



RESEARCH ARTICLE

EFFECT OF CLIMATIC CHANGES ON BACTERIAL AND FUNGAL DIVERSITY OF SIRKAZHI
TALUK NAGAPPAINAM DISRICT, TAMILNADU, INDIA

Tamilkodi, R., *Victoria, J. and Dr. V. Dhivaharan

Department of Microbiology, Sundarakkottai, Mannargudi, Tamilnadu, India

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ABSTRACT

The present study deals with the diversity and distribution of fungal and bacterial population in various seasons (Monsoon, Premonsoon, Summer and Postmonsoon) from three different places of Sirkazhi Taluk (Thenpathi (S1), Kovilpathu (S2) and Sirkazhi (S3)). The physicochemical parameters of such soils were identified. The physical parameters include the analysis of Soil Color, Texture, Electrical Conductivity (mmhos/cm.), P^H, Moisture content (%) and Temperature (°C) of the soils. The chemical parameters include the analysis of Calcium (mg/g), Magnesium (mg/g), Zinc (ppm), Ferrous (ppm), Manganese (ppm), Organic Carbon (ppm), Nitrogen (kg/ac), Potassium (kg/ac) and Phosphorus (kg/ac) present in three crop land soils collected. Totally, 19 different species of soil bacteria were observed from the soil samples. The highest percentage of frequency of bacteria isolated from all the soil samples were recorded, in which, *Sreptococcus*, *Pseudomonas fluorescens*, *Flavobacterium* and *Neisseria* were predominant followed by the other species of bacterium. Totally 19 different species of soil fungi were observed from the soil samples. The highest percentage was recorded for *Aspergillus flavus*, *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus oryzae* and *Trichoderma viridae* followed by the other fungal species.

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INTRODUCTION

The term, soil refers to the outer, loose material of earth's surface, a layer distinctly different from the underlying rocky strata. Agriculturally, it is the region supporting plant life and from which plants obtain their nutrients and mechanical support. From the microbiologist point of view the soil environment is unique in several ways; it contain different groups of microorganisms, viz., bacteria, fungi, actinomycetes, algae and protozoa; it is the site of biological interaction in nature; and most of the biochemical reactions concerned in the decomposition of organic matter, weathering of rocks and nutrition of agricultural crops occur only in this region (Stotzky, 1997a). Soil may be defined as a thin layer of earth's crust which serves as a natural medium for the growth of plants. It is the unconsolidated mineral matter that has been subjected to, and influenced by genetic and environmental factors – parent material, climate organisms and topography all acting over a period of time. Soil differs from parent material in the morphological, physical, chemical and biological

properties. They serve in varying degree as a reservoir of nutrients and water for crops, provide mechanical anchorage and favorable tilth. The components of soils are mineral material, organic matter, water and air, the proportions of which vary and which together form a system for plant growth; hence there is the need to study the soils in perspective.

Types of soil

Soil generally contains less than 1-4 per cent organic matter, but they may consist of 20 percent 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, silt, fine sand, coarse sand, stones and gravel.

S.No	Name of the particle	Diameter range (mm)
1	Clay	Less than 0.002
2	Silt	0.002-0.02
3	Fine sand	0.02-2.0
4	Coarse sand	0.20-2.0
5	Stones and Gravel	Above 2.0

*Corresponding author: Victoria, J.

Department of Microbiology, Sundarakkottai, Mannargudi, Tamilnadu, India.

Soil is classified into specific category by the quantities of mineral separates contained (Fanning and Fanning, 1989).

