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RESEARCH ARTICLE

Diversity Status of Beneficial Microorganisms in Heavy Metal Polluted Tannery Effluent Treatment Area in Dindugal, Tamil Nadu

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Abstract

Industrial waste is one of the most essential sources of contamination in the surface environment. Among different industries, tannery industry releases huge amount of pollutants into the ecosystem. Long term disposal of the tannery wastes has resulted in wide contamination of agricultural land and water sources in different parts of India. An attempt was made to study the diversity status of different microbial organisms in tannery effluent treated samples in Dindugal, Tamil Nadu. It was found that Electrical Conductivity (EC) and heavy metal contents were higher and population density of different beneficial microbes found better. Among different microbes isolated, phosphate solubilizing microbes (PSB) was found maximum which is followed by fungi and actinomycetes. The population density of non-symbiotic and symbiotic nitrogen fixers were found to be low in numbers when compared to other samples screened. Similarly, occurrence and distribution of AM fungal spores were also found low in heavy metal polluted samples as compared to the samples collected from non-polluted outside tannery effluent treatment area. Among different Arbuscular Mycorrhizal (AM) fungi, *Glomus* species was found to be dominant in the samples collected from outside tannery effluent treatment area as compared to tannery effluent samples.

Keywords: AM fungi, *Acaulospora, Glomus,* rhizobacteria, heavy metals, tannery effluent.

Introduction

Industrial wastes are usually generated from different industries; as a result the amount and toxicity of wastes released from industrial activities vary with the industrial processes. Leather tannery effluents are ranked as the highest pollutants among all the industrial wastes, which leads to an introduction of novel chemicals into the ecosystem. Tannery waste is generated in huge amounts during the process of tanning by leather industries throughout the world (Singh *et al*., 2011). Because of high cost implication, <5% of the industries in the world have adopted the sufficient measure for treatment of effluent while most have ignored it (Khopkia, 2009; Precthi *et al*., 2009). When tannery wastes are used in cultivable lands or when the lands are irrigated with such wastes, the fertility of the soil is drastically affected (Aina *et al*., 2007). Tannery effluents are mainly characterized by high salinity, high organic loading and specific pollutants such as chromium. Different chemicals used in tanning are lime, sodium carbonate, sodium bi-carbonate, common salt, sodium sulphate, chrome sulphate, fat liquors, vegetable oils and dye (Banuraman and Meikandaan, 2013). The released pollutants such as organic compounds and heavy metals exercise negative impact on environment causing toxicity to flora and fauna. Microbial population was found to be diverse in different ecosystems, based upon the nature of soil such as industrial, agriculture, forestry etc.

Most of the microbes have the ability to adapt the new environment by changing their physical and chemical characters. Below ground microbiota are particularly sensitive to changes in soil physico-chemical properties such as temperature, moisture, pH, nutrient availability or clay content (Lauber *et al*., 2008; Teklay *et al*., 2010; Balogh *et al*., 2011). These factors directly control the abundance and activity of microbes and indirectly affect them through regulating the bioavailability of the contaminants (Vig *et al*., 2003; Giller *et al*., 2009). Such alleviation of the negative effects of heavy metal pollution on soil biota may be the cause for the weak or absent correlations between trace metal concentrations and microbial activity reported by some authors (Schipper and Lee, 2004; Niklinska *et al*., 2005). However, the addition of sludge considerably increases the amount of heavy metals in soil, causing changes in soil properties which could be toxic for soil microorganisms (McGrath *et al*., 1995). Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants (Birch and Bachofen, 1990). In every step of tanning process a considerable amount of waste water is released contain huge amount of chemicals. Most of the industries have effluent treatment plants but there is no complete removal of chemicals and they were found in the treated samples.

Table 1. Samples collected for the study.

Sl. No.	Samples collection site	Nature of sample
1.	Filtration tank (D1)	Effluent
2.	Clarified tank (D2)	Effluent
3.	Aerobic lagoon (D3)	Soil
4.	Rhizosphere soil from the plants grown in aerobic lagoon (D4)	Soil and root
5.	Treated sludge (D5)	Soil
6.	Industrial field rhizosphere soil (D6)	Soil and root

Considering the problem, the present study aims to determine the status of physico-chemical parameters and different beneficial microbial population in soil and effluent samples collected from tannery treatment area in Dindugal, Tamil Nadu.

Materials and methods

Collection of soil samples: Six different samples were collected from a common tannery effluent treatment plant situated at Dindugal in Tamil Nadu, India (Table 1). All the samples were immediately transported to laboratory for further analysis. The samples were kept in refrigerator at 4ºC until further use.

