Population dynamics of bacteria and physiochemical properties of soil during different seasons in rice fields of Tiruvarur district, rice bowl of Tamil Nadu.

Deepan, B[1], Prakash, P[2], Ambikapathy, V[1*], Gomathi, S[1] and Panneerselvam, A[1]

[1*]Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi-613503.
(Affiliated to Bharathidasan University, Tiruchirappalli - 24), Thanjavur, Tamil Nadu, India.

[2]Indian Biotrack Research Institute, Thanjavur – 613 005. Tamil Nadu, India.

[*]Corresponding Author E.mail: amirthaneet@gmail.com &drva1967@gmail.com

Abstract:

Rice plants are in close contact with the soil through their roots. Microbes inhabited on the root surface (rhizoplane), inside the root (endosphere), and in the soil surrounding the root surface (rhizosphere) play a crucial role in plant immunity and yield. But there is very little information regarding the microbial consortia in the rhizosphere during different seasons of rice cultivation. Therefore, the objective of our study was to assess, contrast and correlate the bacterial population observed during different seasons among various places in Tiruvarur district along with their physiochemical parameters. This study revealed 21 bacterial species that belong to three major phyla, four classes, eight orders, ten families, and fifteen genera. In the Tiruvarur rice fields, bacterial genera such as *Aeromonas*, *Azotobacter*, *Azospirillum, Bacillus, Bradyrhizobium, Enterobacter*, *Escherichia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia, Shigella, Vibrio, and Yersinia* were found. The soil physiochemical characteristics like pH, electrical conductivity, organic carbon, available nitrogen, phosphorus, potassium, zinc, copper, iron, and manganese were studied during the premonsoon, monsoon, postmonsoon, and summer seasons in five places in the Tiruvarur rice field. Additionally, the Pearson correlation coefficient analysis was also done on the obtained databases and the level of significance was seen at $P < 0.05$. The results of the study demonstrate that a special community of bacteria inhabited the rice soil during different seasons which may help to improve crop yield.

Keywords: Rhizosphere, Rice field, Physiochemical Parameters, Bacteria, Monsoon

Introduction:

Rice is the most important crop agriculturally as well as economically feeding over half of the world's population [1]. In Asia and developing nations, rice consumption is very high.[2]. Rice is an annual monocot grass that belongs to the family Poaceae. It contains abundant carbohydrates and lot of vitamins, minerals and polyphenols. Only two primary rice species are widely farmed out of the 23 different varieties. They are *Oryza glaberrima*, or African rice, and *Oryza sativa*, or Asian rice. *Oryza sativa* is grown all throughout the world, but *Oryza glaberrima* is only grown in Africa. Indica, which has long grains, and Japonica, which has round grains, are the two primary subspecies of *Oryza sativa*. Indica rice types are extensively farmed in Asia, but Japonica rice is mostly grown and consumed in Australia, China, Taiwan, Korea, Europe, Japan, Russia, Turkey and the United States. [3].

Rice is the most important staple food in india which meets around 60% of daily energy requirements or 41% of the nation's total grain production from 35% of the country's grain-producing land. Rice is therefore crucial for ensuring national food security. India is the world's second-largest producer of rice and rice's top exporter. 120 million tonnes of rice would be produced in India in FY 2020–21, up from 53.6 million tonnes in FY 1980 [4, 5]. In India rice is grown under widely varying conditions of altitude and climate. Rice cultivation in India extends from 8 to 35ºN latitude and from sea level to as high as 3000 meters. Rice crop needs a hot and humid climate. It is best suited to regions which have high humidity, prolonged sunshine and an assured supply of water. The average temperature required throughout the life period of the crop ranges from 21 to 37º C. Maximum temp which the crop can tolerate 40^0C to 42 ^{0}C .

