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Phytoremediation of textile effluent contaminated soil using neem leaf extracts

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ABSTRACT

Phytoremediation is an alternative low cost approach for treatment of heavy metal polluted soil. This study demonstrates the phytoremediation potential of neem leaf extracts on textile effluent contaminated soil. The textile effluent contaminated soil was treated with three different concentrations (0.25mg/ml,0.5mg/ml and 0.75mg/ml) of neem leaf extracts and overnight soaked *Vigna radiata* seeds were germinated in treated soils. Germination percentage was recorded and soil and plant analysis were carried out after radical emergence. pH of leaf extracts treated soils were decreased and catalase activity and microbial biomass carbon levels in treated soil, germination percentage, root and shoot length of *Vigna radiata* plants grown in treated soils were significantly ($p<0.05$) increased. Growth of *Vigna radiata* plants grown in untreated soil was impaired. Total protein level, chlorophyll contents, peroxidase activity, superoxide dismutase activity, and ascorbate level in roots and shoots of *Vigna radiata* plants grown in treated soil mainly 0.5mg/ml and 0.25mg/ml treated soil only slightly decreased and lipid peroxidation levels were slightly increased compared to roots and shoots of *Vigna radiata* plants grown in normal soil. These results imply the positive effect of neem leaf extract on textile effluent contaminated soil.

KEYWORDS

Phytoremediation;
Microbial biomass carbon;
Environmental pollution;
Textile effluent;
Mung bean.

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of modern world[1]. The textile dyeing industries in Tirupur uses bleaching liquids, acids, dyes and chemicals in dyeing and bleaching processes. All this leads to the effluents and having high salinity-sodility content in the river Noyyal where it flows. It is threat to crop yield when used for irrigation. Organo chlorides used are a known potent carcinogen which degrades agricultural lands[2]. The untreated textile effluents that are released and disposed in open land and into aquifer are high in conductivity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and also contain high concentration of heavy metals such as chromium, cadmium, lead, selenium etc[3]. Excessive metal concentration in contaminated soil can result in decreased soil microbial activity, soil fertility, and yield losses[4]. Heavy metal pollution of soil enhances plant uptake causing accu-

mulation in plant tissues and eventual phytotoxicity and change of plant community[5]. Plants have developed complex antioxidative defense systems to alleviate the damage caused by reactive oxygen species and the degree of damage depends on the balance between the formation of reactive oxygen species and its removal by the antioxidative scavenging systems that defend against them[6,7].

The phytoremediation of metal-contaminated soils offers a low cost method for soil remediation and some extracted metals may be recycled for value[8]. High-biomass crops can be considered as an alternative to hyper accumulator plants to phytoremediate soils contaminated by heavy metals[9]. Neem (*Azadirachta indica A. Juss*) is perhaps the most useful traditional medicinal plant in India[10]. Neem leaf extract and oil-seed cakes can reduce the plant damage produced by root-knot nematodes even in the soils polluted with heavy metals[11].

In the present study it is aimed to determine the potential of neem leaf extracts to eliminate the heavy metal toxicity in textile effluent contaminated soil by using certain biological methods.

EXPERIMENTAL

Soil collection and processing

The textile effluent contaminated soil was collected from the area nearer to textile dyeing factory in Tirupur, Tamil Nadu, India. The normal soil which is not contaminated with heavy metal was collected from the agricultural land, Pollachi and it is constituted as control. After collection, these soil samples were brought to the laboratory and hand picked to remove discrete plant residues and large soil animals (earth worms, etc). Soil samples were air dried at room temperature and sieved to remove debris and this soil was filled in four polythene bags. These soils were treated with water and three different concentrations of fresh neem leaf extracts (0.25 mg/ml, 0.5 mg/ml and 0.75 mg/ml) which were collected from Karpagam University campus, Coimbatore, Tamil Nadu, India.

Seed collection and processing

The seeds of Mung bean (*Vigna radiata*) were collected from the local market, Coimbatore. Healthy seeds of Mung bean (*Vigna radiata*) were selected for uniformity and were surface sterilized with 0.1 % mercuric chloride and then washed with distilled water. After washing thoroughly, these seeds were soaked in water and three different concentrations of fresh neem leaf extracts (0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml) for over night.

After soaking, the seeds were transferred to soil filled polythene bags. During the vegetation period, the plants were irrigated with corresponding solutions. When the plants reached the phase of stem extension, they were used for biological analysis.