Physico-chemical parameters of different samples: The effluent water, sludge and soil samples collected from different study locations were analyzed for their physico-chemical parameters such as, pH, color, odor, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Electrical Conductivity (EC), Bulk density, Organic carbon, available Nitrogen (N), Phosphorus (P), Potassium (K), Calcium, Magnesium, Copper, Zinc, Manganese, Iron, Carbonates, Bicarbonates, Chlorine and presence of heavy metal (APHA, 1992).

Isolation and identification of different microbes from various samples: Diversity status of different microbes in tannery effluent and soil samples was studied using pour plate technique described by Subba Rao (2007). For isolation of Phosphate Solubilizing Bacteria (PSB), samples were pour plated into Pikovskaya's agar medium; for isolation of non-symbiotic nitrogen fixing organisms, samples were pour plated into Jensen's agar and Rojo Congo agar media; for isolation of symbiotic nitrogen fixing organisms, samples were pour plated into Yeast Extract Mannitol Agar (YEMA) medium, for isolation of Actinomycetes, samples were pour plated into Starch casein agar medium and for isolation of fungi, samples were plated in Potato Dextrose Agar (PDA) medium. All the plates were then incubated at 28±2ºC for 3-7 d and microbial population count was calculated by the following formula (Reddy and Reddy, 2004):

$$\text{Colony forming unit (cfu/mL)} = \frac{\text{No. of colonies x Dilution factor}}{\text{Amount of soil (g)}}$$

Isolation and identification of AM fungal spores from different soil samples: Soil samples collected from tannery effluent treatment and outside effluent treatment areas were subjected to isolation of AM fungal spores using wet sieving and decanting method described by Gerdemann and Nicolson (1963) and species level identification was done by using standard keys (Schenck and Perez, 1987).

Results and discussion

Physico-chemical parameters of different samples: The results of the physico-chemical parameters are presented in Table 2. The effluent from tannery industrial filtration tank and clarified outlet was brownish yellow in color and had an unpleasant odor. The color and odor of the effluent may be due to the presence of large amount of chemicals used during processing of skin and hides. The pH of the samples ranged between 7.4 and 7.7 in all the samples analyzed which was under the tolerance limit of 5.5 to 9.0 prescribed by the Bureau of Indian Standards (1991). The EC was found to be high range in filtration tank (D1) (17.4 dSm^{-1}) followed by clarified outlet (D3) (15.0 dSm^{-1}), when compared to all other samples. The rhizosphere soil collected from aerobic lagoon (D4) showed less EC (4.8 dSm^{-1}). EC is the best indicator of salinity, EC above 4 is taken as highly saline, and hence, the soil is not suitable for plant growth. In the present study, the effluent samples had EC greater than 4. In leather industry, huge amount of salt (NaCl) is used to preserve the skins from decomposition immediately after they are stripped in the slaughterhouse and subsequently the excess of salt has to be removed in the tannery before further processing. Akan *et al.* (2008) reported that the higher conductivity alters the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna.

The effluent showed a higher level of total dissolved solids (TDS) (21675 ppm). Similar observation was made by Bhalli and Khan (2006) in liming section of tanning process, protein, hair, skin and emulsified fats are removed from the hides, which are released in the effluent causes increase in total solids. In soil samples, the bulk density was found in the range between 0.8 and 1.4 g/cc. The present organic carbon was higher in D3 (2.46%) followed by D6 (2.10%) as compared to other samples. The level of sodium was found to be maximum in effluent sample D1 (6474 ppm).

Table 2. Physico-chemical parameters of soil samples.

