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RESEARCH ARTICLE

AMELIORATION OF TANNERY EFFLUENT POLLUTED SOIL AND ITS RESPONSE OF BLACK GRAM (*Vigna mungo)*

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ABSTRACT

In this study, a higher amount of various elements get deposited in the soil and make them polluted. Since, this polluted water reduces the crop production as well as the soil properties. To measure the bioremediation of some tree species like *Pongamia glabra, Polyalthia, longifolia, Hesperia populnea, Pithecolobium dulci, Mangifera indica, Moringa olefera, Tamarindus indica, Acacia nilotica, Samanea saman and Azadirachta indica* were grown in the polluted soil upto 90 days. The treatment in which all the tree species are grown in one pot and it showed a remarkable reduction of pollutants. The bioremediated soil showed a better germination in black gram seeds. The polluted soil were mixed with organic amendments like vermicompost, biofertilizer (*Rhizobium*) and FYM to improve the soil fertility and their toxicity was tested by growing black gram in that soil. Among the amendments, the vermicompost mixed polluted soil showed good results in morphological, biochemical and yield parameters.

INTRODUCTION

Environment pollution is becoming the global problem in which water pollution is an important issue as water is used directly for various purposes. The major sources of water pollution are industrial effluents and the wastewater generated from various industries which is being discharged to the common drainage (or) nearby soil (Lokhande and Vaidya, 2004). Pollution is the greatest threat posed to humanity and even to the whole biosphere. There is a growing concern for the environmental pollution caused by wastewaters especially in the developing countries like India. In most pats of India, the liquid wastes are either discharged into the water courses (or) on to the land causing severe pollution problems. The recycling of wastes can be considered a great boon in combating the pollution problems in the present situation of resource scarcity where important components of wastewater can be recycled in various ways. The use of waste water for irrigation has emerged in the recent past is an important way of utilization of wastewaters taking the advantage of the presence of considerable quantities of calcium, potassium and magnesium along with some other essential elements. The other advantage of wastewater irrigation includes an important aspect of pollutant removal. The pollutants are partly taken up by the plants and transformed in the soil to harmless forms while some be held in the soil without causing any damage. The use of wastewaters for irrigation may be much more beneficial, especially in the arid and semi-arid regions of the world including India. Though, it seems quite promising to use wastewaters for agriculture, it is marred by several constraints due to various problems like soil salinity, interaction of chemical constituents of the wastes with the uptake of nutrients and changes in soil property and microflora. More than fifty percent of the tanneries in the country exist in Tamil Nadu. There are about 25 tanneries operating in and around Tiruchirappalli city, Tamil Nadu, India. In India leather industry contributes significantly towards exports, employment generation and occupies an important place in Indian economy. The tanning industry is one of the major consumers of water and most of it is discharged as wastewater, which contains high amounts of heavy metals (Sinha *et al.,*2002). In India, tannery industries play a prominent role as it contributes fifteen percent of the total production capacity of the world. These tanneries specialize in processing hides into heavy leather [sole, harness and industrial leather]. The enormous pollution load along with the toxic nature of wastewater makes the tanneries a potential threat to the areas in the vicinity of their location. The tannery wastewater is being contaminated with high levels of metals [Fe, Cu, Zn, Mn, and Cr], which when consumed causes serious health hazards.

This necessitates a detailed scientific study before any specific waste can be used for irrigation (or) a particular