S.No	Textural groups	Relative proportion of different sized mineral particles
1	Sandy Soil	85% sand +15% Clay or silt or both
2	Loamy sand	70% sand+30% clay or slit or both
3	Silt	90% silt+10% sand

Soil bacteria

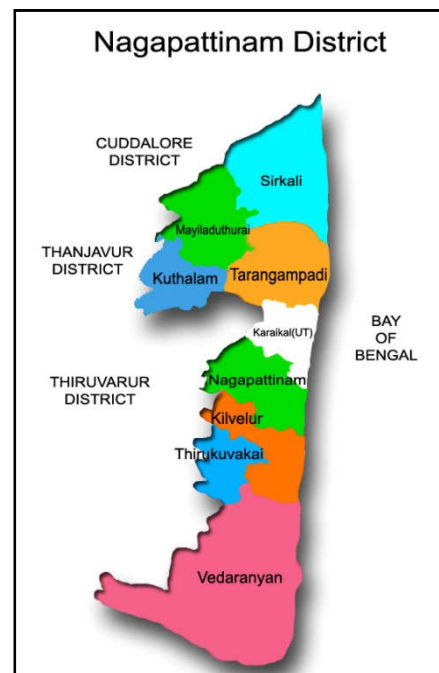
Soil bacteria play pivotal roles in various biogeochemical cycles (BGC) and responsible for the cycling of organic compounds. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition, plant health. Bacteria is the most numerous organisms in the soil, averaging between 10^6 to 10^9 organisms per gram of soil. Due to their small mass they only account of the small amount of the biomass of the soil. Nonsporulating rods, pseudomonades and actinomycetes are the most common bacteria in the soil.

Soil fungi

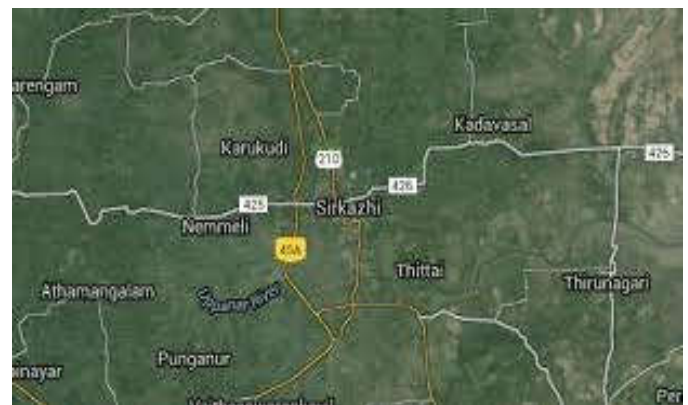
Soil is a complex ecosystem composed of multiple minute habitats and harbors almost all major taxonomic groups of fungi. The soil fungi vary from bacterium-engulfing slime molds, mycorrhizae and root pathogenic basidiomycetes (Stotzky, 1997b). Soil fungi range from microscopic in which the whole organisms consists of a single cell dry weight of less than 1×10^{-12} to the immense, which filaments of a single colonial microorganism can occur never an area of 15 heat are and the individuals has been estimated to have a weight of 1×10^7 g (Smith *et al.*, 1992).

Description of study site

Nagapattinam District in Tamilnadu State of India is spread over eight Taluks with a total geographical extent of 2715.83 sq.km. with the head quarter at Nagapattinam. This District lies on the shores of the Bay of Bengal between Northern Latitude 10.7906 degrees and 79.8428.Degrees Eastern longitude with eight Taluks and eleven panchayat unions. The study area is spread over in 2,32,257 hectares of land and has 499 revenue villages. Sirkazhi is located on the main route between Mayiladuthurai and Chidhambaram, Shri Arulmigu Sattanatha Swami temple has many wonderful architectural and sculptural features. This temple has been glorified in the divine songs of Thevaram. One of the four great divine poets, the Saiva Saint Thirugnana Sambandar was bestowed with the divine grace by Lord Siva and Parvathy here. Every year in the Tamil month of Chithirai, Thirumulaippal festival is celebrated in a grand manner.



Sirkazhi taluk



Geography and Climate Nagappattinam district

It is Located at Latitude-10.7, Longitude-79.8. Nagapattinam District is sharing border with Cuddalore District to the North, Thanjavur District to the west, Thiruvarur District to the west. It's in the 8 meters to 11 meters elevation range. This District belongs to Southern India. It is a Coastal district, a beach also there Nagapattinam District is sharing border with bay of bengal. It is Hot in summer. Nagapattinam District summer highest day temperature is in between 28° C to 36° C. Average temperatures of January is 26° C, February is 26° C, March is 28° C, April is 30° C, May is 31° C.