Sl. No.	Physico-chemical parameters	D1	D2	D3	D4	D5	D6
1.	pH	7.4	7.6	7.4	7.4	7.7	7.7
2.	Electrical conductivity (dSm^{-1})	17.4	15.0	13.9	4.8	9.8	8.2
3.	Bulk density (g/cc)	-	-	0.9	0.8	0.7	1.4
4.	Organic carbon (%)	-	-	1.74	1.38	2.10	2.46
5.	TDS	21675 ppm	-	NA	NA	-	-
6.	Sodium (ppm)	6474	754.2	-	-	-	-
7.	Available Nitrogen (kg/ha)	-	-	174.5	268.2	171.7	154.0
8.	Available Phosphorus (Kg ha^{-1})	-	-	23.81	26.46	24.26	21.53
9.	Available Potassium (ppm)	80.2	14.3	258.8	382.2	475.8	284.3
10.	Calcium (meq/100 g)	1.04	0.48	3.8	7.6	4.2	4.8
11.	Magnesium (ppm)	0.11(meq/100 g)	0.12(meq/100 g)	0.40	0.60	0.20	0.40
12.	Carbonates (meq/L)	Absent	Absent	-	-	-	-
13.	Bicarbonates (meq/L)	8.4	5.6	-	-	-	-
14.	Arsenic (ppm)	0.2	0.02	0.2	0.2	0.2	0.2
15.	Cadmium (ppm)	0.1	0.1	0.1	0.1	0.1	0.1
16.	Copper (ppm)	0.05	0.5	0.05	0.05	0.05	0.05
17.	Lead (ppm)	0.2	0.02	0.2	0.2	0.2	0.2
18.	Mercury (ppm)	0.02	0.2	0.02	0.02	0.02	0.02
19.	Zinc (ppm)	0.5	0.5	0.5	0.5	0.5	0.5
20.	Chromium (ppm)	0.2	0.2	0.2	0.2	0.2	0.2

D1–Filtration tank effluent; D2–Clarified outlet effluent; D3–Aerobic lagoon soil; D4–Rhizosphere soil sample collected from aerobic lagoon; D5–Sludge; D6–Industrial field soil.

Rasmussen (2000) reported that the use of huge salts in different stages of tanning process which was the reason for high concentrations of sodium and chloride in the effluent. Kolay (2000) described that the high amount of sodium in water breaks the soil aggregates and blocks the soil pores in irrigated fields. The available phosphorus was maximum in D4 (26.46 Kg ha^{-1}) followed by D5 (24.26 Kg ha^{-1}). The available nitrogen was higher in D4 (268.2 kg/ha) followed by D3 (174.5 kg/ha). The level of heavy metals such as arsenic and lead was found to be same in D1, D3, D4 and D5 samples (0.2 ppm) and in case of D2 it was found to be 0.02 ppm. The presence of cadmium was in the range of 0.1 ppm in all the samples analyzed. The occurrence of copper was 0.05 ppm in all the samples except D2 it was 0.5 ppm. The presence of mercury was found to be 0.02 ppm in all the samples except D2 (0.2 ppm). The amount of zinc was found to be in range of 0.5 ppm in all the samples. Mohanta *et al.* (2010) reported that the tannery wastewater is being contaminated with higher levels of metals (iron, nickel, chromium, zinc, cadmium, manganese and copper) during different process and their irrigation contaminates the soil, which affects vegetable and crops, it leads to health hazards to the consumers.

The level of chromium was found to be 0.2 ppm in all the analyzed samples, which was higher than the amount prescribed by BIS (2.0 mg/L or 0.002 ppm). Chromium is the major chemical used in tanning process and hence, its discharge into the waste effluent or sludge was found to be high. In accordance with the present work, Krantz and Kifferstein (2002) reported that the extremely high concentrations of chromium, sodium, magnesium, calcium and ammonia were detected in the tannery effluent.

Fent (2004) reported the continuous discharge of chromium in low concentration which is toxic to aquatic life and has been shown to disrupt the aquatic food chain. The results clearly assert the fact that soil in and around the tannery industrial effluent treatment plant experiences changes in physico-chemical parameters.

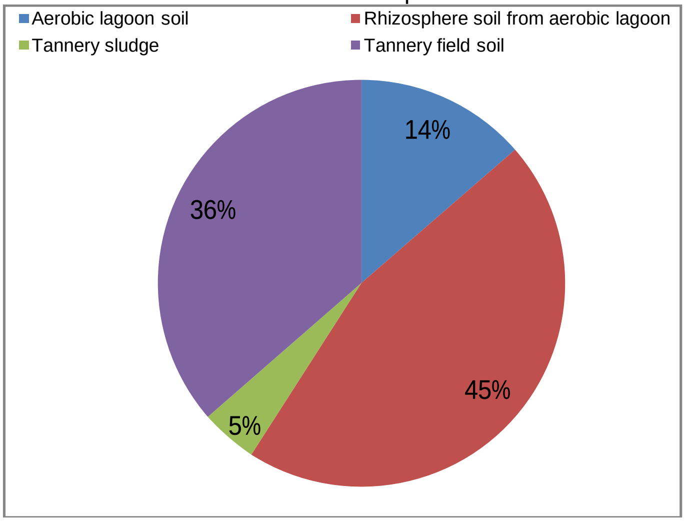
Status of population density of microbes in different samples: Data on the diversity status of different microbial population in various samples collected from common effluent treatment plant is presented in Table 3. It was found that, soil collected under rhizosphere of plants grown in contaminated area show considerable microbial population especially PSB ($38x10^6$) followed by fungi ($18x10^3$) and actinomycetes ($14x10^5$) when compared with other samples. The presence of symbiotic and non-symbiotic nitrogen fixers is found less in number. Metals are toxic to all biological systems including microbes, plants and animals. The microorganisms are highly as compared with other systems due to their small size and direct involvement with their environment (Gilled *et al*., 1998; Patel *et al*., 2007). Breathy *et al*. (2011) reported that the metallic contaminants like Hg, BP, Zn, As, Cod, Cr, Na, K, Cu etc. destroy bacteria and other beneficial microorganisms in the soil. Heavy metals tend to precipitate phosphatic compounds and catalyze their decomposition. These metals are considered to be indestructible poisons and their accumulation in soil for a long period may be highly fatal to living organisms. In addition, high salinity may also be the reason for decrease of population count of microbes. Most of the microbes have an ability to adapt for its new environment by using different genetic modifications; this may be the reason for the presence of some microbes in this highly polluted soil and effluent samples.