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crop with specific soil and climate (Arindam, 2001; Singh and Sinha, 2004; Senthil Kumar and Sekar, 1998). Bioremediation is the use of biological agents like bacteria, fungi and plants to remove (or) degrade the pollutants from the contaminated soil. This technology has appeared to reduce the enormous costs and environmental disturbance that are associated with current clean up polluted soil on agricultural crops before are used for crop cultivation (Panwar *et al.,*2002). Phytoremediation is an emerging technology which uses plants and their associated microbes to remove, degrade the polluted medium. If has been successfully employed for remediation of different kind of contaminants. Tree species play a major role in reclamation of pollutants. Growing of tree seeding is one of the most promising and potentially effective techniques for the removal of pollutants (An *et al.,*2006 and Sankar Ganesh, 2008). The toxic compounds are trapped into the trunks of such tree species which will remain for a longer time and will not come to the food chain as well. It is an affordable technology that is most useful when the contaminates are with the root zone of plants. Amendments act as vital remediation measures. Primarily the amendment micro-organisms are found to be very effective to degrade, the environmental contaminants into less toxic forms. There are number of previous literature available regarding the bioremediation of industrial polluted soil (Bentjen Isteve, 2002; Shimp *et al.,*1993). So, the present work deals with bioremediation of tannery effluent polluted soil by growing tree species and amendments mixed polluted soil and its response on blackgram [*Vigna mungo* L.].

MATERIAL AND METHODS

Tannery industry effluent Polluted soil

The tannery industry effluent polluted sample was collected in polythene bags from Prime Tannery Industry, Sempattu, Tiruchirappalli district of Tamil Nadu, India. The polluted soil was analysed for its various physico-chemical parameters. The seeds of blackgram were collected from Tamil Nadu Agricultural University, School of Genetics, Department of Pulses, Coimbatore, Tamil Nadu, and India.

Remediation of Polluted Soil

The seedlings of various tree species like *Pongamia glabra, Polyathia longifolia, Thespesia populnea, Pithecolobium dulei, Mangifera indica, Moringa olefera, Tamarindus indica, Samanea saman, Azadirachta indica and Acacia, nilotica,* collected form Forest Research Station, Coimbatore were used for this bioremediation study. Pots were filled with 5Kg of tannery effluent polluted soil. The seeds of tree species were sown in pots separately and also in combination viz., each tree species in removing pollutants. All species were allowed to grow in polluted soil upto 90 days. The soil samples were collected from the pot at 90 days and they were analysed to know their properties.

Amendments

Amendments like Vermicompost, *Rhizobium* and FYM [Farm yard manure] were collected from Faculty of Agriculture, Annamalai University, Tamil Nadu, India. Polluted soil and amendments [2:1 ratio] were mixed and used to study the germination, growth and yield of black gram.

Germination studies

The bioremediated soils were analysed and they were used for germination studies of *Vigna mungo* L. The morphological parameters like germination percentage, seedling length, seedling fresh and dry weight, Vigour index, tolerance index and percentage of phytotoxicity were calculated on 15th day old seedlings.

Morphological studies

Germination Percentage

The number of seeds germinated in each treatment was counted on 7th DAS and the germination percentage was calculated by using following formula.

$$\text{Germination \%} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seedsown}} \times 100$$

Root and Shoot length

Five seedlings were taken from each treatment and their root and shoot length [cm / seedling] were measured by using a scale and these values were recorded.

Fresh and Dry Weight

Five seedlings were collected from each treatments and their fresh weight [g/seedling] were measured with the help of an electrical single pan balance. Their dry weight [g / seedling] were taken after keeping them in a hot air oven at 80°C for 24 hours by using an electrical single pan balance.

Total leaf area

The leaf area was calculated by measuring the length and breath of the leaf as described by Yoshida *et al.,*1972. Leaf area [cm^2] = k x length x breath; Where, k = Kemp's constant [for dicot leaves] = 0.66

Yeild [kg/ m^2]

Five plants samples were taken from each treatment at the time of harvest for the observation of the yield parameters such as number of pods per plant, number of seeds per pod, 100 seed weight and yield of plant [kg/ m^2].

Biochemical analysis

Chlorophyll and carotenoid

0.5g of fresh leaf material was taken and ground with the help of pestle and mortar with 10ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved and the residue was re-extracted with 10ml of 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. The absorbance was read at 645nm for chlorophyll 'a' 663nm for chlorophyll 'b' and 480nm for total chlorophyll in UV-Spectrophotometer. The carotenoid content of the extract was read at 450nm. The values were calculated using the formula proposed by [Arnon, 1949] for total chlorophyll and [kirk and Allen, 1965] for carotenoid contents.

