO₂ uptake, *Leucaena leucocephala* (Subabool), *Melia azedarach* (Wild Neem), metalloids, Phytoremediation, Stress biomolecules, Tannery sludge

INTRODUCTION

According to the estimate in 2004 - 2005, India is the third largest producer of leather bumping in US \$ 2.4 billion (Koshy, 2006) to process finished leather from animal hides and skins. The discharge of wastes from the tanning units, without proper treatment, has already led to pollution of many rivers and lands. During the treatment of tannery effluent, a huge amount of tanned solid particles are separated as “sludge”. Researchers all over the world are currently directing their efforts to find an alternate way of treating the effluent and sludge discharge. “Phytoremediation”, is an emerging technology in which the plants are employed to absorb and bio-magnify elements from a polluted environment and metabolize them into various biomolecules in their tissues (Garbisu *et al.*, 2002). This technology is considered as an alternative to the cost-involving physico-chemical methods that have been used over

the years. Phytoremediation technology is highly promising because it is cheaper and is able to minimize the contaminating levels of pollutants. In this work, the tree saplings like *Azadirachta indica*, *Melia azedarach* and *Leucaena leucocephala* were employed as phytoremediators and the biochemical changes occurring after 6 months of exposure of plants to the tannery sludge were studied.

MATERIAL AND METHODS

Tannery sludge was collected from the effluent treatment plant at Sembattu, Trichy (Tamil Nadu, India), sun dried, crushed and filled in earthen pots (30 cm height and 30 cm diameter). The pots filled with the garden soil collected from Bharathidasan University botanical garden served as the control. One month old tree saplings namely *Melia azedarach* Linn. (Wild Neem), *Azadirachta indica* A. Juss. (Neem) and *Leucaena leucocephala* (Lam) de Wit. (Subabool) procured from the Forest Range Office Nursery at Kalamavoor (Pudukkottai District) were planted in both the sludge-

Correspondence and reprint requests : V. Sakthivel
Ph.: +91 431 98656 89400

and garden soil-filled earthen pots (in triplicate). The pots were periodically watered (Bharathidasan University bore well water) by avoiding running down of water from the pots. The plants were allowed to grow in outdoor conditions and leaf samples were collected randomly and pooled, from the 6 months old tree saplings for the following biochemical analyses: Total chlorophyll (Arnon, 1949), total soluble sugars (Dubois *et al.*, 1956), sucrose (Van Handel, 1968), total soluble starch (Mc Cready *et al.*, 1950), free amino acids (Troll and Canan, 1953), total phenols (Swain and Hillis, 1959), total soluble proteins (Lowry *et al.*, 1951), free proline (Bates *et al.*, 1973), total nitrogen (Umbreit *et al.*, 1972), nitrate (Wolley *et al.*, 1960), total soluble proteins were analysed using SDS-PAGE (Laemmli *et al.*, 1970) and light dependent CO₂ uptake was carried out using Infrared gas analyser (Licor Inc, USA) by following Geiger and Fondy (1979).

The sludge and soil samples were dried separately in a hot air oven at $100 \pm 2^\circ\text{C}$ for 48 h and then sieved (0.2 mm) to produce a homogeneous sample. 1.0 g of sludge and soil was taken separately in a boiling test tube to which 15.0 ml of triple acid mixture consisting of 10.0 ml of concentrated nitric acid, 4.0 ml of perchloric acid (PCA) and 1.0 ml of concentrated hydrochloric acid (HCl) was added. The tubes were heated until complete cease-over of brown fumes. The samples were cooled to room temperature and diluted to 100 ml using double glass-distilled water. The samples were filtered through Whatman No.1 filter paper. The filtered samples were analysed (APHA, 1992) for the following elements through Atomic Absorption spectrophotometer Varion (SpectrAA 220, Australia), Calcium (Ca), Phosphorus (P), Potassium (K), Sodium (Na), Magnesium (Mg), Manganese (Mn), Copper (Cu), Zinc (Zn), Iron (Fe) and Chromium (Cr). The elemental status of tannery sludge and garden soil is given in Table 1. Elemental status of the water used for irrigation was also measured (Data not presented). Statistical analyses of the data (Students t-test and level of significance) were done according to the method of Mead and Curnow (1983).

