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Research Article

INVESTIGATION OF SOIL CHARACTERS AND MICROBIAL DIVERSITY OF PERAVURANI TALUK SOIL OF THANJAVUR DISTRICT, TAMILNADU

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ABSTRACT

Soil contains many kinds of microorganisms. Soil is fundamental and irreplaceable; it governs plant productivity of terrestrial ecosystem and it maintains biogeochemical cycles because microorganisms in the soil degrade, sooner or later, virtually all organic compounds including persistent xenobiotics and naturally occurring polyphenolic compounds. This study deals with the microbial diversity at three sites of traditional paddy field in Peravurani taluk, Thanjavur district, Tamil Nadu. Study period was one year covering all the four seasons viz, Monsoon (October to December), Post monsoon (January – March), Summer (April to June), Pre monsoon (July to september). The physico-chemical properties of collected soil were analyzed. About, 47 different colonies were isolated from the 12 soil samples. The colonies distributed in 24 different bacterial species belonging to the 16 genera were isolated by using Nutrient agar medium and identified by colony, morphological and biochemical test and 23 different fungal species belonging to 9 genera were isolated by using Rose Bengal Agar medium and identified by Lacto phenol cotton blue staining. The predominant bacterial species isolated were *Bacillus* and *Pseudomonas* and the predominant fungal species isolated were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Aspergillus sydowi, Aspergillus nidulans.* From this study inferred the seasonal variation in the nutrient availability, soil microbial population and physico-chemical parameters to comparable levels of microbial richness were observed.

INTRODUCTION

Soil is the mixture of minerals, organic matter, gases, liquids and the countless organisms that together support life on Earth. Soil is a natural body known as the pedosphere and which performs four important functions such as it is a medium for plant growth, water storage, supply and purification. It is a modifier of Earth's atmosphere. It is a habitat for organisms; all of which, in turn, modify the soil.

Soil is considered to be the "skin of the Earth" and interfaces with its lithosphere, hydrosphere, atmosphere and biosphere. The term pedolith, used commonly to refer to the soils, literally translates 'level stone'. Soil consists of a solid phase (minerals and organic matter) as well as a porous phase that holds gases and water. Accordingly, soils are often treated as a three state system of solids, liquids and gases.

In the soil microbes are dynamic equilibrium. The microbes require some basic nutrients such as nitrogen, phosphorous and potassium for normal growth. The fertility level of soil is influenced by the availability of these elements. The temperature changes can also influences microorganisms both quantitatively and qualitatively. The organic matter influences the nature and properties of soil and affect the activity of microorganisms in the soil and soil pH also place an important role in microbial activity.

Types of Soil

Soil generally contains less than1-4 per cent organic matter, but they may consist of 20 per cent colloidal organic matter. Additionally an organic layer up to 30 cm deep may be found on the soil surface (Visser and Parkinson, 1992). According to the size, soil particles are graded into clay, slit, fine sand, coarse sand, stones and gravel.

Name of the particle	Diameter range (mm)
Clay	Less than 0.002
Silt	0.002-0.02
Fine sand	0.02-2.0
Coarse sand	0.20-2.0
Stones and Gravel	Above 2.0

Soil is made up of many things like weathered rock particles and decayed plant and animal matter with varying ratios of

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minerals, air, water and organic materials. Soil fertility is an important factor, which determines the growth of plant. Soil fertility is determined by the presence or absence of nutrients. i.e. macro and micronutrients

Nitrogen, Phosphorous and potassium are the major nutrients considered as the macro nutrients. These nutrients are playing an important role in ecosystem and plant promoting factors. Organic carbon is the major source of environmental factors. Organic carbon which enters the soil from plant sources (e.g., as cellulose) is usually released as carbon dioxide or methane. Disturbance in soil carbon cycling, which has been observed under field conditions, is the result of several environmental factors.