Protein

Protein content was determined by the method of Lowry *et al.,*1951. 0.5g of plant sample was homogenized in 10mL of 20% TCA trichloroacetic acid. The homogenate was centrifuged at 800 rpm for 10 minutes. The supernatant was discarded and the pellet was re-extracted with 5mL of 0.1N NaOH. 1mL of the

Table 1 : Physico - chemical properties of polluted soil and bioremediated soil

S.No.	Name of the organisms used for bioremediation	Soil Properties											
		P^H	Ec	N	P	K	Na	Ca	Mn	Cr	Fe	Cu	Zn
1	Polluted soil [control]	8.0	0.53	96	60	460	45	82	16.74	21.01	22.38	0.85	7.38
2	All tree plants in one pot	8.6	0.36	73	2	70	26	54	4.87	16.19	5.86	0.62	0.60
3	*Pongamia glabra*	8.5	0.46	77	60	42	32	66	2.46	8.66	17.41	0.70	1.88
4	*Polyalthia longifolia*	8.5	0.40	70	44	53	21	45	4.00	13.82	18.94	0.69	2.01
5	*Thespesia populnea*	8.3	0.28	78	62	50	36	70	2.23	6.05	12.19	0.68	1.72
6	*Pithecolobium dulci*	8.3	0.20	74	58	58	27	56	3.56	12.90	6.22	0.71	2.01
7	*Mangifera indica*	8.2	0.46	66	42	39	18	38	2.05	4.27	11.50	0.65	1.50
8	*Moringa olefera*	8.6	0.32	72	27	98	24	51	3.38	11.34	13.29	0.80	1.91
9	*Tamarindus indica*	8.8	0.55	87	26	114	40	78	5.43	17.82	22.60	0.72	0.94
10	*Acacia nilotica*	8.2	0.48	74	29	70	28	60	4.32	15.70	14.64	0.79	7.13
11	*Samanea saman*	8.8	0.57	75	20	95	30	63	3.30	10.17	18.51	0.69	7.10
12	*Azadirachta indica*	8.8	0.22	84	44	48	39	74	3.25	9.38	7.47	0.73	4.62

Table 2 : Germination studies of blackgram [*Vigna mungo* L] grown in the bioremediated soil

S. No	Name of the organisms used for bioremediation	Germination Percentage (%)	Seedling growth (cm/seedling)	Seedling dry weight (g / seedling)	Vigour index	Tolerance index	Percentage of Phytotoxicity
1	Polluted soil [control]	65 ± 3.25	13.6 ± 0.68	0.65 ± 0.032	786.4 ± 39.32	--	--
2	All tree plants in one pot	98 ± 4.90	30.7 ± 1.53	0.998 ± 0.049	2674 ± 133.7	1.4246 ± 0.071	0.2620 ± 0.013
3	*Pongamia glabra*	96 ± 4.80	28.3 ± 1.41	0.926 ± 0.046	2412 ± 120.6	1.2662 ± 0.063	0.2001 ± 7.010
4	*Polyalthia longifolia*	96 ± 4.80	29.9 ± 1.49	0.878 ± 0.043	2393 ± 120.6	1.2053 ± 7.060	0.9228 ± 0.046
5	*Thespesia populnea*	92 ± 4.60	26.0 ± 1.3	0.760 ± 0.038	2634 ± 131.7	1.4136 ± 0.070	0.2045 ± 7.010
6	*Pithecolobium dulci*	92 ± 4.60	27.9 ± 1.39	0.820 ± 0.041	2304 ± 115.2	1.2028 ± 0.060	0.8938 ± 0.044
7	*Mangifera indica*	90 ± 4.50	23.7 ± 1.18	0.628 ± 0.031	1720 ± 86.0	0.9911 ± 7.049	0.6122 ± 0.030
8	*Moringa olefera*	89 ± 4.45	25.9 ± 1.29	0.725 ± 0.036	2150 ± 107.5	1.1858 ± 0.059	0.8788 ± 0.043
9	*Tamarindus indica*	95 ± 4.75	29.3 ± 1.46	0.874 ± 0.043	2425 ± 121.25	1.2099 ± 7.060	0.2034 ± 0.010
10	*Acacia nilotica*	88 ± 4.40	24.6 ± 1.23	0.716 ± 0.035	1988 ± 009.4	1.0884 ± 0.054	0.8592 ± 0.042
11	*Samanea saman*	76 ± 3.80	20.4 ± 1.02	0.601 ± 0.030	1692 ± 84.6	0.7642 ± 0.038	0.4671 ± 7.023
12	*Azadirachta indica*	86 ± 4.30	22.8 ± 1.14	0.702 ± 0.035	1984 ± 99.2	1.0088 ± 0.050	0.6880 ± 0.034