RESULTS AND DISCUSSION

The physical and chemical composition of tannery sludge is given in Table –1. The porosity (75.09 %) and water holding capacity (76.50 %) of the tannery sludge were higher than that of the garden soil. The pH of the sludge was 8.16, whereas the EC value of the sludge ($2275 \mu\text{S cm}^{-1}$) was several-fold higher, compared to that of the garden soil ($86.57 \mu\text{S cm}^{-1}$). The concentrations

of elements such as Ca, total N, NO₃ and Mg were higher in the sludge than in the garden soil. In addition, the sludge as expected also contained higher levels of Cr (26 mg g⁻¹ sludge), Na (208 mg g⁻¹ sludge) and Cl₂ (71 mg g⁻¹ sludge). Hence it was found necessary to employ moderately stress tolerant and fast growing tree species

Table 1. Physical and chemical properties of the garden soil and tannery sludge

Parameters	Garden soil	Tannery sludge
PHYSICAL		
Colour	Red	Brown
Odour	Nil	Foul
pH at 25°C	8.26 ± 0.18	8.16 ± 0.17
EC at 25°C ($\mu\text{S cm}^{-1}$)	86.57 ± 0.95	2275 ± 5.20
Bulk density (g cm ⁻³)	1.91 ± 0.25	0.66 ± 0.13
Real specific gravity (g cm ⁻³)	1.35 ± 0.11	0.58 ± 0.12
Porosity %	27.92 ± 1.56	75.09 ± 1.98
Water holding capacity %	31.1 ± 1.21	76.5 ± 2.18
CHEMICAL (mg/g)		
Calcium	44.86 ± 2.89	320.93 ± 20.12
Total Nitrogen	2.18 ± 0.37	8.60 ± 0.77
Phosphorus	75.56 ± 3.45	25.08 ± 1.22
Potassium	19.86 ± 0.98	21.85 ± 1.05
Nitrate	0.77 ± 0.04	5.99 ± 0.45
Sodium	20.25 ± 1.18	207.75 ± 15.81
Magnesium	57.19 ± 2.92	170.52 ± 12.05
Manganese	5.57 ± 0.33	3.13 ± 0.32
Copper	2.65 ± 0.54	1.36 ± 0.27
Zinc	2.46 ± 0.42	3.33 ± 0.39
Iron	181.71 ± 14.89	78.21 ± 2.91
Chromium	Nil	25.66 ± 1.19
Chloride	4.25 ± 0.65	70.91 ± 1.95

Table 2. Leaf biomolecules of 6 months old tree saplings grown over garden-soil and tannery-sludge independently.

Leaf Biomolecules (mg/g dw)	Garden Soil-Grown Tree Saplings (Control)			Tannery Sludge-Grown Tree Saplings (Treatment)		
	<i>Melia azedarach</i>	<i>Azadirachta indica</i>	<i>Luecaena leucocephala</i>	<i>Melia azedarach</i>	<i>Azadirachta indica</i>	<i>Luecaena leucocephala</i>
Chlorophyll a	6.07 ± 0.28	6.97 ± 0.23	6.07 ± 0.23	9.0 ± 0.83** (156.44)	11.08 ± 0.22* (158.95)	10.11 ± 0.38* (166.88)
Chlorophyll b	2.54 ± 0.32	3.10 ± 0.29	1.25 ± 0.24	3.88 ± 0.54** (152.65)	5.02 ± 0.34** (161.62)	2.05 ± 0.29* (170.34)
Chlorophyll a/b ratio	2.38	2.44	2.24	2.20	5.02	4.92
Total chlorophyll	8.61 ± 0.29	10.07 ± 0.30	7.26 ± 0.28	13.38 ± 0.64** (155.32)	16.09 ± 0.32* (159.78)	12.16 ± 0.35** (167.45)
Total soluble sugars	124.09 ± 1.23	128.52 ± 1.54	130.18 ± 1.67	177.94 ± 1.29** (143.40)	196.18 ± 1.47** (153.12)	229.26 ± 2.46* (176.11)
Sucrose	21.64 ± 0.13	18.87 ± 0.08	18.28 ± 0.11	29.25 ± 0.20** (135.19)	29.76 ± 0.19** (157.65)	31.02 ± 0.21** (169.63)
Total soluble starch	20.66 ± 0.15	18.28 ± 0.12	24.13 ± 0.17	27.91 ± 0.15* (135.09)	28.01 ± 0.17* (153.18)	38.65 ± 0.25** (160.15)
Free amino acids	16.72 ± 0.07	17.84 ± 0.09	10.07 ± 0.08	24.06 ± 0.13* (143.91)	28.32 ± 0.16** (158.73)	17.40 ± 0.09* (172.89)
Total phenols	4.68 ± 0.08	5.71 ± 0.09	4.42 ± 0.08	6.07 ± 0.08* (129.81)	7.64 ± 0.06* (133.76)	6.93 ± 0.08* (156.58)
Total soluble proteins	129.93 ± 1.05	126.28 ± 1.07	131.94 ± 1.16	184.07 ± 1.65* (141.67)	199.21 ± 2.32* (157.75)	228.24 ± 2.83** (172.99)
Free proline	11.22 ± 0.08	12.69 ± 0.09	9.42 ± 0.08	15.37 ± 0.09* (136.99)	18.97 ± 0.09* (149.43)	16.04 ± 0.09* (170.26)
Total nitrogen	20.58 ± 0.12	20.25 ± 0.17	22.85 ± 0.22	29.40 ± 0.29* (140.96)	31.52 ± 0.25* (155.61)	36.72 ± 0.19** (160.66)
Nitrate	1.22 ± 0.07	1.27 ± 0.03	1.30 ± 0.04	1.56 ± 0.03* (127.64)	1.70 ± 0.04* (133.14)	1.83 ± 0.04* (140.51)