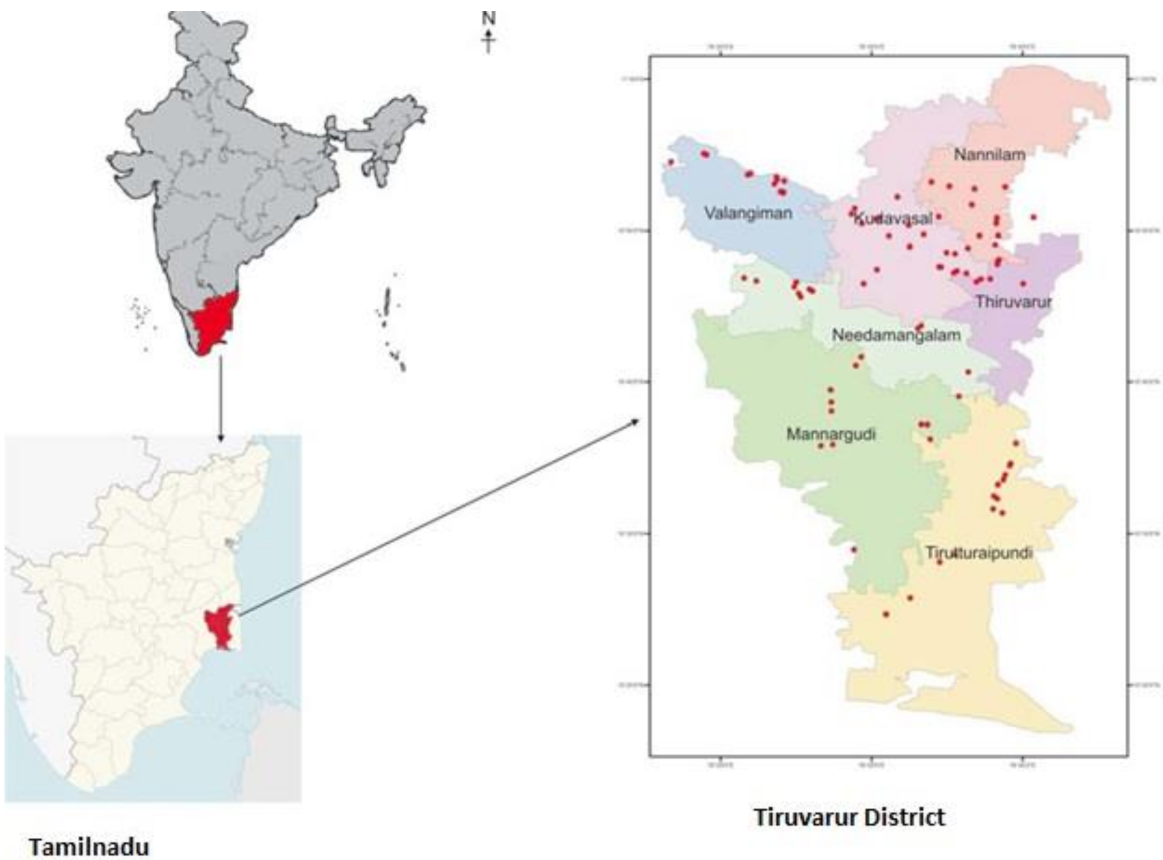
Soil microbiota play a key role in enhancing the soil's inherent ability to inhibit diseases that affect plants on the soil [6]. In agroecosystems, the dynamics of soil organic matter (SOM) and plant nutrient availability are significantly influenced by microbiota [7, 8]. The diversity and activity of bacteria is regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure and temperature) factors. This work reports and discusses the most reliable findings in relation to a comprehensive understanding of soil bacteria pertaining to rice crop and their correlation with various physiochemical parameters.

Materials and Methods:

Study site and sample collection

The Kaveri delta often known as the "Rice Bowl of Tamilnadu," is located in the Thiruvarur District of Tamilnadu, India (Fig.1). The district is located between latitudes 100 16' and 110 50' north and longitudes 79°27' and 79°50' east. The Thiruvarur district covers a total area of 2377 km^2. The district is bordered on the east by Nagapattinam District, the north by Mayiladuthurai District, the west by Thanjavur District and the south by Palk Strait. The region experiences hot, tropical weather with daily highs and lows between 26.39°C and 35.19°C [9].

Fig.1. Geographical location of the study area.



The soil samples were taken during Premonsoon (Aug-Sep, 2022), Monsoon (October-december, 2022) and Postmonsoon (Jan- Feb, 2023) periods in various rice fields of of Tiruvarur district ie.Thiruthuraipoondi, Tiruvarur, Mannargudi, Needamangalam and Nannilam. Three subsamples of 200-250g soil were collected around the root at a depth of 15cm to form a composite sample (n=45). The samples were placed in Ziplock bags and were stored at−80°C until further analysis.

Physiochemical Analysis

The soil samples were suspended in distilled water and the particles were allowed to settle down. The pH values, electrical conductivity, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed. Using a pH meter (Duralab, India), the suspension pH was determined. The electrical conductivity of the soil was measured using a conductivity meter in the water extract filtrate. The macro nutrients such as Nitrogen by Alkaline permanganate method [10], phosphorous by Olsen method [11], potassium (neutral normal ammonium acetate method), organic carbon by Walkley and Block method[12] and micro nutrients such as copper, iron, manganese and zinc were analyzed by DTPA extract method using atomic absorption spectrophotometer [13].