Table 1. Geographical extent of the Taluks in Nagappattinam District

S.No	Name of the Taluks	Name of the Panchayat Unions	Extent (ha)	Total Percentage	No. of Revenue Villages
1	Nagapattinam	1. Nagapattinam	30,231	13.02	84
		2. Thirumarugal			
2	Kilvelur	3. Kilvelur	27,445	11.82	53
		4. Keelaiyur			
3	Thirukkuvalai	5. Vedaranniyam	14,040	6.04	35
		6. Sirkazhi			
4	Vedaranniyam	7. Kollidam	47,029	20.25	51
		8. Sembanarkoil			
5	Sirkazhi	9. Mayiladuthurai	44,214	19.04	87
		10. Talainayar			
6	Tharangambadi	11. Kuthalam	27,726	11.94	67
7	Mayiladuthurai		24,485	10.54	67
8	Kuthalam		24,485	7.35	55
	Total		2,32,257	100.00	499

MATERIALS AND METHODS

Sample collection

Soil samples were collected from the villages namely Thenpathi (S1), Kovilpathu(S2) and Sirkazhi (S3) in Sirkazhi taluk of Nagapattinam District. They were geographically with dry lands, urban and flood affected area thereby with different variety of soil viz, clay loam and sandy clay soil. A V- shaped cut was made with a spade to remove 1 to 2 cm slice of soil. The sample was collected on the blade of the spade and put in a bucket. In this way all the samples were collected from all the spots marked for one sampling unit. The soil was poured from the bucket on a piece of clean paper or cloth and mixed thoroughly. The soil was spreader evenly and divided it into 4 quarters. Two opposite quarters was rejected and the rest of the soil was mixed again. The process was repeated till left with about half kg of the soil and collected and put in a clean cloth bag. Each bag should properly mark to identify the sample. The brought to the laboratory in sterilized polythene bags handpicked, air dried and stored in containers for further investigation.

Physicochemical Parameters

The physical parameters includes soil color, texture, p^H , moisture content, temperature and electrical conductivity and the availability of macronutrients such as organic carbon, nitrogen, phosphorus, potassium, magnesium, phosphate and calcium and Micro nutrients (Alloway, 1995) such as copper, iron, manganese and zinc was analyzed by relatively in present in sirkazhi Taluk of three villages such as Thenpathi (S1), Kovilpathu (S2), Sirkazhi (S3) of Nagapattinam District in Postmonsoon, Summer, Premonsoon and Monsoon season. Physical parameter was analyzed by using standard method and micro nutrients analyzed by Atomic Absorption Spectrophotometer (Saucer, 1980).

Isolation of bacteria and fungi

Serial dilution was performed by using the collected soil sample to isolate the fungal and bacterial population from the soil samples. The soil samples were diluted with conical flask containing 90ml of sterile distilled water and mixed thoroughly to make 1:10 dilution (10^{-1}). Then 10 ml of diluted sample was transferred to the next conical flask and serially diluted into the series of conical flask having 90ml of sterile distilled water with sterile pipettes, up to 10^{th} dilutions. Here, 10^4 to 10^7 dilutions were taken for the bacterial isolation and 10^2 to 10^5 dilution were taken for the fungal isolation (Ronald Atlas, 1998). Nutrient agar and Rose Bengal agar medium used for bacterial and fungal isolation respectively. Nutrient agar and Rose Bengal agar medium was prepared and sterilized at 121°C for 15 minutes at 15 lbs pressure. Petri plates were sterilized and properly labeled 1ml of sample from different dilution was transferred into the respective plates. Finally, the cooled medium was poured into the sample containing plates and incubated at 37°C for 24 hours for bacteria and 25°C for 72-96 hours for fungi and the colonies were counted. Different colonies were observed and transferred to their specific media for identification.

Conidial population

The number of Colony Forming Units (CFU) present in 1 gram of the soil samples were determined by multiplying the number of colonies with dilution factors.