Data are the mean values of three different experiments ± standard error.
Percentage over control is given in parenthesis, * = P < 0.05, ** = P < 0.01

for biodegradation of tannery wastes. Plants such as *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* were employed. During the entire period of study, there was no application of any kind of fertilizer either to the tannery sludge-grown / garden soil-grown plants. As observed in earlier studies (Hemphill et al., 1985; Mazur and Koc, 1976 and Wickliff et al., 1981), the application of tannery sludge was found to promote all morphometric parameters, namely shoot length, root length, above and underground biomass, dry weight, moisture content (Data not shown). Since the plants raised over the sludge were always found to be dark green compared to the control, the levels of leaf biomolecules were determined in 6 months old tree saplings. The following leaf biomolecules such as chlorophyll a, chlorophyll b, total chlorophyll, total soluble proteins, free amino acids, total phenols, total soluble sugars, sucrose, total soluble starch, free proline, total nitrate and total nitrogen were quantified using standard methods. As reported in Table 2, in 6 months old tree saplings chlorophyll content as well as the chlorophyll a/b ratio increased by 2-fold over the control. Similar increase in chlorophyll content was also reported in some other context when plants were raised over the distillery sludge (Sureshbabu and Vivekanandan, 1997). In various amendments of tannery waste, Singh *et al.* (2004) observed an increase in the chlorophyll content of *Lycopersicum esculantum* when the plants were grown in tannery sludge-amended soil. In consonance with the increased chlorophyll contents, total soluble proteins, free amino acids, total phenols, total soluble sugars, sucrose, total soluble starch and free proline also increased significantly in the treated plants. A similar observation of increased leaf biomolecules was also observed in many of the legumes and monocotyledonous crops when plants were raised over the distillery sludge/biocompost (Sureshbabu and Vivekanandan, 1997). Singh and Sinha (2004) observed increased protein content in *Helianthus annuus* L. when grown over the tannery sludge. The increased content of leaf biomolecules as well as crop yield was reported by various workers by application of paper mill sludge and sewage sludge (Phillips *et al.* 1997; Wong and Su, 1997). The total N content of plants also increased. In view of increased protein content in the leaves of the treated plants, the total soluble leaf proteins were isolated from *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* and the electrophoretic profile was prepared using 5 to 20 % gradient SDS PAGE (Plate 1), and the protein profile was further characterized

Fig. 1. Light dependent CO₂ uptake of the 6 months old tree saplings grown over the garden soil and tannery sludge. Data are the mean values of three different experiments. ± Standard error.

using gel documentation. There was a significant amplification of a few proteins more particularly in the region between 100 and 105 KDa in all the treated plants except *Melia azedarach*. Another significant increase

Plate 1. Electrophoretic protein profile of 6 months old garden soil- and tannery sludge-grown *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* with their respective control.

Fig. 2. Chromium assimilation in leaves of 6 months old tree saplings grown over tannery sludge. Data are the mean values of three different experiments. $\pm$ Standard error.

was observed in the region between 49 and 55 KDa. In other stresses such as dehydration, salinity and heavy metals (metalloids), new polypeptides have been reported to be synthesized, as well as amplification of certain other polypeptides (Barathi *et al.* 2001; Chaitanya *et al.* 2001; Sundar *et al.* 2003).

The light-dependant CO_2 absorption as measured by IRGA in 6 months old tree saplings is presented in Figure 1, which clearly illustrates increased photosynthetic CO_2 uptake in *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* by 54 %, 63 % and 70 % respectively as compared to the control.

Uptake of chromium by the three tree species was investigated. A significant uptake of Cr was detected in all the tree species (Figure 2). Assimilation of chromium in the leaves of *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* was 2.39, 3.45 and 3.43 mg/g dry weight respectively. Suggesting that uptake, assimilation and translocation of Cr occurred in the three plant species and that they could be implemented in phytoremediation of chromium.

The present work portends that sturdy and stress enduring fast growing tree species such as *Melia azedarach*, *Azadirachta indica* and *Leucaena leucocephala* can be employed for reclamation of tannery polluted soil through a simple, economically viable and pollution free technology called Phytoremediation or Green Technology. It may also be inferred from the study that any agricultural land polluted

by tannery effluent / sludge can be reclaimed through phytoremediation making the land suitable for cultivation in the course of time.

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