SCIENTIFIC ARTICLE

Study on the status of beneficial microbes from afforested textile and urban waste water polluted sites in Tirupur district, Tamil Nadu, South India

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Abstract

Utilization of efficient microbes and suitable plant species may provide an alternative method for bioremediation of polluted sites. Attempt was made to investigate the status of beneficial microbes *viz*., Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal (AM) fungi from the rhizosphere soil and root samples of six different tree species *viz., Acacia nilotica, Azadirachta indica, Casuarina equisetifolia, Eucalyptus tereticornis*, *Pongamia pinnata* and *Thespecia populnea* in afforested sewage effluent sites in Tirupur, Tamil Nadu. It was found that all the tree species had AM fungal colonization in the roots and soil spore population of AM fungi and PGPR population in the rhizosphere soils but variation among different samples screened. Maximum population density of PGPR was found from the rhizosphere of *Azadirachta indica* and *Casuarina equisetifolia.* Similarly, maximum number of AM fungal spores was observed from the rhizosphere of *Azadirachta indica,* followed by *Eucalyptus tereticornis* and *Casuarina equsetifolia*. The physico-chemical parameters of the afforested sewage effluent sites were studied and the nutrient status was improved after afforestation.

Keywords: Phosphate solublizing bacteria*, Azotobacter* spp.*, Azospirillum* spp., Arbuscular Mycorrhizal fungi

Introduction

Rapidly increasing urban population and industries had lead to the production of contaminated waste water. Discharge of untreated waste water from industries and urban waste water add 30,000 million liter per day of pollution into Indian rivers. This has lead to the pollution of soil, water and ground water sources used for agriculture and human consumption. Irrigation of untreated waste water in agriculture fields cause contamination of land, surface water and ground water with heavy metals like nitrates, fluorides etc.

Environmental pollution is an extremely important issue today, affecting all of us in one way or the other. In the past few decades, the disposal of sewage and industrial effluents to water bodies from uncontrolled urbanization has caused serious pollution problem. The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generates huge amounts of waste water (Hai *et al.,* 2006). More than 700 industries including dyeing units are discharging large amounts of industrial effluents regularly in Tirupur and surrounding areas (Jayashree *et al.,* 2011). The industrial effluents discharged from the textile dyeing units contain higher amount of heavy metals especially chromium, copper and lead which ultimately leaches to ground water and lead to contamination due to accumulation of toxic metallic components and resulted in a series of well documented problems in living beings because they cannot be completely degraded (Malarkodi *et al*., 2007). Hence, industrial effluents create lot of environmental problems and health hazards and are becoming more complex and critical not only in developing countries like India but also in developed countries. The Indian textile industry is the world's second largest after China. In the present study, attempts were made to determine the physico-chemical property of afforested soil and also to isolate and identify the status of beneficial microbes present in the rhizosphere of different tree species available in the afforested sites.

Materials and Methods

Sample collection

The Tamil Nadu Forest Department (TNFD) has done afforestation of textile industry waste water and urban sewage effluent polluted areas in Sarkarperiyapalayam and Kasipalayam, Tirupur district, Tamil Nadu, Southern India, by planting trees such as *Azadirachta indica, Casuarina equisetifolia, Eucalyptus tereticornis*, *Pongamia pinnata* and *Thespecia populnea.* The roots and rhizosphere soil samples were collected from the root zone of the above mentioned trees in zib lock poly bags and brought to the laboratory for further analysis. All the root samples were washed gently with tap water and immediately fixed in formalin- acetic acid- alcohol (FAA-50% 100 ml ethyl alcohol + 5ml glacial acetic acid + 13 ml formalin) and the soil samples were kept under refrigerator until the spores of AM fungi were processed.

Two sample locations where the TNFD had done afforestation works in textile industry waste water and urban sewage effluent polluted water areas *viz*., Sarkarperiapalayam (S1) and Kasipalayam (S2) respectively in Tirupur were chosen for the present study.

Physico-chemical analysis

Physico-chemical parameters like pH, Electrical Conductivity (EC), presence of Phosphorus, Potassium, Calcium, Magnesium, Manganese, Iron and heavy metals such as Copper and Zinc were analyzed from the collected soil samples based on APHA (1992).

Isolation and identification of beneficial microbes from rhizosphere soil sample

Serial dilution and plating techniques as described by Subba Rao (2007) was adopted for enumerating the population of beneficial bacteria. These isolates were further identified up to genera level according to Martin *et al.* (2006).