Number of CFU's of fungi per gram dry weight of soil

$$\frac{\text{Meanno of colonies} \times \text{dilution factor}}{\text{Dryweight of the soil}}$$

In order to assess the dominance of individual species site percentage contribution was worked out as follows,

$$\% \text{ contribution} = \frac{\text{Total number of colonies}}{\text{Total number of individual colonies}}$$

Identification of bacteria and fungi

Gram staining (Han's Christian Gram, 1884), motility test and biochemical tests, Indole, MR-VP, Citrate Utilization test, Oxidase test, Catalase test, Triple Sugar Iron test and Carbohydrate Fermentation test were used to identify bacterial species and confirmed by using Bergey's Manual of Determinative Bacteriology. Lactophenol cotton blue technique was used to identify the fungi and by the confirmed fungal Manual, Dematiaceous Hypomycetes (Ellis, 1976).

RESULTS AND DISCUSSION

The environmental factors such as p^H , moisture, electrical conductivity and temperature play an important role in the distribution of microflora (Christensen, 1989). The positive correlation was observed with p^H , organic, inorganic compounds and moisture content of the soil on the population of fungi and bacteria. In the present investigation, the physico chemical parameters of crop land soil were observed, maximum level of temperature 47°C , p^H 7.87, moisture 55.2%, carbon 1.26 ppm, zinc 3.1 ppm, potassium 72.1 kg/hectare, and nitrogen 95.2 kg/hectare. Since, the moisture content is more in paddy crop when noted in Thenpathi (S1), Kovilpathu (S2) and Sirkazhi (S3) villages, contain high number of *Aspergillus*, *Trichoderma*, *Bacillus* and *E.coli*. The presence of some chemicals may enhance the growth of certain fungal species at Thenpathi village like *Aspergillus niger*, *Aspergillus oryzae*, *Saccharomyces* and *Rhizopus oryzae* and bacteria like *Bacillus cereus*, *Vibrio sps*, *Proteus vulgaris*, *Enterococcus sps* and *Streptococcus sps*. At kovilpathu village, higher ferrous content may induce the growth of fungi like *Fusarium oxysporum*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Rhizopus stolonifer*, *Penicillium citrinum*, *Penicillium conidia* and bacterial species like *Flavibacterium sps*, *Bacillus mesentericus*, *Pseudomonas aeruginosa*, *E. coli*, *Pseudomonas sps*, *B. coagulans* and *Micrococcus sps*. The presence of some chemicals may enhance the growth of certain species of bacteria like *Streptococcus sps*, *Flavibacterium sps*, *Pseudomonas aeruginosa*, *Micrococcus sps* and *Bacillus licheniformis*. Fungi like *Aspergillus repens*, *Absidia glaura*, *Trichoderma viridae*, *Alternaria sps* and *Cladosporium sps*.

Table 2. Physical parameter of soil from Sirkazhi Taluk

Season	pH			Moisture			Temperature			Electrical Conductivity		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Monsoon	7.38	7.85	7.14	35.5	40.5	39.2	36	25	26	1.2	2.1	1.2
Postmonsoon	7.28	7.69	7.87	49.5	50.5	49.2	34	35	36	2.2	2.4	2.3
Premonsoon	7.74	7.14	7.17	45.7	49.2	50.7	34	36	35	2.3	2.3	1.6
Summer	7.74	7.12	7.18	33.2	31.5	33.5	45	47	45	1.6	1.9	1.8

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi

Table 3. Percentage of frequency of bacteria from three different villages of Sirkazhi Taluk

S. No.	Field	Average	B.c	Vib	P.v	Ent	Stre	B.s	P.f	A.ae	Sta.s	N	S.a	P. m	Flavi	B. m	Pseu	E.c	B.c	Mi.sp
1	S1	35	3	4	3	2	3	4	5	1	2	2	2	1	2	2	-	-	-	-
2	S2	34	-	-	-	-	6	-	5	-	-	-	-	-	7	2	5	6	2	2
3	S3	33	-	-	-	-	7	-	3	-	5	6	-	-	-	-	4	-	5	3
Total = 102			3	4	3	2	16	4	13	1	7	8	2	1	9	4	8	6	7	3
% of contribution			2.9	3.9	2.9	1.9	15.6	3.9	12.7	0.98	6.8	7.8	1.9	0.98	8.8	3.9	7.8	5.8	6.8	2.9