Biological analysis

Soil analysis

The soil pH was determined in a 1:2.5 soil to water ratio using the glass electrode pH meter[12]. The catalase activity was measured by the titrimetric method of Euler and Josephson[13]. Soil microbial biomass carbon was estimated by extracting 30 gm of oven dried soil samples in 0.5 M K_2SO_4 (1:4 W/V), known as the chloroform-fumigation-extraction method, described by Vance, Brookes, and Jenkinson[14]. Microbial biomass carbon was calculated by measuring the difference in extractable organic carbon between the fumigated and unfumigated soils, which are simply formulated as equation 1,

Biomass carbon = 2.64 × EC

Where EC refers to the difference in extractable organic carbon between the fumigated and unfumigated treatments; 2.64 is the proportionality factor for biomass carbon released by fumigation extraction. The catalase activities of the test and control samples were measured at intervals by the method of Euler and Josephson[13].

Plant analysis

The percentage of germination was considered after radical emergence. Morphological parameters including shoot and root length of the plants were measured with the help of meter scale. Chlorophyll a, b and total chlorophyll were calculated by the method of Witham, Blaydes, and Devlin[15]. Total protein was estimated by Lowry's method[16]. Lipid peroxidation was estimated by thiobarbituric acid assay method[17]. The enzymatic antioxidant peroxidase activity was ascer-

tained by the method of Addy and Goodman[18], the superoxide dismutase activity was ascertained by the method of Misra and Fridovich[19] and the non-enzymatic antioxidant ascorbic acid was estimated by the method of Omaye, Turabull, and Sauberlich[20].

Statistical analysis

Statistical analysis was carried out using SPSS 11.0 package program. The effect of neem leaf extracts on textile effluent contaminated soil was determined by student 't' test. A 95% confidence limit ($p<0.05$) was chosen to indicate the significance between the control soil and leaf extracts treated and untreated soils.

RESULTS AND DISCUSSION

Soil analysis

The change in pH of control and effluent contaminated soils treated with three different concentrations of neem leaf extracts (0.25, 0.5 and 0.75mg/ml) treated soil is shown in Figure 1.

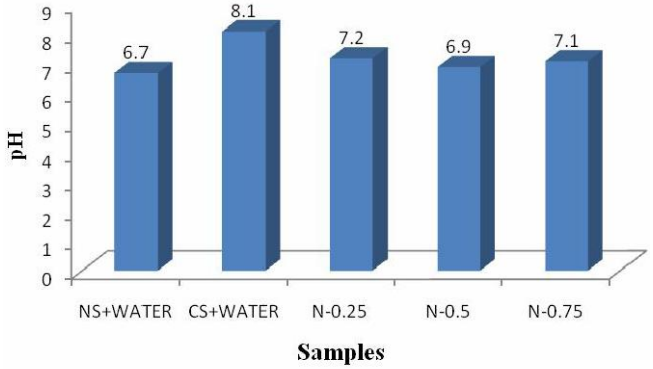

Figure 1 : Effect of three different concentrations of neem leaf extracts (0.25, 0.5 and 0.75 mg/ml) on the pH of textile effluent contaminated soil. NS- normal soil; CS-contaminated soil; N- neem.

The pH of control soil was 6.7, which were slightly acidic, and the untreated contaminated soil was 8.1, which were moderately alkaline. The irrigation water analysis showed that the pH of textile waste water is 8.01 to 8.09 which are slightly alkaline in nature[21]. In this study, the neem leaf extracts mainly N-0.5 greatly reduced the pH of effluent contaminated soil and this pH values were nearer to pH value of normal control soil.

C_{mic} had been suggested as possible indicator of soil environmental quality in toxicity assays[22] and enzymatic activities can sensitively reflect the biological situation in the soil[23]. Microbial biomass is a sensitive parameter and can be used as an indicator of changes in organic matter composition earlier than it could be registered in another way. TABLE 1 shows the C_{mic} level and catalase activity in control and leaf extracts treated soils.

