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Research Article

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Phytoremediation of Tannery Waste Polluted Soil using *Hyptis suaveolens* (Lamiaceae)

P. Sivakumar*, M. Kanagappan and S. Sam Manohar Das
Department of Zoology, Scott Christian College (Autonomous), Nagercoil, Tamilnadu, India
*Corresponding Author E-mail: capeparam@rediffmail.com

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ABSTRACT

Phytoremediation is a new technology in which plants are used to remove pollutants from water and soil. Interaction between plants and heavy metals is based on the ability of plants to absorb and accumulate metals from soil. FTIR spectra also confirmed the interaction of chromium ions with the hydroxyl, carboxyl and amino groups that are present on the plant biomass used for phytoremediation of tannery effluent. The high removal efficiency and heavy chromium accumulation capacity of Hyptis suaveolens makes an excellent choice for phytoremediation of tannery effluent. The Atomic Absorption Spectrophotometric (AAS) analysis of tannery wastewater revealed that the samples were heavily contaminated with heavy metals, mainly with chromium. The roots of Hyptis suaveolens accumulated more heavy metals than the stem and leaves. The experiment results that the Hyptis suaveolens can be used for removal of heavy metal from tannery contaminated soil.

Key words: *Hyptis suaveolens, Tannery Effluent, FTIR, Phytoremediation*

INTRODUCTION

The indiscriminate use of untreated wastewater will be considered as one of the most significant sources of environmental pollution that may directly affect the human health via crops and soil[29,3]. The presence of heavy metals in tannery effluent affects soil fertility and depletes the nutrients of the soil[1].

Phytoremediation is an eco-friendly technology that uses plants to degrade, transform, immobilize or stabilize various organic and inorganic pollutants present in soils, muds or wastewaters[8]. Many industrial wastewater contains heavy metals like cadmium, lead, zinc, cobalt and chromium. Among heavy metals chromium plays a major role in polluting our water environment[4].

Proper selection of plant species for phytoremediation plays an important role in the development of remediation methods[21].Phytoremediation takes advantage of the unique, selective and naturally occurring uptake capabilities of plant root systems, together with the translocation, bioaccumulation and pollutant storage or degradation abilities of the entire plant body. Soil contamination has become a serious problem in all industrialized areas of the country. Soil is equally regarded as the ultimate sink for the pollutants discharged into the environment[24].

The tannery industry which generates huge amount of effluent and sludge with very high concentrations of Cr causes soil and water pollution and creates large number of wastelands in the form of sludge[23].

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Several aquatic species have been identified and tested for phytoremediation of heavy metals from the polluted water[12]. The roots of Indian mustard are found to be effective in the removal of cadmium, chromium, lead and zinc[30].

Chromium accumulates mainly in roots and shoots; however roots accumulate the major part, only a small part translocated to the shoots[9,26] . In pea plants exposed to Cr there was an increase in concentration of Cr in different parts of the plant with the increase of Cr supply. Accumulation of Cr in different parts of the plant was in the following order roots>stem >leaves >seed[27] and the bean seeds accumulated about 0.1% Cr, while roots accumulated 98%[5].

The effect of tannery effluent on leaf area and biomass reported that all the concentrations tested showed decreased leaf area[6]. Cr applied at 60 mg kg^{-1} and higher levels reduced the leaf size, caused burning of leaf tips or margin and slowed leaf growth rate[25]. Cr had a pronounced effect on leaf growth and preferentially affected young leaves in tomato plants[10]. Reduction in leaf biomass was correlated with the oxalate acid extractable Cr in *P. vulgaris*[11].

Soil pollution by toxic metals is a serious problem for the environment and also one of the environmental stresses for higher plants. Metal toxicity is related to bioavailability rather than the total concentration of metal in the soil and may reduce both quality and productivity of plants[7]. Use of plants that have constitutive and adaptive mechanisms for tolerating or accumulating high metal contents in their rhizosphere and tissues, is the emerging *in situ* remediation technology employed in the cleanup of soils, sediments and water that have been polluted by organics, salinity and heavy metals[13,16,17].

In this study, the American mint (*Hyptis suaveolens* L.) was grown in soil artificially contaminated with tannery effluent for a period of 90 days and the accumulation of the metal in the plant was analysed after 3 months.