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi

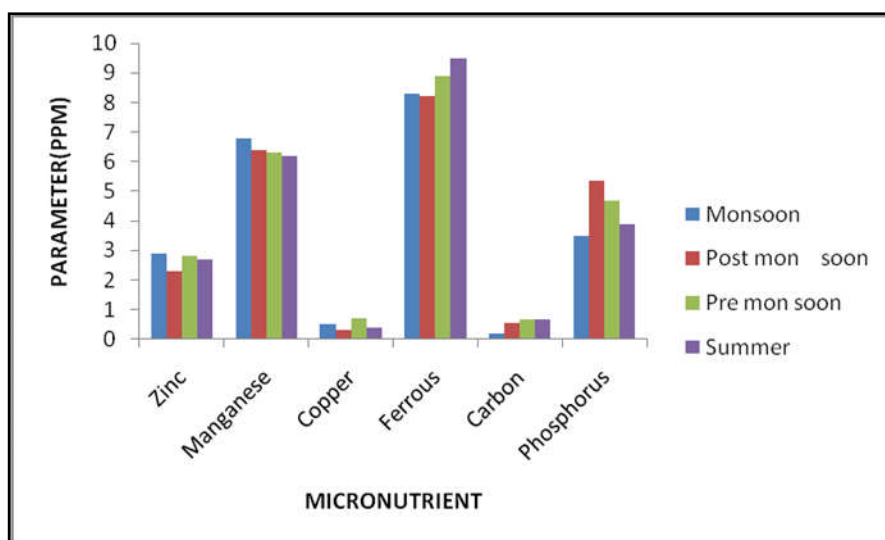


Figure 1. Micronutrient of soil in different village

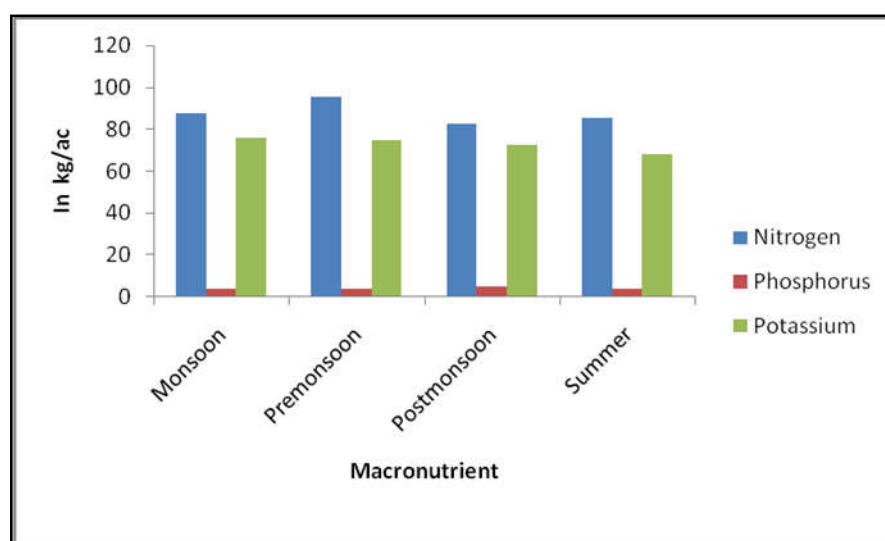
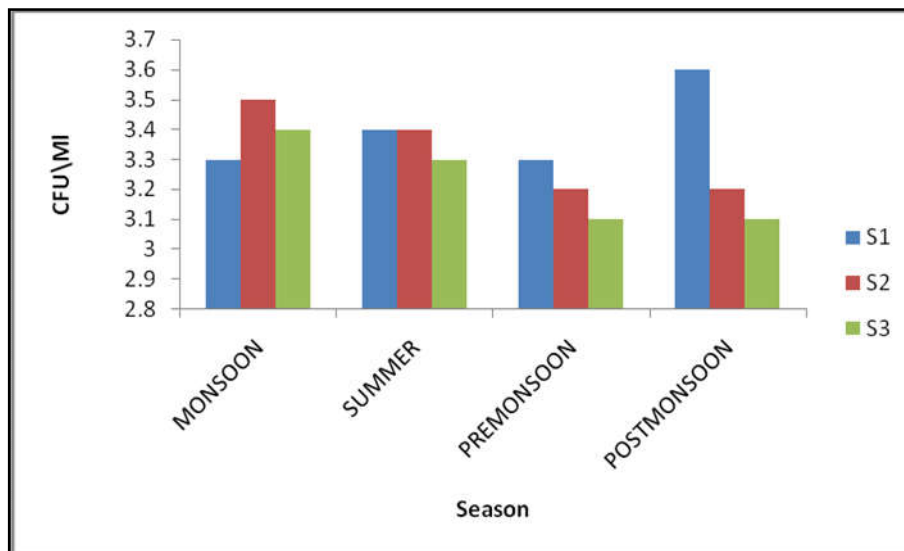
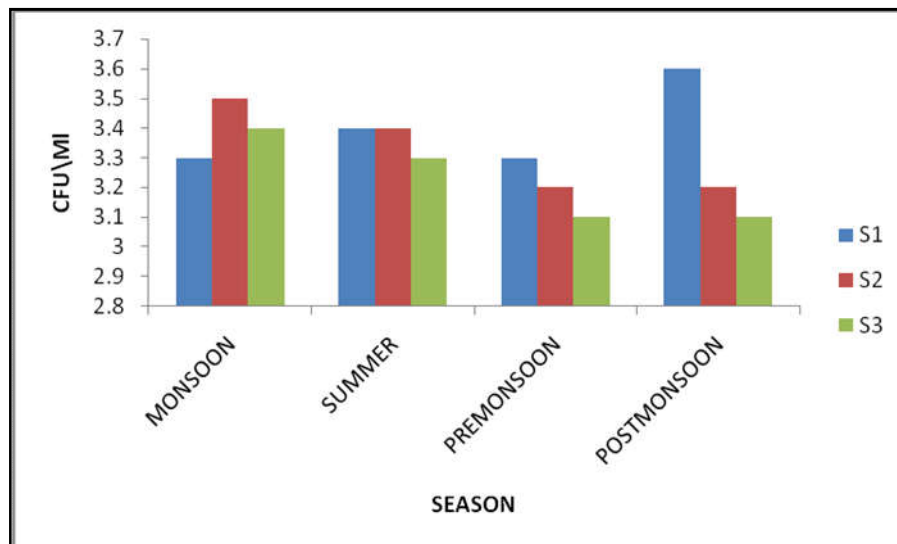


Figure 2. Macronutrients (NPK) of soil in different village



S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi

Figure 3. Colony forming unit of bacteria in different villages



S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi

Figure 4. Colony forming unit of bacteria in different villages

Decrease in fungal and bacterial population along soil depth confirms the finding of Tiwari *et al.*, (1987) and Shukla (1992). Surface layer of soil is usually provided with high organic matter which in presence of adequate moisture supply is acted upon by microorganisms to decompose the complex organic residues into simple forms; hence, the species of microorganisms are high on surface layer of the soil. Generally fungi and bacteria found in deep layer or slow growing due to unavailability of mineral nutrients and compaction of soil along depth (Dkhar and Mishra, 1992). One gram of the soil sample thus prepared was added to 10 ml sterilized distilled water and serial dilutions were made (Johnson, 1957).