Nitrogen cycling includes processes like mineralization, immobilization, nitrification, denitrification, and nitrogen fixation, of which nitrogen fixation and nitrification are most easily disrupted. Nitrifying bacteria are reported to be sensitive to acidic environment and require aerobic conditions. Waterlogged soils can become anoxic and thus may not support nitrification. Symbiotic nitrogen fixation by bacteria in root nodules of legumes is known to be a delicate and complicated phenomenon, and can be disrupted by some kinds of pollutants.

Phosphorous cycling can be affected by processes that interfere with mycorrhizal fungi. Cycles involving sulphur, iron, manganese and other elements also depend upon the significant roles played by microorganisms.

Micronutrients Zinc (Zn), Copper (Cu), Magnesium (Mg), Calcium (Ca), Ferrous (Fe), Molybdenum (Mo), Boron (B) are referred as micronutrients. These elements are required in minute quantities for plant growth, but have the same agronomic importance as macronutrients have and play a vital role in the growth of plants. Micronutrients also increase plant productivity, leaf and grain yield. Most of the micronutrients are associated with the enzymatic system of plants. Whenever a micronutrient is deficient, the abnormal growth of plant results which sometime cause complete failure of plants. Grains and flower formation does not take place in severe deficiency. The main sources of these micronutrients are parent material, sewage sludge, town refuse, farmyard manure (FYM) and organic matter.

Biodiversity is defined as, “the variability among the living organisms from all sources including terrestrial, soil, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species and between species of ecosystems” (Breure, 2004). Biodiversity is extremely, complex, dynamic and varied like no other feature of the Earth. Its innumerable plants, animals and microbes physically and chemically unite the atmosphere (the mixture of gases around the Earth), ecosphere (the solid part of the Earth), and hydrosphere (the Earth’s water, ice and water vapour) into one environmental system which makes it possible for millions of species, including people, to exit. At the same time, no other feature of the Earth has been dramatically influenced by man’s activities. By changing biodiversity, we strongly affect human well-being and the well being of every other living creature.

Bacteria are the most dominant group of microorganisms in soil, water and probably equal one half of the microbial biomass in soil. They are present in all type of soil but the population decreases as the depth of soil increases. Under anaerobic conditions, bacterial dominate the scene and the microbiological activities in soil since fungi and actinomycetes do not grow in absence of oxygen.

Type of cultivation and crop management practices found to have greater influence on the activity of soil microflora. Fungi are an important component of soil microbiota more in abundance than bacteria, depending on soil depth and nutrient conditions. Different soils have specific fungal flora, but the majority of species found in them are cosmopolitan (Ainsworth and Sussman, 1968).

Fungi are fundamental for soil ecosystem functioning (Warcup, 1951), especially in forest and agricultural soils, they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization. It was estimated 1.5 million fungal species are present in natural ecosystems, but only 5-10% has been described formally (Hawksworth, 2001).

Microfungi play important role in nutrient cycling by regulating soil biological activity (Arunachalam *et al*., 1997). However, the rate at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions. There have been number of studies on the distribution of soil microfungi in agricultural field. Some studies dealt with the influence of plant community (Chung *et al*., 1997) and other attempted to examine seasonal trends (Kennedy *et al*., 2005).

The objectives of the study are to collect the soil samples from different location of Peravurani Taluk, and to analyse the physico-chemical properties of the soil. Heavy metals of the soil were analyzed by using Atomic Adsorption Spectrophoto metric method. To isolate and identify the bacteria and fungi from soil by standard methods and to study the statistical values for above research.