TABLE 1 : Effect of neem leaf extracts on catalase activity and biomass C in textile effluent contaminated soil

Samples	Catalase activity (Mm of H_2O_2 $min^{-1}g^{-1}$)	Biomass C ($\mu g\ g^{-1}$)
Control	0.43 ± 0.036	110.9 ± 0.45
CS + Water	0.03 ± 0.01	7.2 ± 0.2
CS + N- 0.25	0.25 ± 0.09[a]*	72.9 ± 0.45 [a]*
CS + N- 0.5	0.36 ± 0.013[a]*	76.0 ± 0.5 [a]*
CS + N- 0.75	0.23 ± 0.02[a]*	38.0 ± 0.5

CS-contaminated soil; N-neem; 0.25, 0.5 and 0.75-concentration of neem leaf extracts(mg/ml); Values represent mean ± S.D (n = 5);[a] – Comparison between control and different concentrations of neem leaf extracts; * - indicates significant at p< 0.05 level

N-0.25 and N-0.5 treatment highly increased the C_{mic} level and in N-0.75 treatment moderately increased the C_{mic} level in textile effluent contaminated soils when compared to untreated soil. In contaminated soils, microorganisms need more energy to survive in unfavorable conditions. Similar result was reported in that inhibition of carbon biomass by heavy metals is even higher than 80%[24]. Effluent contamination decreased the catalase activity in soil and different concentrations of neem leaf extracts treatment increased its activity at different level. N-0.5 treatment greatly increased and it was nearer to normal soil. N-0.25 and N-0.75 treatment moderately increased the catalase activity.

Crop growth is affected when the concentration of heavy metals were increased[25]. It is well known that heavy metals cause several toxic effects on plants such as inhibition of seed germination[26] and metabolic disturbances by altering the essential biochemical reactions[27]. The germination percentage of *Vigna radiata* grown in control and treated soil is shown in Figure 2.

The normal soil showed 100% germination and in untreated soil, the germination percentage was only 30. Among the leaf extracts treated soil, maximum germination percentage (95%) was recorded in N-0.25 treated soil. Untreated soil contains high heavy metal concentration which affects the seed germination. The

growth of the germinated seeds was impaired in untreated soil and it showed clear phytotoxic symptoms. The neem leaf extracts treatment reduced the heavy metal toxicity in effluent contaminated soil.

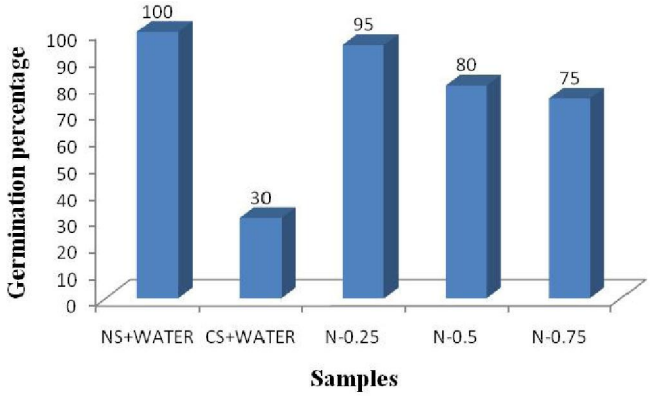

Figure 2 : Effect of three different concentrations of neem leaf extracts (0.25, 0.5 and 0.75 mg/ml) on germination of *Vigna radiata* seeds in textile effluent contaminated soil. NS-normal soil; CS-contaminated soil; N- neem.

The root and shoot length and chlorophyll contents of *Vigna radiata* grown in control and treated soil is shown in TABLE 2.

Heavy metal toxicity depresses the seedling growth and neem leaf extracts improved the seedling growth in terms of shoot and root length. The shoot and root length of *Vigna radiata* plants grown in normal soil was 14.14 cm and 3.74 cm respectively. N-0.5 caused a considerable increase in root and shoot length even under stress. The root and shoot length was also improved in N-0.25 and N-0.75 treated soil when compared to shoot and root length of *Vigna radiata* seeds germinated on untreated soil which was impaired due to the high heavy metal concentration. This result is in accordance with the result of[28] in which growth and biomass of *Cicer arietinum* were decreased by distillery effluent. Impaired chlorophyll development due to heavy metals may be the result of interference with protein. The heavy metal treatments presumably blocked the synthesis and activity of enzyme proteins responsible for chlorophyll biosynthesis[29]. In this study, *Vigna radiata* plants grown in N-0.5 treated soil was showed high levels of chlorophyll a, b and total chlorophyll contents and it was nearer to *Vigna radiata* plants grown in normal soil. These levels were slightly reduced in leaves of *Vigna radiata* plants grown in N-0.25 and N-0.75 treated soil. The results of the present study are also in accordance with the earlier reports of[30] in which the effluent receiving plants showed retarded photosynthetic pigment activity.