Nutrient agar medium was used to isolate the bacterial species from the soil. Here, 10^4 to 10^7 dilutions were taken for the bacterial isolation. Fungal population present in the soil sample

were determined by plating the soil dilution of 10^{-2} to 10^{-5} dilution over solidified Rose Bengal Agar medium and Potato Dextrose Agar medium. In the present study, totally 19 bacterial species were isolated from the paddy field soil by dilution plate technique. The bacterial species are identified their morphological character, biochemical and Bergey's manual of determinative bacteriology. The bacterial species are *Bacillus cereus*, *Vibrio sps*, *Proteus vulgaris*, *Enterococcus sps*, *Streptococcus sps*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Aerobacter aerogens*, *Aeromonas sps*, *Staphylococcus sps*, *Neisseria sps*, *Staphylococcus aureus*, *Proteus mirabilis*, *Flavibacterium sps*, *Bacillus mesentericus*, *Pseudomonas aeruginosa*, *E. coli*, *Pseudomonas sps*, *B. coagulans*, *Micrococcus sps*, *Bacillus licheniformis*. In the present study, totally 19 fungal species were isolated from the paddy soils by dilution plate technique.

Table 4. List of bacterial species isolated from three different villages of Sirkazhi Taluk

S. No.	Organisms	Study site		
		S ₁	S ₂	S ₃
1	<i>Bacillus cereus</i>	+	-	-
2	<i>Vibrio sps</i>	+	-	-
3	<i>Proteus vulgaris</i>	+	-	-
4	<i>Enterococcus sps</i>	+	-	-
5	<i>Streptococcus sps</i>	+	+	+
6	<i>Bacillus subtilis</i>	+	-	-
7	<i>P.fluroscens</i>	+	+	+
8	<i>Aerobacter aerogens</i>	+	-	-
9	<i>Aeromonas sps</i>	+	-	-
10	<i>Staphylococcus sps</i>	+	-	+
11	<i>Neisseria sps</i>	+	-	+
12	<i>S. aureus</i>	+	-	-
13	<i>Proteus mirabilis</i>	+	-	-
14	<i>Flavibacterium sps</i>	-	+	+
15	<i>Bacillus mesentericus</i>	-	+	-
16	<i>P. aeruginosa</i>	-	+	+
17	<i>E. coli</i>	-	+	-
18	<i>Pseudomonas sps</i>	-	+	+
19	<i>B. coagulans</i>	-	+	-

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi (+) – present, (-) - absent

Table 5. Percentage of frequency of fungi from three different villages of Sirkazhi Taluk

S.No.	Field	Average	A.o	A.n	A.f	A.fl	A.ni	Are	A.r	Sac	Ver	F.o	R.s	R.o	P.c	P.co	A.b	T.v	T.hor	Cl	A.l
1	S1	34	2	4	5	-	4	-	-	4	-	4	-	3	-	-	-	4	-	-	-
2	S2	33	-	-	6	-	3	4	-	-	-	5	4	-	4	6	-	-	4	-	-
3	S3	33	-	-	4	4	-	-	3	-	3	-	-	4	-	-	5	3	-	3	4
Total = 100			2	4	15	4	7	4	3	4	3	9	4	7	4	6	5	7	4	3	4
% of contribution			2	4	15	4	7	4	3	4	3	9	4	7	4	6	5	7	4	3	4

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi

List of fungal species isolated from three different villages of Sirkazhi taluk

S. No	Organisms	Study site		
		S ₁	S ₂	S ₃
1	<i>Aspergillus niger</i>	+	-	-
2	<i>Aspergillus oryzae</i>	+	-	-
3	<i>Saccharomyces</i>	+	-	-
4	<i>Rhizopus oryzae</i>	+	-	+
5	<i>Verticillium sps</i>	-	-	+
6	<i>Fusarium oxysporum</i>	+	+	-
7	<i>Aspergillus fumigates</i>	+	+	+
8	<i>Aspergillus nidulans</i>	+	+	-
9	<i>Rhizopus stolonifer</i>	-	+	-
10	<i>Penicillium citrinum</i>	-	+	-
11	<i>Penicillium conidia</i>	-	+	-
12	<i>Aspergillus flavus</i>	-	-	+
13	<i>Aspergillus ruber</i>	-	+	-
14	<i>Aspergillus repens</i>	-	-	+
15	<i>Absidia glaura</i>	-	-	+
16	<i>Trichoderma viridiae</i>	+	-	+
17	<i>Alternaria sps</i>	-	-	+
18	<i>Cladosporium sps</i>	-	-	+
19	<i>Trichoderma harzianum</i>	-	+	-