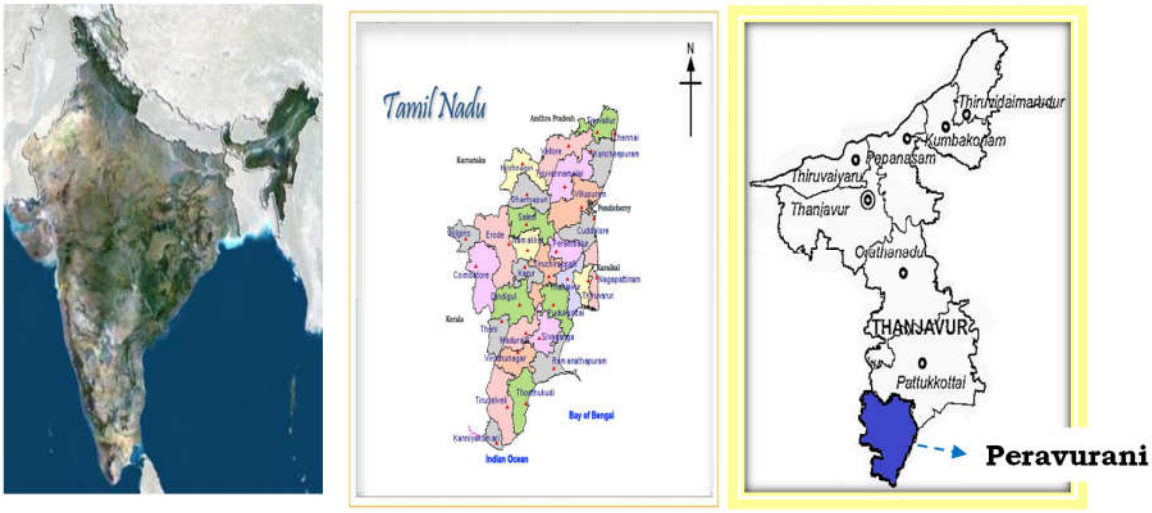
MATERIALS AND METHODS

Description of the study area

The present study focused on the area in Peravurani taluk at Thanjavur district in Tamil Nadu state. The study area is situated at 10.3^0 North latitude, 79.18^0East longitude and 18 meters elevation above the sea level. Peravurani is a Panchayat town in Thanjore district in the Indian state of Tamilnadu having about 21,663 inhabitants. It is the administrative headquarters of the Peravurani Taluk. It is a selection grade town Panchayat. This town lies in the new delta region of River Cauvery.This region is irrigated by Kallanai Kalvai and its distributories. This area mostly consists of Alluvial soil. Agriculture is the main economy of this region. Rice is extensively grown here and is the staple of this people. This area is the highest producer of coconut in the state. Coir industry is also an important livelihood of people here due to the large generation of coconut byproducts. Dairy and dairy products are also a major source of income in this region. In Thanjavur district, 13 soil series were identified. Peravurani soil sereies occupied 2.06 percent. In peravurani soil series paddy and pulses are grown.In Peravurani soil series , include grayish brown, very deep, alkaline, fine loamy, calcarious soils,

setting on the sides of the jungle riverrs having proximity to the sea Sandy loam- cultivated. The study area was designated as S1- Aathalur, S2- Kalathur, S3- Nattanikkottai.

Map of the Study Areas



Collection of Soil Samples

Soil sample was collected from Peravurani taluk, Thanjavur district during the season, January to March- Post monsoon, April to June - Summer, July to September- Premonsoon,October to December – Monsoon.

Soil samples were collected from the selected villages of Thanjavur District, Tamil Nadu, and India. Five spots were fixed in a plot for taking one composite mixture of the soil. The surface of the field was scrapped away to obtain a uniformly thick slice of soil from the plough depth from each place. A V-shaped cut was made with a spade to remove 1 to 2 cm slice of soil. The sample collected on the blade of the spade was put in a clean bucket. In the same way the samples were collected from all the spots selected for one sampling unit. Thus the samples were poured on the clean paper and mixed thoroughly. Then the samples were spread evenly and divided into four equal parts. The two opposite quarters were rejected and the remaining samples were mixed. The same process was repeated until the reach of half kg of soil. The sample was collected in a clean bag and marked properly. The mouth of the bag was tied carefully.