Isolation and Identification of AM spores

Rhizosphere soil (100g) was thoroughly mixed and dispersed in one liter water and the suspension was left undisturbed for 15 minutes to allow the heavier particles to settle. Then the suspension was decanted through 710, 250 and 45 µm sieves and remains on the sieves were washed into beakers (Gerdemann and Nicolson, 1963). After settlement of heavier particles, the supernatant was filtered through girded filter papers. Each filter paper was spread on the petri dish and observed under a dissecting microscope. The intact AM spores were counted and picked up with a wet needle and mounted in polyvinyl glycerol-lactophenol with or without Melzer's reagent on a micro slide for identification. The intact and the crushed spores were examined under a compound microscope and identified as per Trappe, (1982) and Schenck and Perez, (1987) methods.

Estimation of percent root colonization of AM fungi

Root samples were washed gently with tap water to remove FAA solution completely and then processed for estimation of percent root colonization of AM fungi. The root segments were cleared and stained in Trypan blue solution (Phillips and Hayman, 1970). The stained roots were examined with a Nikon compound microscope and the per cent root colonization was estimated according to magnified intersection method (McGonigle *et al.,* 1990).

Results and Discussion

Physico-chemical parameters of soil samples

The soil samples were analyzed for various physico-chemical properties and the data are furnished in Table 1. Both the soil samples (S1 and S2) displayed slightly alkaline pH (8.2 and 8.3). Malarkodi *et al.* (2007) reported that the highest pH values were noticed in the areas nearer to textile and dyeing industries in Tamil Nadu such as Karamadai (8.96), Thenthirupathi (8.96), Ponnaiyarajapuram (9.24) and Thelungupalayam (9.30). This might be attributed to the addition of alkaline earth metals, like Ca, Mg and alkali metals like Na, present in the effluent water in higher proportion. Electrical conductivity which represents total ions concentration ranged from (0.19 dSm^{-1} in S1 and 1.36 dSm^{-1} in S2). The measurement of electrical conductivity can be used as a quick way to locate potential soil and water quality problems. It is commonly used as a measure of salinity of soil (Ishaya *et al*., 2011). A concentration of 26.1 ppm and 22.2 ppm (mean +/- values) of Phosphorus in S1 and S2 was recorded in S1 and S2 respectively. The available potassium was found to be high in both S1 and S2 (241.6 ppm and 281.6 ppm). Available potassium content of soil increased significantly by the waste water application. Calcium and magnesium are very important elements for plant life. In the present study, the concentration of Calcium and Magnesium was found to be 0.40 meq/100g and 0.32 meq/100g calcium; and 0.05 ppm and 0.04 ppm magnesium respectively in both S1 and S2 samples respectively. The range of presence of heavy metals such as Iron, Copper, and Zinc in S1 and S2 was found to be in the concentration of 14.2 ppm and 12.6 ppm; 0.8 ppm and 0.6 ppm; 1.1 ppm and 0.5 ppm respectively.

Table 1: Physico-chemical parameters of soil samples

S. No.	Physico-chemical parameters	Sample locations	
		S1	S2
1.	pH	8.2	8.1
2.	Electrical Conductivity (dSm^{-1})	0.19	1.36
4.	Available Phosphorus($Kg\,ha^{-1}$)	26.1	22.2
5.	Available Potassium (ppm)	241.6	281.6
6.	Calcium (meq/100g)	0.40	0.32
7.	Manganese (ppm)	3.4	4.4
8.	Iron (ppm)	14.2	12.6
9.	Magnesium (ppm)	0.05	0.04
10.	Copper(ppm)	0.8	0.6
11.	Zinc(ppm)	1.1	0.5

S1- Sarkarperiyapalayam soil, S2- Kasipalayam soil

Population density of beneficial microbes

The population density of beneficial bacterial isolates are presented in Table 2 and Fig. 1. The bacterial colonies isolated from soil samples, gave countable colonies, but the growth of the colonies decreased when the dilution factor increased. The rhizosphere soil of *Azadirachta indica* of both S1 and S2 had shown the maximum population density of Phosphate solublising bacteria (PSB), *Azotobacter* sp. and *Azospirillum* sp., followed by *Casuarina equsetifolia.* It is interesting to note that the population density of bacterial isolates was found to be low in other rhizosphere soil samples compared to *Azadirachta indica* and *Casuarina equsetifolia*. The reason for these comparative increase and decrease in population may be due to the phytoremediation of textile sewage contaminated soil by specifies tree species along with beneficial microbes. Comparative studies by Fliessbaach *et al.* (1994) and McGrath *et al.* (1995) had shown reductions in microbial biomass or soil enzyme activities for agricultural soils amended with metal-containing sewage sludge.