MATERIALS AND METHODS

The plant selected for the remediation of tannery effluent is *Hyptis suaveolens* L. The raw tannery effluent was collected from a leather processing industry at Ambur, Vellore district, Tamil Nadu and stored at 4°C for further analysis. The effluent was diluted to 25% concentration for use in pot study. The garden soil samples were collected from nearby places. Seeds were germinated in experimental pots and watered. *Hyptis suaveolens* seedlings were grown in pots filled with garden soil and vermicompost in the ratio of 3:1. The seedlings were collected from uncontaminated soils. The experiment was conducted for 90 days. Then the plants were harvested from pots.The metal uptake was estimated once in every 30 days up to 90 days (3 months).

The sample plants were removed from the pots and washed under tap water and then with distilled water. The collected plants were air dried, then placed in a dehydrator for 2-3 days and then oven dried for four hours at 100ºC. The dried samples of the plant were powdered and stored in polyethylene bags. The powdered samples were subjected to acid digestion. 1gm of the powdered plant materials were weighed in separate digestion flasks and digested with HNO_3 and HCl in the ratio of 3:1. The digestion on hot plate at 110ºC for 3-4 hours were continued till a clean solution was obtained. After filtering the filtrate was analyzed for the metal contents in AAS.

Fourier transform infrared spectroscopy (FTIR) is a powerful technique for studying molecular structures. FTIR is powerful tool for identifying types of chemical bonds in a molecules by producing an infrared absorption spectrum that is like a molecular “finger print”. FTIR is perhaps the most powerful tool for identifying types of chemical bonds (Functional groups) the wave length of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum.

The plant samples were oven dried at 60°C and ground into fine powder through a gate mortar. Two milligrams of the sample were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The pellet was immediately put into the sample holder and FT-IR spectra were recorded in the range 4000–450 cm^{-1} for all samples, the stem, leaves and root powder samples were collected and analyzed for certain biochemical parameters.

The present study is concerned with phytoremediation of *Hyptis suaveolens.* The chemical changes in the functional group of tannery effluent treated chromium were monitored using with a Bruker 55 model FT-IR spectrometer.

RESULTS

The parts of the plant were analyzed to estimate the accumulation of chromium by 30th, 60th and 90th days. *Hyptis suaveolens*s howed a tendency of high absorption of chromium by the root system. The roots have accumulated 41.79 mg/kg (Table.1) of chromium during the experimental period and the accumulation in leaves and stem was low and consistent from 30th day up to 90th day. However, chromium was actively absorbed by the stem and leaves during the first 30 days of the experimental period. The chromium was not translocated above ground plant parts largely and remained in the roots (Fig. 1).

The present study roots of *Hyptis suaveolens* treated with raw tannery effluent were shorter and brownish and presented less number of root hairs (Fig. 2). Thereduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, effluent transport to the aerial part of the plant has direct impact on cellular metabolism of stems contributing to the reduction in plant height (Fig.3). The IR spectrum of plant samples collected are shown in Figures 4A to 4F. The absorption bands and their tentative assignments are given for both the treated and the control sample.

Leaf Control

The present investigation on leaves of *Hyptis suaveolens* plant of FTIR analysis denoted the presence of fatty acids, i.e., a peak of 2920.23 cm^{-1} revealed aliphatic –CH_3 and CH_2 (Chlorophyll) stretching of fatty acids which is followed by the presence of stretching of lipid esters with the peak of 1732 cm^{-1}. Another important peak revealed 1637.56 cm^{-1} indicate the amide I of β pleated sheet structures of proteins. Simultaneously, a peak of 1246.02 cm^{-1} revealed presence of onward stretching of phosphodiesters in phospholipids. The absorption band spectra of 1311.59 cm^{-1} indicated amide III band components of proteins. In control leaf the presence of carbohydrates was indicated due to the peak of 1099.43 cm^{-1} (Fig. 4A).

Treated Leaf

The phytochemical changes pertaining to leaves treated with tannery effluent exhibited different peaks. Peak dimension of 2920.23 cm^{-1} revealed aliphatic CH_3 and CH_2 stretching which represented the chlorophyll is being stretched due to the toxicity of chromium. The absorption band of 1732.08 cm^{-1} indicated C=O vibrations. Leaf treated with effluents revealed a peak of 1668.43cm^{-1} exhibit amide I band components of proteins which are due to remediation of leaf compound when compared to leaf control. Simultaneously, FTIR analysis on treated leaf samples showed a peak of 1541.12 – 1523.76 cm^{-1} indicate amide II band components of proteins. A promising result of leaf treated denoted deformations of CH_2 or CH_3 groups in aliphatic indicate the lignin components present in the leaf(Fig. 4B).