Isolation and identification of soil bacteria

One gram of rhizosphere soil sample taken in 9 ml of sterile distilled water. The samples were serially diluted for six fold and were plated on nutrient agar.Then incubated for 24 hours at 37ºC. After incubation the colonies were purified by the continuous streaking method and characterized on the basis of macroscopic, microscopic and biochemical tests, according to the 9th edition, Bergey's Manual of determinative bacteriology 1923 [14]. The bacterial isolates were identified macroscopically by examining colony morphology, surface pigment, size, margin, surface on nutrient agar plates and microscopic examination including gram's staining behavior, shape and cell arrangement. Motility tests were also performed. The following biochemical tests such as Gram staining [15], Indole test [16], Methylred and Voges proskaure test [17], Citrate utilization test[18], Catalase test[19], Carbohydrate fermentation test[20], oxidase test[21], Triple sugar iron test[22] were performed and their characters were observed and summarized in Table 2.

Statistical analysis

Using SPSS software, the Pearson correlation matrix was generated. For level of significance, correlation coefficients between physicochemical parameters and bacterial populations were recorded.

Results and Discussion:

Taxonomic analysis of Bacterial Community abundance on different rice field soils of Tiruvarur district

Morphological and biochemical characters of isolated bacteria were summarized in table 1 and 2. Microphotography of isolated bacteria from paddy fields were shown in figure 2 and abundance of bacterial communities was examined in different seasons, including monsoon, premonsoon, postmonsoon and summer for all taxonomic levels among different areas of Tiruvarur distric were displayed as graphs (Fig. 3-7)

Table1 :Morphological characteristics of isolated bacteria

Name of the strains	Morphological characters							
	Growth	**Shape**	**Surface**	**Margin**	**Color**	**Elevation**	**Consistency**	**Opacity**
BDVA 1	Rapid	Sperical	Concentric	Lobate	White	Flat	Buttery	Opaque
BDVA 2	Rapid	Circular	Rough	Entire	Creamy white	Raised	Viscous	Opaque
BDVA 3	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque
BDVA 4	Rapid	Rhizoid	Smooth	Lobate	Milky white	Flat	Viscous	Opaque
BDVA 5	Slow	Punctiform	Rough	Irregular	White	Umbonate	Adhesive	Opaque
BDVA 6	Slow	Irregular	Rough	Irregular	Milky white	Raised	Buttery	Opaque
BDVA 7	Slow	Circular	Smooth shiny	Entire	Creamy white	Pulvinate	Buttery	Opaque
BDVA 8	Rapid	Punctiform	Rough	Undulate	White	Pulvinate	Adhesive	Opaque
BDVA 9	Slow	Punctiform	Smooth	Undulate	Pale yellow	Convex	Cluster	Translucent
BDVA 10	Slow	Irregular	Dull	Curled	Creamy white	Raised	Buttery	Opaque

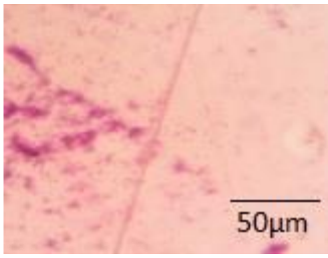
BDVA 11	Slow	Circular	Smooth shiny	Entire	Light orange	Pulvinate	Buttery	Opaque
BDVA 12	Slow	Circular	Rough	Irregular	White	Flat	Viscous	Opaque
BDVA 13	Slow	Vibroid	Smooth	Regular	White	Dense	Adhesive	Opaque
BDVA 14	Slow	Circular	Smooth	Entire	Dark Brown	Raised	Viscous	Opaque
BDVA 15	Slow	Spindle	Wrinkled	Curled	Pale White	Dense	Viscous	Translucent
BDVA 16	Slow	Irregular	Wrinkled	Entire	White	Convex	Viscous	Translucent
BDVA 17	Slow	Irregular	Smooth	Entire	White	Convex	Viscous	Translucent
BDVA 18	Slow	Circular	Smooth	Entire	Creamy white	Convex	Gummy	Translucent
BDVA 19	Slow	Circular	Smooth	Irregular	Greyish white	Convex	Buttery	Translucent
BDVA 20	Rapid	Circular	Smooth	Irregular	Pale yellow	Raised	Buttery	Opaque
BDVA 21	Rapid	Circular	Smooth	Irregular	Greyish white	Convex	Viscous	Translucent