TABLE 2 : Effect of neem leaf extracts on root and shoot length and chlorophyll contents in *Vigna radiata* plants grown in textile effluent contaminated soil

Samples	Shoot length (cm)	Root length (cm)	Chlorophyll a ($\mu g\ g^{-1}$)	Chlorophyll b ($\mu g\ g^{-1}$)	Total Chlorophyll ($\mu g\ g^{-1}$)
Control	14.14 ± 0.02	3.74 ± 0.05	4.84 ± 0.05	1.08 ± 0.031	5.92 ± 0.28
CS + N- 0.25	3.0 ± 0.36	2.33 ±0.04 [a]*	1.46 ± 0.03	0.59 ± 0.04 [a]*	2.0 ± 0.07
CS + N- 0.5	11.6 ±0.45 [a]*	5.1 ± 0.26 [a]*	3.46 ± 0.08 [a]*	0.67 ± 0.042 [a]*	4.13 ± 0.04 [a]*
CS + N- 0.75	3.8 ± 0.21	2.8 ± 0.36 [a]*	1.18 ± 0.053	0.38 ± 0.04	1.56 ± 0.035

CS-contaminated soil; N-neem; 0.25, 0.5 and 0.75-concentration of neem leaf extracts(mg/ml); Values represent mean ± S.D (n = 5); [a] – Comparison between control and different concentrations of neem leaf extracts;* - indicates significant at p< 0.05 level

The protein and TBARS level in roots and shoots of *Vigna radiata* plants grown in control and treated soil is shown in TABLE 3.

Nitrogen is a precursor for the synthesis of amino acids[31]. Since the nitrogen content of the metal treated plants was reduced, ultimately amino acids and protein contents of the plants were also reduced because there was only limited availability of nitrogen for the synthesis of amino acids[32]. In this study, the shoots and roots of *Vigna radiata* plants grown in N-0.5 treated soils were showed higher total protein content compared to roots and shoots of *Vigna radiata* plants N-0.25 and N-0.75 treated soil. This shows that N-0.5 greatly reduces heavy metal toxicity mainly cadmium toxicity in textile effluent contaminated soil. Roots of *Vigna radiata* plants were showed reduced total protein content compared to shoots because roots are easily affected by heavy metals than the shoots. Similar report was reported by[33] who reported that protein content decreased from 71.4-19.0% in metal exposed plants at metal concentrations equivalent to those found in polluted soil. As an indicator of lipid peroxidation, the

content of the thiobarbituric acid reacting substances (TBARS) was measured. The peroxidation of cell membranes severely affect its functionality and integrity and can produce irreversible damage to cell function and can be initiated by ROS species such as $O_2^{\cdot -}$, $OH^{\cdot -}$, H_2O_2 or by the action of lipooxygenase[34]. In this study, the level of TBARS in shoots and roots of *Vigna radiata* plants grown in normal soil was 20nM g^{-1} fwt and 35nM g^{-1} fwt respectively. This level was greatly increased in shoots of *Vigna radiata* plants grown in N-0.75 treated soils, and slightly increased in shoots of *Vigna radiata* plants grown in N-0.25 and N-0.5 treated soils. In roots, the TBARS content is more when compared to shoots. TBARS level was very high in roots of *Vigna radiata* plants grown in N-0.75 treated soil. Roots of *Vigna radiata* plants grown in N-0.25 and N-0.5 treated soils showed slightly increased level of TBARS when compared to roots of *Vigna radiata* plants grown in normal soil. A high level of lipid peroxidation was reported in the case of higher plants under Cr and other heavy metal toxicity[35].

TABLE 3 : Effect of neem leaf extracts on total protein and TBARS levels in *Vigna radiata* plants grown in textile effluent contaminated soil

Samples	Total protein (mg g^{-1})		TBARS level (nM of MDA g^{-1})	
	Shoot	Root	Shoot	Root
Control	165.3 ± 0.49	113.8 ± 0.55	20.0± 0.96	35.0 ± 1.0
CS + N-0.25	103.25 ± 0.58[a]*	92.6 ±1.0 [a]*	32.0±1.29[a]*	56.6 ±1.2[a]*
CS + N-0.5	115 ± 2.5 [a]*	103.2±6.0[a]*	25.4±1.26[a]*	53.0±1.83[a]*
CS + N-0.75	107.2 ± 0.57 [a]*	97.6 ± 1.0[a]*	46.0 ± 0.96	60.6 ± 2.83

CS-contaminated soil; N-neem; 0.25, 0.5 and 0.75-concentration of neem leaf extracts(mg/ml);TBARS- Thiobarbituric acid reactive substances;Values represent mean ± S.D (n = 5); [a] – Comparison between control and different concentrations of neem leaf extracts;*- indicates significant at p< 0.05 level

Antioxidative enzymes are considered to be an important defense system of plants against oxidative stress caused by metals[36]. Peroxidase is thought to be a stress marker enzyme and its higher induction may indicate stress exerted by heavy metal, which can be correlated with amount of the accumulated metal. Superoxide dismutase (SOD) catalyzes the dismutation of $O_2^{\cdot -}$ to H_2O_2 and O_2[37]. Ascorbic acid can efficiently scavenge various oxygen-derived free radicals[38].