extract was taken in a test tube and 5mL of reagent 'C' [Protein reagent] was added. This solution was mixed well and kept in dark for 10 minutes. Later, 0.5mL of folin phenol reagent was added and mixture was kept in dark for 30 minutes. The sample was read at 660nm in the UV-Spectrophotometer.

Total reducing sugars

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10mL of 80 percent ethanol. The homogenate was centrifuged for 10 minutes at 800 rpm. The supernatant was saved. Then, the ethanol is evaporated in a water bath at 50°C. The net content was made upto 20mL with distilled water and the extract was used for the estimation of reducing sugar. One mL of extract was taken in a 25mL marked test tube. 1mL of reagent 'C' was added. Then, the mixture was heated for 20 minutes at 100°C in a boiling water bath, cooled and 1mL of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25ml with distilled water. The sample was read in the UV-Spectrophotometer at 520nm. The sugar contents were expressed in mg/g fresh weight basis.

Amino acid

Amino acid content was determined by the method of Moore and Stein (1948). 0.5g of plant sample was homogenized in 10mL of 80% ethanol. The homogenized was centrifuged 800 rpm for 10 minutes. 1mL of the extract was taken in the test tube and 1mL of 0.1N HCl was added to neutralize the sample. To this, 1mL of Ninhydrin reagent was added and heated for 30 minutes in a boiling water bath. Later 5mL of the diluent solution was added and heated again in water bath for 10 minutes. The test tubes were cooled and read the absorbance at 570nm in a Spectrophotometer.

Catalase

Catalase content was determined by the method of Chance and Machly [1967]. An aliquot of 1mL of the supernatant of the enzyme extract was added to the reaction mixture containing 1mL of 0.01MH_2O_2 and 3mL 0.1 M phosphate buffer. The reaction was stopped after it was incubated at 5 minutes in 20°C by adding 10mL of 1%. H_2SO_4. The acidified medium without (or) with the enzyme extract was titrated against 0.005N $KMNO_4$ and catalase activity was expressed at n moles of H_2O_2 utilized [units minutes $^{-1}$ mg $^{-1}$ protein].

Table 3 : Physico - chemical properties of amendment mixed polluted soil

S.No.	Amendments	P^H	Ec	N	P	K	Na	Ca	Mn	Cr	Fe	Cu	Zn
1	Polluted soil [control]	4.8	0.60	94	70	162	58	52	3.00	21.01	12.54	0.67	2.38
2	Polluted soil + biofertilizer[*Rhizobium*]	6.5	0.30	92	38	82	40	33	2.33	16.20	6.80	0.52	1.32
3	Polluted soil & FYM	7.2	0.34	80	44	90	46	42	2.59	17.80	7.94	0.55	1.70

Table 4 : Growth and yeild of blackgram [*Vigna mungo* L] grown under tannery effluent polluted soil mixed with various soil amendments