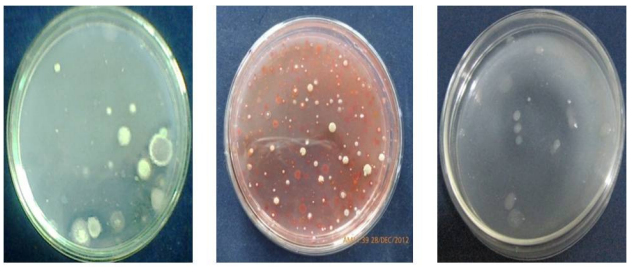
Table 2: Population density of PGPR from various sampling locations*

Sl. No.	Name of the host plant under samples collected	Sample location	Population density of beneficial bacteria (CFU/g)		
			PSB	*Azotobacter* sp.	*Azospirillum* sp.
1.	*Casuarina equsetifolia*	Sarkar Periyapalayam	25 x 10^5	24 x 10^5	26 x 10^5
2.	*Pongamia pinnata,*	Sarkar Periyapalayam	11 x 10^5	16x 10^5	7x 10^5
3.	*Thespecia populnea*	Sarkar Periyapalayam	10x 10^5	8x 10^5	5x 10^5
4.	*Azadirachtaindica*	Sarkar Periyapalayam	22 x 10^5	33 x 10^5	44 x 10^5
5.	*Acacia nilotica,*	Kasipalayam	6 x 10^5	20 x 10^5	14 x 10^5
6.	*Casuarina equsetifolia*	Kasipalayam	33 x 10^5	7 x 10^5	14 x 10^5
7.	*Azadirachta indica*	Kasipalayam	44 x 10^5	22 x 10^5	52 x 10^5
8.	*Pongamia pinnata*	Kasipalayam	5 x 10^5	6 x 10^5	12 x 10^5
9.	*Eucalyptus tereticornis*	Kasipalayam	7 x 10^5	11 x 10^5	13 x 10^5

*Mean of 3 replications

Population density of AM fungal spores and percentage root colonization

Attempt was made to investigate the population density of AM fungal spores and percentage of root colonization in different rhizosphere soil and root samples respectively and it is presented in Table 3 and Fig. 2. It found that AM fungal spores were present in rhizosphere soil of all the samples screened. Maximum number of AM fungal spores was observed in the soil sample collected from the rhizosphere soil of *Azadirachta indica* (180/100gm soil) followed by *Eucalyptus tereticornis* (140/100 gm soil) and *Casuarina equsetifolia* (135/100gm soil). Minimum numbers of spores were seen in the rhizosphere soil samples of *Thespecia populnea* (90/100 gm soil). These studies correlate the work done by Mohan *et al.* (1995) and Mohan and Singh (1996).



PSB *Azospirillum* sp *Azotobacter* sp

Fig. 1: Population density of beneficial PGPR

Percentage root colonization of AM fungi

Data on percent root colonization of AM fungi were recorded in different tree species and shown in (Table 3 & Fig. 3). The persistence of AM fungal colonization was found in root samples of all the tree species screened. Significant per cent root colonization was found in *Azadirachta indica,* (97%) followed by *Casuarina equsetifolia* (91%). The lowest per cent root colonization was observed in *Eucalyptus tereticornis* (35%). A study done by Mohan and Neelam verma (1995) and Mohan *et al.* (1995), the AM fungal association with different tree seedlings in arid zone of Rajasthan and found that the roots of *Azadirachta indica* had greater percent of root colonization.

Table 3: Status of AM fungal spore population and percent root colonization in different samples*.

S. No.	Host plant	Location Name			
		Sarkarperiapalayam		Kasipalayam	
		AM spore population/ 100 g soil	% root colonization	AM spore population/ 100 g soil	% root colonization
1	*Acacia nilotica*	–	–	128	56
2	*Azadirachta indica*	180	60	150	97
3	*Casuarina equisetifolia*	135	90	110	67
4	*Eucalyptus tereticornis*	–	–	140	35
5	*Pongamia pinnata*	100	39	120	45
6	*Thespecia populnea*	90	40	–	–