The enzymic antioxidants peroxidase and superoxide dismutase and non-enzymic antioxidant ascorbic acid levels in roots and shoots of *Vigna radiata* grown in control and treated soil is shown in TABLE 4.

TABLE 4 : Effect of neem leaf extracts on antioxidants peroxidase, superoxide dismutase activity and ascorbic acid level in *Vigna radiata* plants grown in textile effluent contaminated soil

Samples	Peroxidase activity (μM min^{-1} g^{-1})		Superoxide dismutase activity (Units mg^{-1} g^{-1})		Ascorbic acid (mg g^{-1})	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	125 ± 2.1	97.4 ± 2.3	87.3±1.1	30.2±0.53	8.2±0.1	11.2±0.15
CS+N- 0.25	138 ± 2.6 [a]*	118±0.57[a]*	31.8±0.21	17.5±0.25	9.6±0.12	15.5±0.25
CS + N- 0.5	114.6±1.5[a]*	98.4±0.58[a]*	41.3±0.21	19.1±0.1	7.6±0.1	9.8±0.21
CS+N- 0.75	105 ± 0.09	90.4 ± 0.55	28.6±0.3	15.9±0.26	6.3±0.2	9.0±0.32

CS-contaminated soil; N-neem; 0.25, 0.5 and 0.75-concentration of neem leaf extracts(mg/ml); Values represent mean ± S.D (n = 5); [a] – Comparison between control and different concentrations of neem leaf extracts;*- indicates significant at p< 0.05 level

In this study, peroxidase activity was high in shoots of *Vigna radiata* plants grown in N-0.25 treated soils when compared to control. This is an indication of stress of plants by the effluents. In N-0.5 treated soil the peroxidase activity is near normal to that of control soil. Peroxidase activity in roots was low when compared to shoots. The highest peroxidase activity was showed in roots of *Vigna radiata* plants grown in N-0.25 and in N-0.5 treated soils it is near to control soil. The present investigation is also in agreement with[39] who demonstrated that the cadmium treatment significantly decreased the peroxidase activity in *Calamus tenuis* leaves. When compared to control, moderate superoxide dismutase activity was observed in shoots of *Vigna radiata* plants grown in N-0.5 treated soil (41.3 Units mg^{-1} protein) and reduced activity was observed in shoots of *Vigna radiata* plants grown in N-0.75 and N-0.25 treated soils. Superoxide dismutase activity in roots was low when compared to shoots. In roots moderate activity was observed in N-0.25 and N-0.5 treated soils and reduced activity was observed in N-0.75 treated soils. The present results were also evi-

dent from the findings of[40] who has been observed NaCl and Cd induced reduction in SOD activity in various plants. The ascorbic acid level was highest in shoots of *Vigna radiata* plants grown in N0.25 treated soils and this level was higher than that of *Vigna radiata* plants grown in normal soil. The moderate level was observed in shoots of *Vigna radiata* plants grown in N-0.5 and N-0.75 treated soils. Compared to shoots, roots showed high level of ascorbic acid. The highest level was observed in roots of *Vigna radiata* plants grown in N-0.25 treated soil and compared to control moderate level was observed in roots of *Vigna radiata* plants grown in N-0.5 and N-0.75 treated soils. The present findings are supported by[39] who reported that the combination of NaCl and Cd at high concentration decreased ascorbate and glutathione content in *Calamus tenuis* leaves.

CONCLUSION

Based on the result, we conclude that neem leaf extracts reduced the heavy metal toxicity in textile effluent contaminated soil. Neem leaf extracts improved the textile effluent contaminated soil fertility by reducing the pH and increasing the enzyme catalase activity and microbial biomass carbon level and ultimately it improved the germination of seeds and seedling growth. Finally, we can conclude that neem leaf extracts particularly N-0.5 is very effective in phytoremediation of textile effluent contamination. So further research in this area can be carried out like field work to determine the long-term field-scale applicability. Additionally, we can further identify and isolate the compounds responsible for removing the heavy metals.

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