Table 2: Biochemical characteristics of isolated bacteria

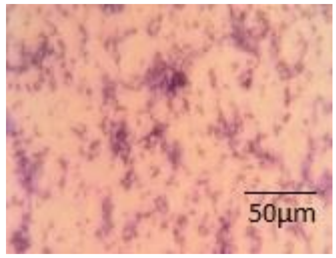
Name of the Biochemical	**Gram Staining**	**Indole broth**	**Methyl Red reaction**	**Voges Proskauer reaction**	**Citrate Utilization**	**Catalase**	**Carbohydrate fermentation**			**Oxidase**	**Triple Sugar Iron Agar**	**Name of the bacteria**
Code of the bacteria							**Glucose**	**Mannitol**	**Sucrose**			
BDVA 1	- ve	+	-	-	+	+	+	Invalid	-	+	A/AH_2S	***Aeromonas* sp.**
BDVA 2	- ve	-	+	+	+	-	-	Invalid	+	+	H_2S	***Flavobacterium odoratum***
BDVA 3	+ ve	-	-	-	+	+	+	+	+	+	A/AH_2S	***Bacillus subtilis***
BDVA 4	- ve	+	-	-	+	-	+	+	+	+	A/AH_2S	***Serratia fonticola***
BDVA 5	- ve	+	+	+	+	+	-	-	Invalid	+	A/AH_2S	***Pseudomonas aeruginosa***

BDVA 6	-ve	+	-	-	-	-	+	+	+	-	A/AH_2S	***P. fluorescence***
BDVA 7	+ ve	-	-	+	-	-	-	-	+	+	A/AH_2S	***Bacillus* sp.**
BDVA 8	- ve	+	+	-	-	+	+	Invalid	Invalid	-	A/A	***Escherichia coli***
BDVA 9	-ve	+	+	-	-	+	-	-	-	+	A/A	***Rhizobium* sp.**
BDVA 10	+ve	+	-	+	-	+	+	+	+	-	-	***Vibrio cholerae***
BDVA 11	+ ve	-	-	+	-	+	Invalid	+	+	-	A/AH_2S	***Bacillus cereus***
BDVA 12	-ve	-	+	+	+	+	+	+	+	-	A/A	***Enterobacter cloacae***
BDVA 13	-ve	-	+	-	+	+	+	+	+	+	A/A	***Azospirillum* sp.**
BDVA 14	-ve	+	+	+	+	+	+	+	+	+	A/AH_2S	***Azotobacter chrococcum***
BDVA 15	-ve	-	+	-	+	+	+	+	+	+	A/AH_2S	***Azospirillum brasilense***
BDVA 16	+ve	-	-	+	+	+	+	+	+	+	A/A	***B.amyloliquefaciens***
BDVA 17	+ve	-	+	+	+	+	+	+	+	-	A/AH_2S	***B.megaterium***
BDVA18	-ve	-	+	-	+	+	-	+	-	+	A/AH_2S	***Bradyrhizobium japonicum***
BDVA 19	-ve	-	+	-	-	+	+	+	-	+	A/AH_2S	***Salmonella typhi***
BDVA 20	-ve	+	+	-	-	+	+	+	+	-	A/A	***yersinia enterocolitica***
BDVA 21	-ve	+	+	-	-	+	-	+	-	-	A/A	***Shigella* sp.**

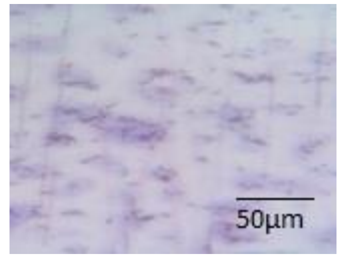
Fig.2. Microphotography of isolated bacteria from paddy fields in different seasons