S.No.	Various soil amendments	Parameters studied							
		Shoot length (cm/plant)	Root length (cm/plant)	Total leaf area (cm^2/plant)	Fresh weight (g/plant)	Dry weight (g/plant)	No. of fruits/ plant	Number of seeds/fruit	Yeild (g/m^2)
1	Polluted soil [control]	18.6 ± 0.93	11.4 ± 0.57	239.20 ± 11.96	2.640 ± 0.132	□□1.269 ± 0.027	16.2 ±0.475	85.5 ± 3.34	685 ± 29.6
2	Vermicompost	35.4 ± 1.77	22.0 ± 1.10	363.92 ± 18.196	7.885 ± 0.394	4.268 ± 0.213	15.2 ± 0.76	79.5 ± 3.97	614 ± 30.7
3	Biofertilizer [*Rhizobium*]	28.5 ± 1.42	18.6 ± 0.93	352.08 ± 17.604	5.286 ± 0.264	3.078 ± 0.153	13.0 ± 0.65	77.2 ±3.86	608 ± 30.4
4	FYM	23.0 ± 1.15	19.0 ± 0.95	348.51 ± 17.425	4.780 ± 0.239	2.570 ± 0.128	12.2 ± 0.61	73.5 ± 3.67	585 ±29.2

Table 5. Biochemical contents of blackgram [*Vigna mungo* L]grown under anneryeffluent olluted soil mixed with various soil amendments

S.No.	Various soil amendments	Biochemical Parameters						
		Chlorophyll (mg/g)	Carotenoid (mg/g)	Protein (mg/g)	Amino acids (mg/g)	Total Reducing sugar (mg/g)	Catalase (unit/g)	Peroxidase (unit/g)
1	Polluted soil [control]	0.031 ± 0.001	0.038 ± 0.001	1.24 ± 0.062	0.99 ± 0.049	000 ± 1.609	000 ± 0.565	□□000 ± 0.045
2	Vermicompost	0.219 ± 0.010	0.094 ± 0.004	3.87 ± 0.193	2.86 ±0.143	138.68 ± 6.934	34.75 ± 1.737	2.52 ± 0.126
3	Biofertilizer [*Rhizobium*]	0.120 ± 0.006	0.067 ± 0.003	2.46 ± 0.123	2.01 ±0.100	112.47 ± 5.623	28.04 ± 1.402	1.80 ± 0.09
4	FYM	0.095 ± 0.004	0.059 ± 0.002	1.98 ± 0.099	1.27 ±0.063	81.02 ± 4.051	21.10 ± 1.055	1.55 ± 0.077

Peroxidase

Peroxidase content was determined by the method of Chance and Machly (1967). 0.5mL of enzyme extract was suspended in reactor mixture containing 2mL of 0.1M phosphate buffer [p^H 6.8] and lmL of 0.01M pyrogallol. 1mL of 0.05M H_2O_2 [5:5 in H_2O_2 and distilled water] was added in the solution and incubated at 25°C for 5 minutes and the reaction was stopped by adding 1mL of 2.5N H_2SO_4 [24.5mL of H_2SO_4 + 100mL of distilled water]. The amount of purpurogalline formed was determined by reading the absorbance at 420nm against a blank prepared by adding the extract after the addition of 2.5N H_2SO_4. The peroxidase activity was expressed in unit 0.1 absorbance min^{-1} mg^{-1} protein.

RESULTS

Physico-chemical analyses of tannery industry effluent polluted soil and bioremediated soil using some tree species are shown in Table 1. It showed a remarkable variation in soil properties when compared to polluted soil. The polluted soil was found with excess pollutants and fewer amounts of micro and macronutrients reduce the plant growth. At the same time, the bioremediated soil analysis reveals that it has reduced the amount of pollutants. The germination was observed in all tree species grown in one pot polluted soil furnished in Table 2. The bioremediated soil may be used for germination studies with blackgram seeds. The blackgram plant growth is increased in bioremediated soil when compared with polluted soil. Plant growing on such polluted soil absorbs nourishments from them and entry