•Mean of 3 replications

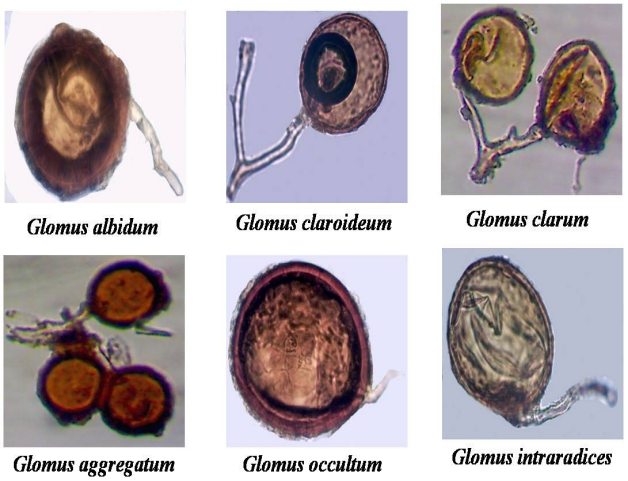


Fig. 2. Diversity of different AM fungal spores

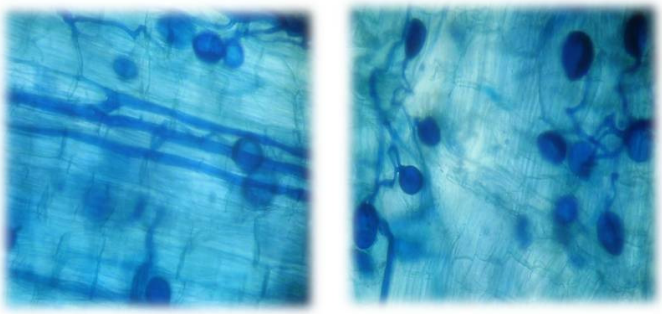


Fig. 3. Root colonization of AM spores in different tree species

Vesicular and hyphal structures in root segments of *Azadirachta indica* (x 100)

Conclusion

The present investigation highlights the presence of different beneficial microbes from the rhizosphere soil analyzed from afforested area in Tirupur district, Tamil Nadu, India. It is found that *Azadirachta indica* and *Casuarina equsetifolia* supported the maximum growth of beneficial microbes including PSB, *Azotobacter* sp., *Azospirillum* sp. and AM fungi in rhizosphere soil. Difference in microbial population is a reflection of many factors such as nutrient, oxygen levels, temperature, pollution, and availability of minerals etc. Further study about the plant and microbial interaction in rhizosphere soil is essential to reveal the fact of diversity status of microbes. These species, therefore, can be used as a potential phytoremediator for polluted sewage contaminated soils and to mitigate soil pollution and can be recommended for afforestation in different polluted areas.

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RESEARCH REPORTS

Bacteria connect to each other and exchange nutrients

It is well-known that bacteria can support each others' growth and exchange nutrients. Scientists at the Max Planck Institute for Chemical Ecology in Jena, Germany, and their colleagues at the universities of Jena, Kaiserslautern, and Heidelberg, however, have now discovered a new way of how bacteria can achieve this nutritional exchange. They found that some bacteria can form nanotubular structures between single cells that enable a direct exchange of nutrients.

Bacteria usually live in species-rich communities and frequently exchange nutrients and other metabolites. Until now, it was unclear whether microorganisms exchange metabolites exclusively by releasing them into the surrounding environment or whether they also use direct connections between cells for this purpose. Scientists from the Research Group Experimental Ecology and Evolution at the Max Planck Institute for Chemical Ecology in Jena, Germany addressed this question using the soil bacterium *Acinetobacter baylyi*and the gut microbe *Escherichia coli*. By experimentally deleting bacterial genes from the genome of both species, the scientists generated mutants that were no longer able to produce certain amino acids, yet produced increased amounts of others.

In co-culture, both bacterial strains were able to cross-feed each other, thereby compensating the experimentally induced deficiencies. However, separating the two bacterial strains with a filter that allowed free passage of amino acids, yet prevented a direct contact between cells, abolished growth of both strains. "This experiment showed that a direct contact between cells was required for the nutrient exchange to occur," explains Samay Pande, who recently obtained his PhD at the Max Planck Institute in Jena on this research project and now started a postdoc at the ETH Zürich.

Observing the co-culture under the electron microscope revealed structures that formed between bacterial strains, which functioned as nanotubes and enabled the exchange of nutrients between cells. Especially remarkable, however, was the fact that only the gut microbe *Escherichia coli* was capable of forming these structures and connecting to *Acinetobacter baylyi* or other *E. coli* cells. "The major difference between both species is certainly that *E. coli* is able to actively move in liquid media, whereas *A. baylyi* is immotile. It may thus be possible that swimming is required for *E. coli* to find suitable partners and connect to them via nanotubes," explains Christian Kost, head of the Research Group Experimental Ecology and Evolution, which is funded by the Volkswagen Foundation.

KNOW A SCIENTIST

Dr. Jonas Salk was an American physician and medical researcher. In 1955 Salk's years of research paid off. Human trials of the polio vaccine effectively protected the subject from the polio virus. When news of the discovery was made public on April 12, 1955, Salk

was hailed as a miracle worker. He further endeared himself to the public by refusing to patent the vaccine. He had no desire to profit personally from the discovery, but merely wished to see the vaccine disseminated as widely as possible. Salk's vaccine was composed of "killed" polio virus, which retained the ability to immunize without running the risk of infecting the patient. A few years later, a vaccine made from live polio virus was developed, which could be administered orally, while Salk's vaccine required injection.

Dr. Salk's last years were spent searching for a vaccine against AIDS. Jonas Salk died on June 23, 1995. He was 80 years old. The 100th anniversary of his birth in 2014 was the occasion for renewed appreciation and celebration of Dr. Salk's contribution to humanity.