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi (+) – present, (-) - absent

The fungal species includes *Aspergillus niger*, *Aspergillus oryzae*, *Saccharomyces*, *Rhizopus oryzae*, *Verticillium sps*, *Fusarium oxysporum*, *Aspergillus fumigates*, *Aspergillus nidulans*, *Rhizopus stolonifer*, *Penicillium citrinum*, *Aspergillus flavus*, *Aspergillus ruber*, *Aspergillus repens*, *Trichoderma viridiae*, *Cladosporium sps* and *Trichoderma harzianum*. The fungal species of *Aspergillus* have been reported that they were the most tolerant one to the adverse conditions in the laboratory (Venkataraman and Rajyalakshmi,

1971) and species of *Aspergillus* and *Penicillium* were tolerant to wide range of environmental conditions. In the present study, 15 fungal species *Aspergillus* and *Trichoderma* were predominant, it produced the black and green colored colonies. The bacterial species *Streptococcus* and *Pseudomonas* were predominant in soil. Totally, 19 different species of soil bacteria were observed from the soil samples. The highest percentage of frequency of bacteria isolated from all the soil samples were recorded, in, which, *Streptococcus*, *Pseudomonas*

fluorescens, *Flavobacterium* and *Neisseria* were predominant followed by the other species of bacterium.

Bacillus cereus –**B.c**, **Vib-** *Vibrio* sps, **P.v-** *Proteus vulgaris*, **Ent-***Enterococcus* sps, **Stre -***Streptococcus* sps, **B.s-***Bacillus subtilis*, **P.f-***Pseudomonas fluorescens*, **A.ac-***Aerobacter aerogens*, *Aeromonas* sps, **Sta.s-***Staphylococcus* sps, **N-***Neisseria* sps, **S.a-***Staphylococcus aureus*, **P. m-***Proteus mirabilis*, **Flavi-***Flavibacterium* sps, **B. m-***Bacillus mesentericus*, **Pseu-***Pseudomonas aeruginosa*, **E.c-***E. coli*, **B.c-** *B. coagulans* and **Mi.sp** –*Micrococcus* sps.

Totally 19 different species of soil fungi were observed from the soil samples. The highest percentage was recorded for *Aspergillus flavus*, *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus oryzae* and *Trichoderma viridae* followed by the other fungal species.

A.n-*Aspergillus niger*, **A.o-** *Aspergillus oryzae*, **Sac-***Saccharomyces*, **R.o** –*Rhizopus oryzae*, **Ver-***Verticillium* sps, **F.o-***Fusarium oxysporum*, **A.f-***Aspergillus fumigates*, **A.ni** – *Aspergillus nidulans*, **R.s** –*Rhizopus stolonifer*, **P.c-***Penicillium citrinum*, **A.fl-***Aspergillus flavus* **A.r-***Aspergillus ruber*, **Are-***Aspergillus repens*, **T.v-***Trichoderma viridae*, **Cla** – *Cladosporium* sps **T.hor** –*Trichoderma harzianum* and **A.l-** *Alternaria*

List of fungal species isolated from three different villages of sirkazhi taluk

S1- Thenpathi, S2- Kovilpathu, S3- Sirkazhi (+) – present, (-) – absent

Conclusion

Thus the present study reveal the presence of bacterial and fungal populations in the soil samples collected from Sirkazhi Taluk. Biodiversity of the soils represents the fertility of the soil. The surface soil consists of high content of organic matter which increases the biodiversity. So, frequent study on the biodiversity keeps up to date knowledge about the fertility. Seasonal climatic changes also influence the biodiversity of the soil by changing the physico chemical parameters. Bacteria and fungi have direct impact on crop production with different environmental conditions. These microbes are able to supply nutrients to crop to encourage plant growth for example, through the production of plant hormones and control or inhibit the activity of plant pathogen.

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