Soil physico-chemical properties

The collected samples were brought to the laboratory by a sterile Polythene bag and sieved through 2mm sieve at field moist conditions and determinations of soil moist content, temperature was analyzed. After removing the debris, the soil samples were suspended in distilled water (1:2 W/V) and allowed to settle down the sand particles. The pH of the suspension was determined using pH meter (Systronics, India). Electrical conductivity of the soil was determined in the filtrate of the water extract using conductivity Bridge as described by Jackson (1973), Organic carbon (OC) content was determined by adopting chromic acid wet digestion method as standard procedure of Walkley and Black (1934), available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956), available Phosphorus by Brayl method (Bray and Kutz, 1945) and available potassium was extracted from soil with neutral 1N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949).

Calcium and magnesium was extracted with neutral 1N ammonium acetate and the available calcium and magnesium in the extract was determined by versenate method (Jackson, 1973).

Heavy metal determination

Available micronutrients such as Zn, Cu, Mn were determined in the diethyl triamine pentaacetic acid extract of soil using Perkin-Elmer model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as Fe were analysed following methods of Bernes (1959); Muthuvel and Udayasoorian (1999).

Isolation of bacteria

Isolation of bacteria from the soil samples were carried out by pour plate method by using Nutrient agar media. The bacteria were identified with the help of standard manual.

Identification of bacteria

The culture were identified at the genus level by using the Gram's staining (Han's Christian Gram, 1884), motility test and biochemical test (Aneja, 2002) including indole, MR-VP, Citrate, Urease, Catalase, Oxidase test.

Isolation of fungi (Warcup, 1955)

Isolation of fungi from the soil samples were carried out by soil dilution plate method by using Rose Bengal Agar media. The fungi were identified with the help of standard manuals (Barneutt, 1998; Ellis, 1993; Gillman, 2001; Raper and Fennel 1965 and Subramanian, 1971).

Identification of fungi (Zafar et al., 2006)

The culture were identified at genus level on the basis of macroscopic (colony morphology, color, texture, shape and appearance of morphology) and microscopic characteristics (saptation in mycelium), presence of specific reproductive structures, shapes and structure of conidia.

RESULT AND DISCUSSION

Analysis of Physico-chemical Parameters

In the present study, pH, moisture, temperature, organic carbon, Electrical conductivity were analysed. The maximum pH range (8.2- S3) was observed, in post monsoon period. The maximum temperature (60^0- S3) was observed, in summer season. The maximum moisture content (55%-S3) was observed, in monsoon season. The maximum electrical conductivity (0.42 dsm^{-1}- S2) range was observed, in post monsoon season (Table – 1)

The chemical parameters, the maximum organic carbon content (0.46%- S2) was noticed in pre monsoon season. The maximum level of N content (0.99%- S3) was noticed in premonsoon season. The high level of phosphorus content (0.154%-S1) was noticed in monsoon season. The maximum level of potassium content (1.82%-S1) was noticed in monsoon season. The maximum level of calcium range (10.2%-S1) was noticed in post monsoon season. The high level of magnesium level (10.3%-S1) was observed in monsoon season (Table – 1).

The environmental factors such as p^H, moisture, electrical conductivity and temperature play an important role in the distribution of microflora (Christensen, 1989; Bisset and Parkinson, 1979)

The distribution of soil microbial population is determined by a number of environmental factors (Kennedy *et al*., 2005). The diversity and distribution of different organisms in the marine environment are influenced by the physic-chemical properties (Rani and Pannerselvam, 2010).

The results of physico-chemical properties of soil samples from 10 different locations of Thiruvarur district were analysed, out of 10 soil samples, 5 samples were sandy clay loam soil, while 3 samples were loamy soils and 2 samples were sandy loam. The maximum pH (8.1) of the soil was recorded at Muthuppetai and maximum pH (7.2) was recorded at Kudavasal. The bulk density of the soil was maximum $1.60g/cm^3$ recorded at Needamangalam and minimum was $1.11g/cm^3$ recorded at Kottur. The maximum (33.41%) water holding capacity (WHC) of the soil was recorded at Thiruthuraipoondi and maximum 18.31% was recorded at Kudavasal. The electrical conductivity of soil was maximum (0.89) recorded in Kudavasal and maximum was (0.24) in Mannargudi (Senthilkumar and Pannerselvam, 2013).