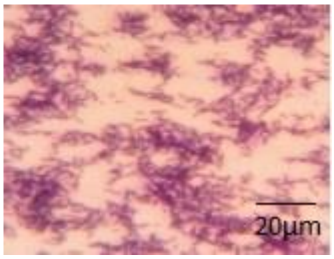
***Aeromonas* sp.**



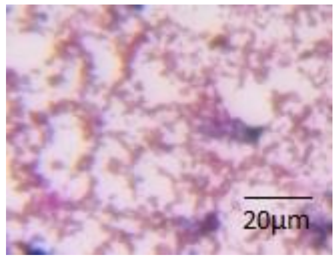
Flavobacterium odoratum



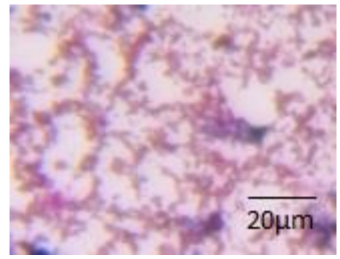
Bacillus subtilis



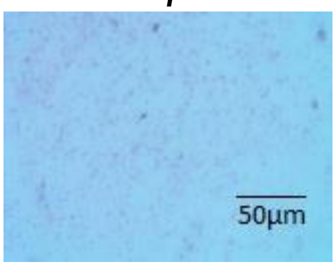
Serratia fonticola



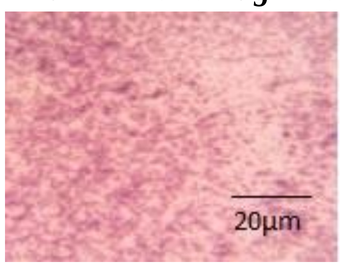
Pseudomonas aeruginosa



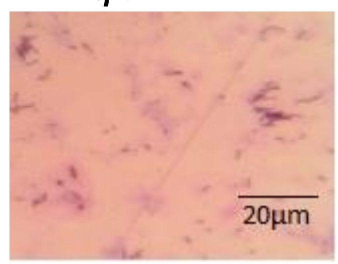
P.fluorescence



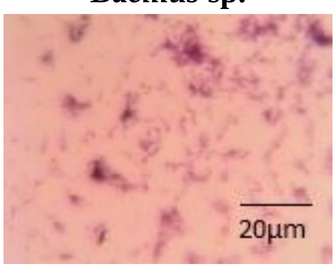
***Bacillus* sp.**



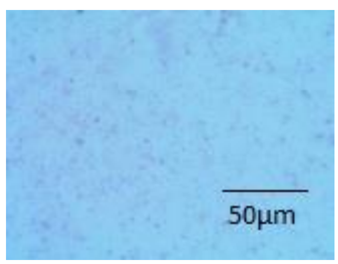
Escherichia coli



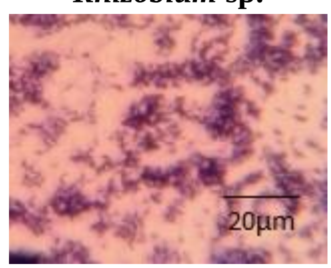
***Rhizobium* sp.**



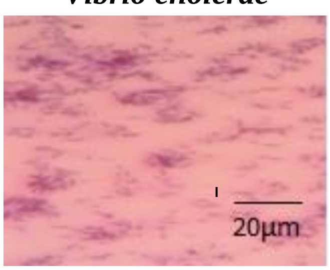
Vibrio cholerae



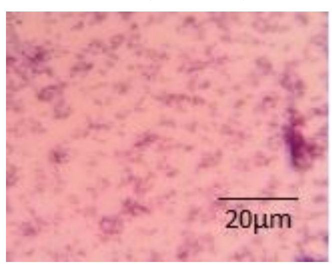
Bacillus cereus



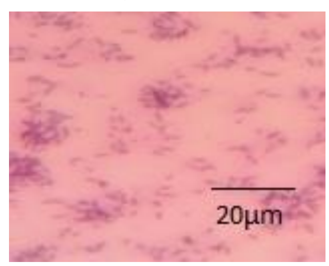