Table 3. Population density of different microbes isolated from different samples.

Sl. No.	Soil samples	Total population of microbes (CFU/mL)				
		Phosphate solubilizing microbes	Symbiotic nitrogen fixer	Non-symbiotic nitrogen fixer	Actinomycetes	Fungi
1.	Filtration tank	$22X10^6$	$4X10^6$	$2X10^6$	$10X10^5$	$12X10^3$
2.	Clarified outlet	$27X10^6$	$7X10^6$	$5X10^6$	$9X10^5$	$10X10^3$
3.	Aerobic lagoon	$29X10^6$	$7X10^6$	$6X10^6$	$12X10^5$	$13X10^3$
4.	Rhizosphere soil from aerobic lagoon	$38X10^6$	$11X10^6$	$13X10^6$	$14X10^5$	$18X10^3$
5.	Tannery sludge	$10X10^6$	Nil	Nil	$2X10^5$	$8X10^3$
6.	Tannery field soil	$23.2X10^6$	$10X10^6$	$9X10^6$	$7X10^5$	$9X10^3$

Mean of three replicates*.

Fig. 1. Population density of AM spores in different soil samples.



Mythili and Karthikeyan (2011) reported that several organisms in the tannery effluent utilize phenol and other pollutants as their carbon source to produce metabolic energy. Zahoor and Rehman (2008) reported that bacteria, yeast, algae, protozoa and fungi found in effluents have developed capabilities to protect them from the chemical constituents and heavy metals present in the effluents. Murugan *et al.* (2011) isolated *Aspergillus niger* capable of producing tannase which was able to degrade tannin, a major constituent of tannery effluent.

Status of population density of AM fungal spores: In the present study, the presence of AM fungal spores was found to be maximum in rhizosphere soil (100/100 g soil; 45%), when compared with tannery field soil (80/100 g soil; 36%), aerobic lagoon treated tannery soil (30/100 g soil; 14%) and tannery sludge (10/100 g soil; 5%) (Fig. 1). Among the isolated AM fungi, *Glomus* species was found in high number. The study is corroborated with the findings made by other researchers. Del Val *et al.* (1999) reported that the long term sludge application with increasing concentrations of heavy metals produced a significant decrease in both the size and diversity of AM fungal populations in soil.

The total number of AM fungal spores strongly decreased with the addition of increasing amounts of heavy metals, but the AM fungal propagules never disappeared completely in soils amended with the highest rates of sludge, suggesting a certain adaptation of these indigenous AM fungi to such environmental stress.

Conclusion

Diversity status of different microorganisms such as plant growth promoting rhizobacteria (PGPR), phosphate solubilizing bacteria, *Azotobacter* spp., *Azospirillum* spp., Actinomycetes and Arbuscular Mycorrhizal (AM) fungi from various samples collected from tannery effluent treatment and outside treatment areas was investigated. The physico-chemical parameters of different samples revealed that the effluent samples showed higher level of TDS, sodium and heavy metals. It was observed that the soil in and around tannery effluent treatment areas experiences change in physico-chemical parameters. The soil samples collected under rhizosphere of plants grown in heavy metal polluted areas revealed considerable amount of microbial population during the investigation. In the present study, the tannery pollutant changes the physico-chemical properties and microbial population in different samples screened. Exploitation of these beneficial microbes as bio-inoculants for production of quality tree seedlings in nurseries and out planting performance of these saplings in various heavy metal polluted areas of the country should be undertaken.

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