Similarly, a peak of 1097.5 cm^{-1} indicate C-H deformation which results due to stretching of carbohydrates due to phytoremediation. FTIR analysis of leaf treated sample indicated a peak of 1035.77 cm^{-1} revealed asymmetric stretching of polysaccharides (Starch and silicate impurities).

Stem Control

In control treated bark of the plant revealed a peak of 2916.37cm^{-1}– stretching vibration of CH_3 and CH_2 groups indicated the presence of chlorophyll. Another peak of 2856.58 cm^{-1}–C-H symmetric stretching of >CH_2 in fatty acids whereas the other peak represent 1637.86 cm^{-1}– ketonic C=O stretching followed by prominent peaks of 1631.78 cm^{-1} –ketonic C=O stretching, 1417.68 cm^{-1} -C-O-H in plane bending in Carbohydrates, DNA/ RNA backbone, proteins, 1109.07 cm^{-1} -C-O, or C-C stretching pertaining to carbohydrates, 1028.53 cm^{-1} -C-O stretching of polysaccharides, 605.65 cm^{-1} represents C-O-O and P-O-C bonding of aromatic compounds (Phosphates). The above results indicated the presence of aromatic compound in the plant sample(Fig. 4C).

Stem Treated

The absorption band spectra of stem treated with tannery effluent revealed a prominent peak of 2920.23 cm^{-1}, aliphatic stretching vibration of CH_3 and CH_2 groups indicated the presence of chlorophyll. Similarly, another peak of 1641.42 cm^{-1}, this band is due to the stretching vibration of carbonyl groups characteristic of secondary amides and other compounds containing C=O group. It is presumed that the absorption peak of 1247.94 cm^{-1} indicate the amide III band components of proteins whereas analysis revealed another peak 1107.13 cm^{-1}asymmetric C-O, C-C dominated by ring vibrations in various polysaccharides. The prominent peak 1041.56 cm^{-1} was confirmed as C-O stretching pertaining to carbohydrates. Whereas a peak of 605.65 cm^{-1} represented C-O-O and P-O-C bonding of aromatic compounds (Phosphates) (Fig. 4D).

Root Control

The study pertaining to analysis of plant roots of *Hyptis suaveolens* denote a peak of 3444.87 cm^{-1} indicate N-H stretching vibrations characterized by presence of amino acids which is followed by another important peak of 3336.85 cm^{-1} indicates N-H stretching of amides. The absorption band spectra revealed a peak of 1730.15 cm^{-1} represent the presence of esters. A peak of 1645 cm^{-1} corresponds to N-H bending of amines. The tufted roots of the plant sample denoted another peak of 1381.03 cm^{-1} indicated the stretching of aromatic amine groups (Fig. 4E).

Root Treated

The present study of phytoremediation represents the following peaks of root treated with tannery effluents indicate the presence of 3441.01 cm^{-1} denoting NH_2 stretching which is followed by similar peak of 3325.28 cm^{-1} -N-H stretching. The absorption bands of spectra represent a peak of 2922.16 cm^{-1} indicate stretching vibration of $>CH_2$ in fatty acids. FTIR analysis of root treated with tannery effluent exhibit another prominent peak of 1637.56 cm^{-1} – bands are due to the stretching vibration of carbonyl group characterized of secondary amides and other compounds containing C=O groups. Another peak revealed 1103.28 cm^{-1} indicates the presence of C-O-C (carbohydrates) groups. Finally the root treated sample denoted another peak of 1030.77 cm^{-1} indicated the presence of starch (Fig. 4F).

The results revealed that heavy metals concentrations in *Hyptis suaveolens* depend significantly on the kind of plant organs. Underground organs showed greater capacity of accumulation as compared to the stem and leaves.