Enterobacter cloacae



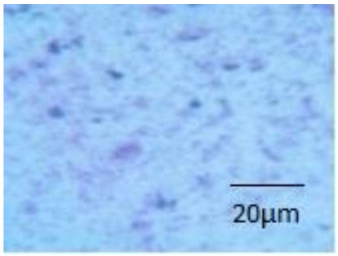
***Azospirillum* sp.**



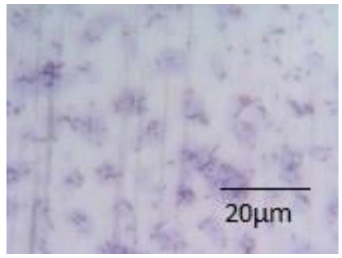
Azotobacter chrococcum



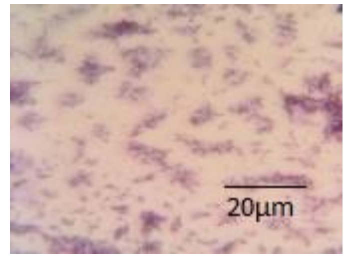
Azospirillum brasilense



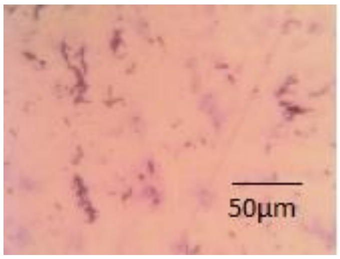
B.amyloliquefaciens



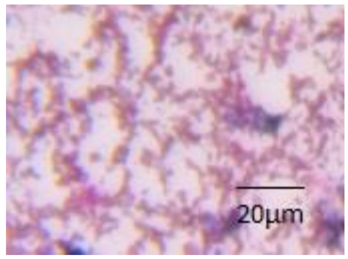
B.megaterium



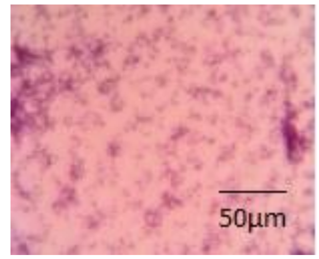
Bradyrhizobium japonicum



Salmonella typhi



Yersinia enterocolitica



***Shigella* sp.**

Fig.3 Bacteria of paddy fields in Thiruthuraipoondi region of Tiruvarur district during different seasons

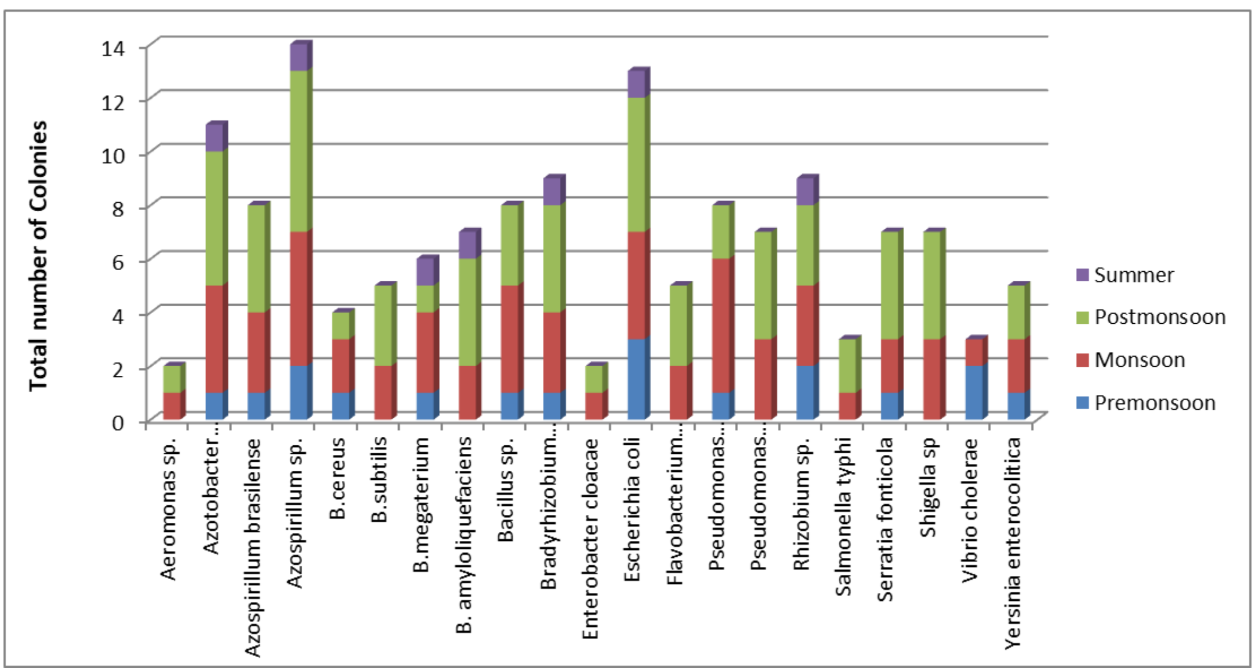


Fig.4 Bacteria of paddy fields in Mannargudi region of Tiruvarur district during different seasons