of pollutants to the plant system during the process of absorption may not be ruled out. The physico-chemical analysis of tannery industry effluent polluted soil revealed in Table 3. Its contains the higher amount of minerals and toxic pollutants and soil organic matter. This polluted soil is not suitable for cultivation. So it decided to give certain soil amendments like vermicompost, biofertilizer [*Rhizobium*] and FYM by mixing with polluted soil to improve the soil fertility. It was reported that the application of Vermicompost increased the value of plant height, leaf area, dry matter content and yield [Table: 4]. Vermicompost has been reported to contain large number of nitrogen fixing phosphate solubilizing and other beneficial micro-organisms which have favourable effect on growth and yield of plant. At the same time, the application of soil amendments improved the soil fertility and plant growth. Table 5 reveals the total chlorophyll, carotenoid, total reducing sugar, protein, aminoacids, catalase and peroxidase contents in the blackgram plant sample under polluted soil mixed with vermicompost, biofertilizer [*Rhizobium*] and FYM. Among them Vermicompost gives best results when compared with other amendments.

DISCUSSION

The removal of elements from polluted soil may be due to hyperaccumulation of the contaminants by tree species and they translocate from the root to shoot and leaves (Cheny *et al.,*1997). This process can be compared to the solar driven pumps which can extract and concentrate certain elements from their environment (Salt *et al.,*1995). Pollutant accumulating contaminants from the soil to the shoot above (Raskin *et al.,*1994). This may be assumed that the lowering the pollution level of the soil. Species tolerant to the stress has been proposed as the indicators and bioaccumulators of the pollutants (Kumar *et al.,*1995). The amendment mixed soil analysis reveals that it contained the required amount of organic matters and neutralize the minerals and nutrients (Nagarajan *et al.,*2005).

The blackgram seedlings grow vigorously in the amendments mixed with polluted soil. Among the amendments the soil mixed with vermicompost shows higher growth performance of blackgram seedlings when compared with other amendments. The similar findings are in conformity with our results (Cunningham *et al.,*1995; Vijayapriya, Muthukaruppan, 2005 and Sankarganesh, 2008). This may be due to the higher rate of multiplication of soil microbes leading to improvement in physical properties of soil (Vidya and usha, 2007).

It has been observed that photosynthetic efficiencies of plant growing under-metal stress decreased with a concomitant change in the composition of photosynthetic pigments (Sharma and Hall, 1992). The total chlorophyll, carotenoid and protein shows the amendments Vermicompost increases and decreases with other amendments when compared to polluted soil. Singh and Sinha (2005) reported the metal accumulation and its tolerance in the plants of blackgram seedlings which grown on various amendment of polluted soil. They recorded an increase in the total chlorophyll, carotenoids, total reducing sugar, protein, amino acid, catalase and peroxidase contents at the vermicompost which was similar to the results of the present study. The greatest response to polluted soil indicates the metal stress and due to the presence of large amounts of various cations and anions (Ram and Jha, 1998).

CONCLUSION

Besides the blackgram plants can be cultivated in the tannery effluent contaminated soil. This plant might be used to remediate the contamination. This vegetation would play a vital role in emediating the polluted system and preventing further contamination. The tree species like *Pongamia glabra, Polyalthia longifolia, Thespesia populnea, Pithecolobium dulci, Mangifera indica, Moringa olefera, Tamarindus indica, Acacia nilotica, samanea saman and Azadirachta indica* were allowed to grow in the polluted soil upto 90 days. Among bioremediation treatments, the polluted soil treated with all tree species showed the high degree of pollutants reduction, then the soil treated with a single tree species. The bioremediated soil showed a better performance in germination studies than that of polluted soil. Among the soil amendments, Vermicompost showed a best result of reclamation of polluted soil and also increased the germination, growth, yield and biochemical parameters of crop plant when compared to other amendments. It is also advisable to farmers to give remediation treatment to polluted soil before sowing, for getting higher yield in agriculture.

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