Heavy metal analysis

The present study investigation that heavy metal analysis from the soil samples was the maximum level of Zn (1.96%-S1) in summer season. The maximum level of Cu (3.27%-S1) in post monsoon season. Fe (9.90%-S1) in monsoon season. Mn level (4.68%-S3) in post monsoon (Table – 2).

The available micronutrients like Zn of the soil were recorded maximum as 2.02% and minimum as 1.06% was at Vellamperambur. The Cu content of the soil was maximum (3.78%) at Thittakudi and minimum 1.27% was in Raramuthirakottai soil. The Fe content of the soil was maximum (10.47%) at Vallam and minimum (7.10%) in Thittakudi soil. The Mn content of the soil maximum as 5.95% was recorded in Peravurani soil and minimum (2.66%) was at Saliyamangalam soil. The maximum content of B (0.594%) was recorded in Soorakottai and Nallavanniyankudikadu soils and minimum 0.28% was in Thiruvalanjul soil (Kanimozhi and Paneerselvam, 2011)

Isolation of Bacteria

Nutrient agar medium was used to isolate the bacterial species from the soil. Here, 10^{-4} to 10^{-6}dilutions were taken for the bacterial isolation. Totally, 24 different species of soil bacteria were observed from soil samples. The bacterial species were identified by their morphological, biochemical character, using Gram's staning and Bergey's manual of determinative bacteriology.

The bacterial species were *E.coli*, *Pseudomonas spp, Staphylococcus spp, Bacillus cereus, Micrococcus, Proteus, E.aerogens, B.subtilis, P.syringae, P.alcaligens, Azospirillium spp, Rhizobium spp, B.circulans, P.putida, Enterobacter spp, Agrobacterium, Streptococcus spp, Brevibacterium, P.fluroscence, Favibacterium spp, P.aerogens, Alcaligens spp, B.mucoides, Azotobacter* recorded the predominant bacterial species were *Bacillus* and *Pseudomonas* in soil (Table-3).

Kanimozhi and Panneselvam (2011) reported that highest population density of Azospirillium was observed in sandy loam soil. Senthil kumar *et al*., 2013 reported that total number of soil 10 morphologically distinct *Azospirillium* isolates were isolated. For enumeration of population density, the number of colonies on the plates was counted in the range of 76-190 colonies. The highest population density was observed in sandy loam soil at kudavasal. The lowest population density was observed in loamy soil at Thiruthuraipoondi.

In the present study, total number of 24 morphologicaly distinct bacterial species isolates was isolated and tabulated. For enumeration of population density, the number of colonies on the plates was counted in the range of 310-380 colonies. The highest population density was observed in sandy loam to sandy clay soil (S1- Aathalur) in post monsoon season. The lowest population density was observed in sandy loam to sandy clay soil (S3-Nattanikkottai) in Monsoon season (Table 4).

The percentage contribution of the bacterial species were *E.coli* (2.9%), *Pseudomonas spp* (3.9%), *Staphylococcus spp* (2.9%), *Bacillus cereus* (1.9%), *Micrococcus* (0.98%), *Proteus* (3.9%), *E.aerogens* (15.6%), *B.subtilis* (3.9%), *P.syringae* (1.9%), *P.alcaligens* (7.8%), *Azospirillium spp* (12.7%), *Rhizobium spp* (1.9%), *B.circulans* (0.98%), *P.putida* (1.9%), *Enterobacter spp* (0.98%), *Agrobacterium* (6.8%), *Streptococcus spp* (7.8%), *Brevibacterium* (7.8%), *P.fluroscence* (3.9%), *Favibacterium spp* (6.8%), *P.aerogens* (5.8%), *Alcaligens spp* (8.8%), *B.mucoides* (3.9%), *Azotobacter* (2.9%). The highest percentage contribution was observed by this study *P.alcaligens* (7.8%) (Table-5).