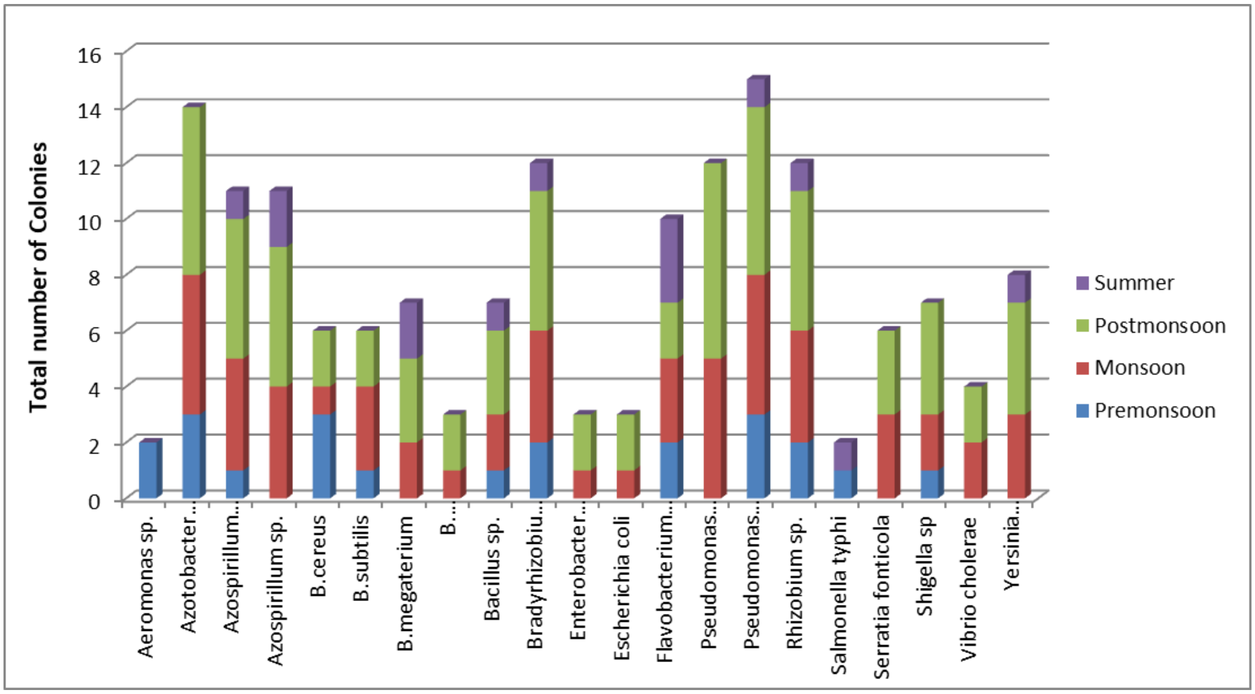


Fig.5 Bacteria of paddy fields in Tiruvarur region of Tiruvarur district during different seasons