Fig. 1: Accumulation of chromium in *Hyptis suaveolens* during the experimental period

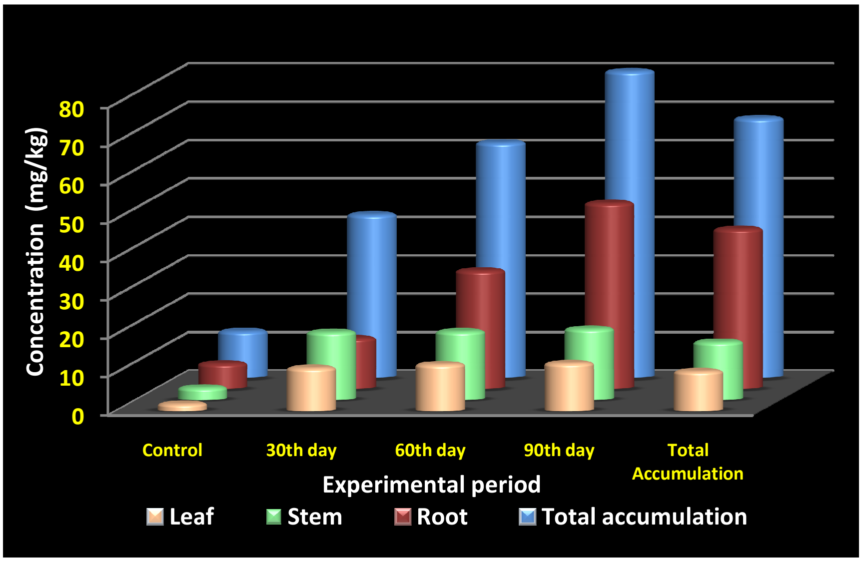


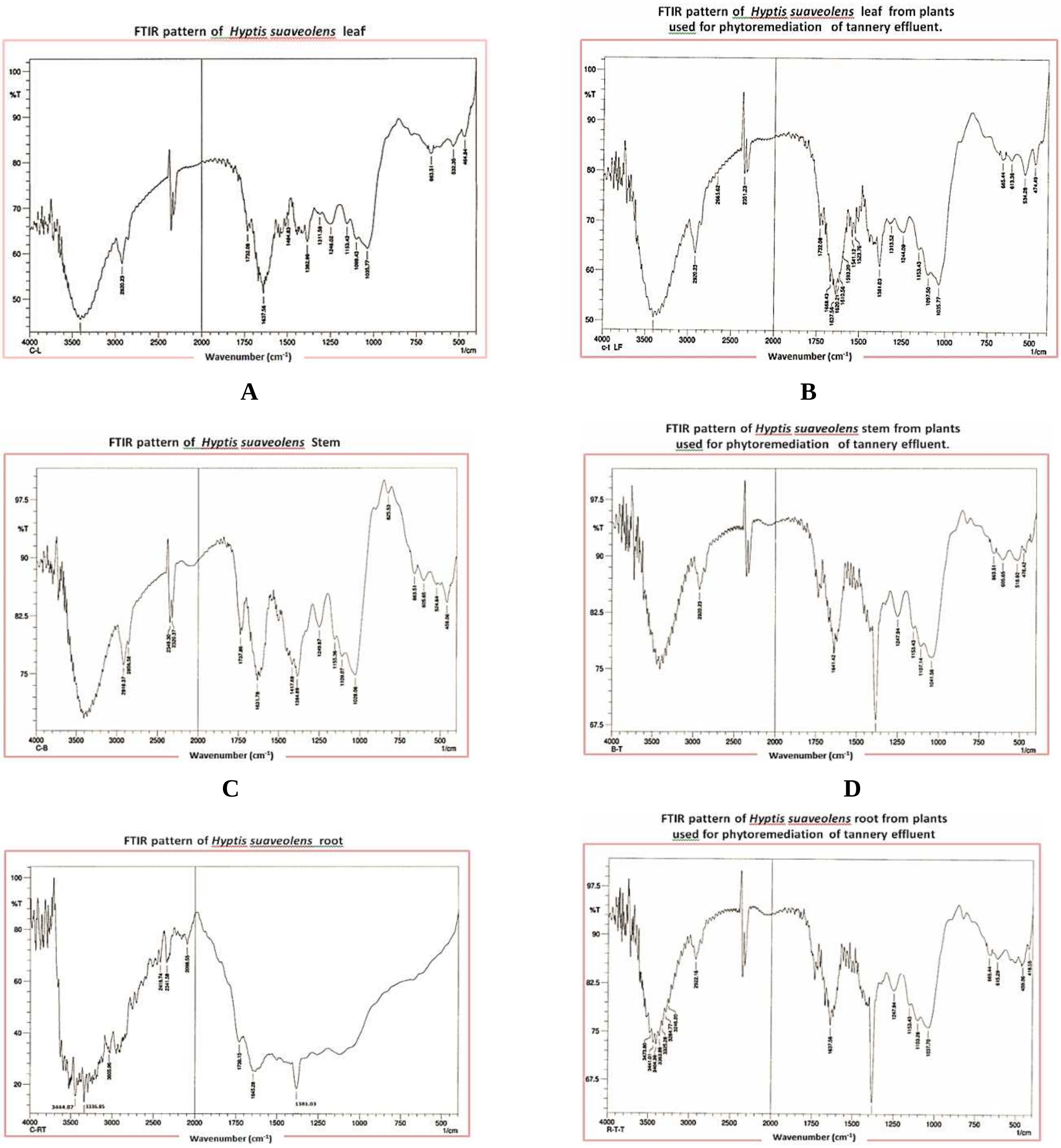
Fig. 2: *Hyptis suaveolens* Roots

Control **90 – days Treated**

Fig. 3: Effect of tannery effluent on the growth of *Hyptis suaveolens* after different days of esxposure

30 – days **90 – days** **90 – days Control**

Fig. 4: FT-IR spectrum of plant samples



A B

C D

E F

Table 1. Accumulation of Chromium (mg/kg biomass) in differentplant parts Of *Hyptis suaveolens* during the experimental period

Plant part	Control	30th day	60th day	90th day	Total Accumulation
Leaf	2.10±0.02	11.45±0.07	12.38±0.03	12.68±0.03	10.58
Stem	3.40±0.05	17.87±0.04	18.04±0.05	18.72±0.08	15.32
Root	6.75±0.03	13.32±0.08	30.95±0.02	48.54±0.03	41.79
Total accumulation	12.25	42.64	61.37	79.94	67.69

DISCUSSION

Phytoremediation is a developing technology which uses plants and their associated microbes for the remediation of soil contamination. This process is cost effective without creating disturbance to the soil. Plant based environmental remediation has been widely pursued by academic and industrial scientists as a favourable low–impact uncontaminated technology applicable in both developed and developing nations[14].

Results indicated the reduction in the measured growth parameters. Sensitive Mungbean cultivars also showed decreased root growth when exposed to Cr(VI)[19]. Metals would have a toxic effect on the normal metabolism of the plant. Plant growing on soils with higher metal value, exhibit signs of unhealthy growth[20]. The morphology of the plants differs to some extent from the plants growing on normal soil. Leaves of these plants are greenish yellow in color, less glossy, more fragile and have crenulations of higher amplitudes along the leaf margins compared to the leaves of the similar species growing on soils without any metal enrichment. Leaf number per plant reduced by 50% in wheat when 0.5mm chromium was added in nutrient solution[22]. The roots of plants that grow on high metals content soil are smaller dimensions with relatively thinner barks and in lesser quantities than from stems of those plants that growing on normal soils [2].

The reduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. Chromium transport to the aerial part of the plant can have a direct impact on cellular metabolism of stems contributing to the reduction in plant height.

In this study, the fact that *Hyptis suaveolens* L. roots showed high accumulation of elements could imply relatively high availability in the soil. Plants can tolerate high heavy metals concentration from soil by two strategies. The first strategy is called accumulation strategy where metal can accumulate in plants at both high and low concentration from soil. These plants are capable of rendering the metals in various ways, for instance by binding them to cell walls, compartmentalizing them in vacuoles or complexing them to certain organic acids or proteins[18]. The second strategy is called exclusion strategy, where transport of heavy metals in stems and leaves is limited over a wide range of metal concentrations in the soil. Some of the plants make stable metal complexes in the root cells to prevent metal translocation from the roots to above ground tissues [28].

The *Hyptis suaveolens* L. has a kind of special ability to accumulate higher amounts of chromium and it may be under stress condition[15]. Among several remediation methods, phytoremediation is a promising one. It is a plant-based technology for contaminated soil cleanup for both organic and inorganic pollutants.

This study shows that *Hyptis suaveolens* has a good potential to takeup and accumulate the toxic heavy metals from tannery polluted soil. It also pave a way for the development of an economically viable technology, involving phytoremediation by plants.

CONCLUSION

From the above studies it is concluded that *Hyptis suaveolens* plant can be used for effective phytoremediation of chromium, the major metal constituent of tannery effluent. Phytoremediation is cost-effective, efficient and easy to use processes, with potential for future applications.

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