Isolation of fungi

Fungal population present in the soil sample were determined by plating technique the soil dilution of 10^{-2} to 10^{-5} dilution over solidified Rose Bengal Agar medium. Totally, 23 different species of soil fungi were observed from the soil samples collected from three different localities. The colonies showed a characteristic color of black, green, white and brown and they

were confirmed by identifying their morphological characters and by Lactophenol cotton blue method. The isolated organisms were identified by using Manual of Soil Fungi (Gillman, 1957), Dematiaceous Hypomycetes (Ellis, 1971), more Dematiaceous Hypomycetes (Ellis and Ellis, 1976), Hypomycetes (Subramaniam, 1971).

The fungal species were *Aspergillus niger, Penicillium spp, Alternaria alternatae, Aspergillus nidulans, P.citrinumn, Mucor, Fusarium sp, P.bovis, Aspergillus terreus, Rhizopus stolinifer, Aspergillus oryzae, Saccharomyces spp, Aspergillus sydowi, Cladosporium sps, Verticillium sps, Trichoderma viridiae, P.conidia, P.chrysogenum, Aspergillus fumigates,Aspergillus granulates, Fusarium solani, Trichoderma harizanum, Aspergillus flavus.* The fungal species were *Aspergillus* and *Penicillium spp* were predominant in soil (Table-6).

In the present study, totally 23 morphologicaly distinct fungal isolates were isolated and tabulated. For enumeration of population density, the number of colonies on the plates was counted in the range of 310-360 colonies. The highest population density was observed in sandy loam to sandy clay soil (S3- Nattanikkottai) in summer season and sandy loam to sandy clay soil (S1- Aathalur) in monsoon season. The lowest population density was observed in S1-Nattanikkottai in Monsoon season (table 7).

Kanimozhi and Paneerselvam (2011) were reported, a total number of 30 morophologically distinct *Azospirillium* isolates were isolated and tabulated. For enumeration of population density, the number of colonies on the plates was counted in the range of 68-210 colonies. The highest population density was observed in sandy loamy soil at Thitubuvanam. The lowest population density was observed in sandy clay loamy soli at Thiruvakanjuli.

The percentage contribution of the fungal species were *Aspergillus niger* (2%), *Penicillium spp* (4%), *Alternaria alternatae* (15%), *Aspergillus nidulans* (4%), *P.citrinumn* (4%), *Mucor* (7%), *Fusarium sp* (4%), *P.bovis* (3%), *Aspergillus terreus* (4%), *Rhizopus stolinifer* (3%), *Aspergillus oryzae* (9%), *Saccharomyces spp* (4%), *Aspergillus sydowi* (7%), *Cladosporium sps* (4%), *Verticillium sps* (6%), *Trichoderma viridae* (5%), *P.conidia* (7%), *P.chrysogenum* (4%), *Aspergillus fumigates* (3%), *Aspergillus granulates* (7%), *Fusarium solani* (7%), *Trichoderma harizanum* (4%), *Aspergillus flavus* (3%). The high level of percentage contribution was observed *Aspergillus oryzae* (9%) (Table-8)

The soil microflora in different crop fields like Paddy, Pulses, Ragi, Sugarcane, Vegetable and Banana were observed. The *Curvularia lunata* (6.8%), *Alternaria alternate* (6.2%), *Penicillium fumiculosum* (13.6%), *Penicillium chrysogenum* (11.1%), *Fusarium solani* (8.1%), *Rhizopus stolonifer* (3.1%), *Mucor spp* (13.1%), *Aspergillus flavus* (16.1%), *Aspergillus terreus* (8.7%) *and Aspergillus niger* (13.1%) were isolated and characterized (Chandrashekar *et al*., 2014)

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