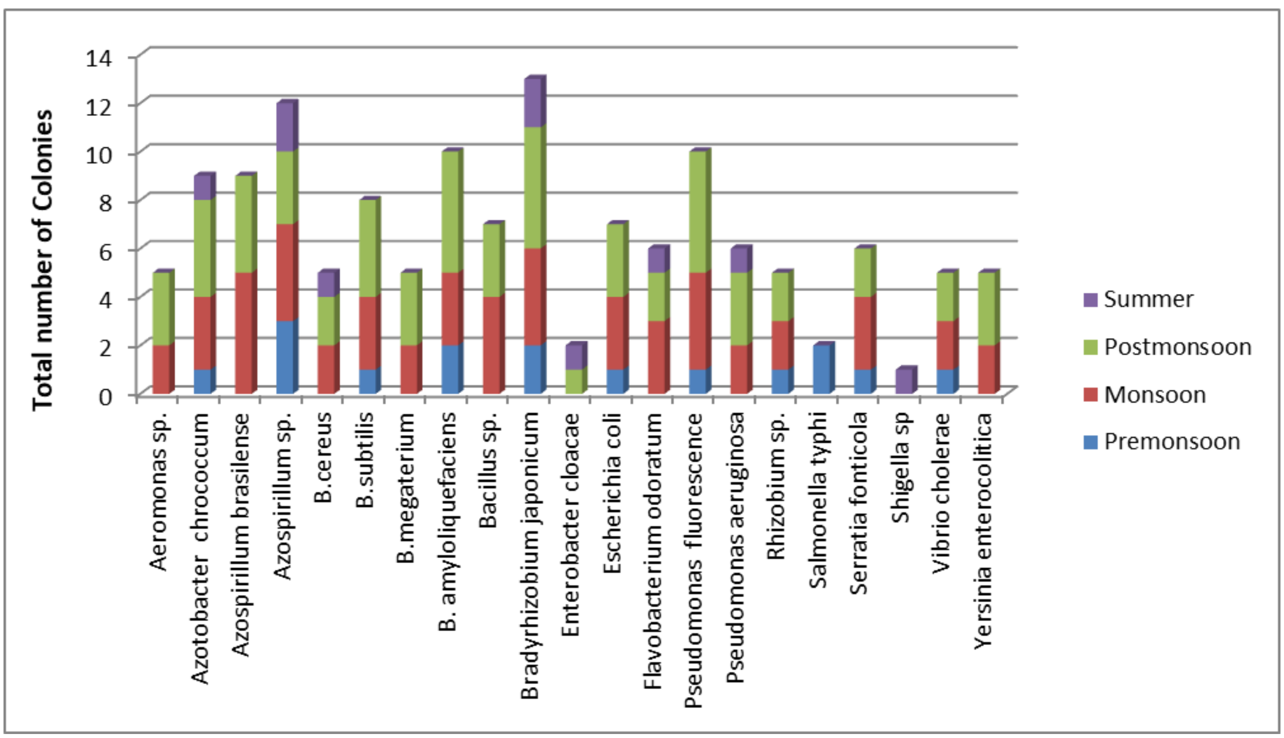


Fig.6 Bacteria of paddy fields in Nannilam region of Tiruvarur district during different seasons

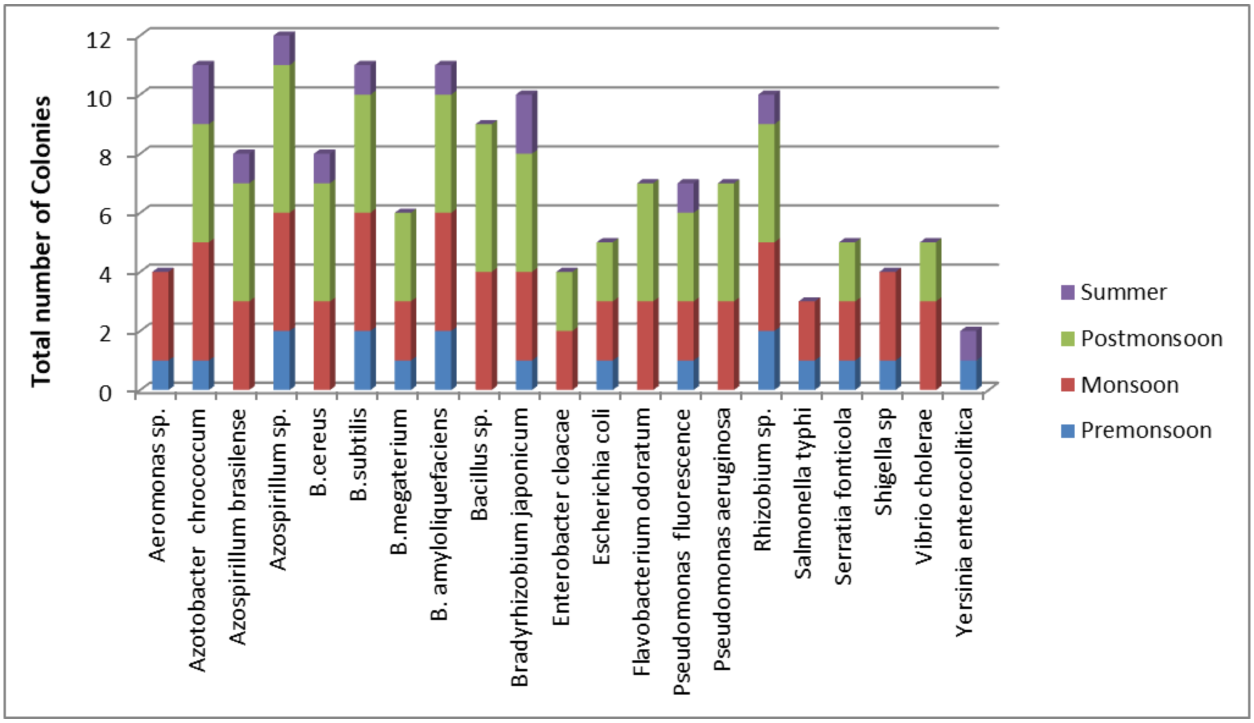
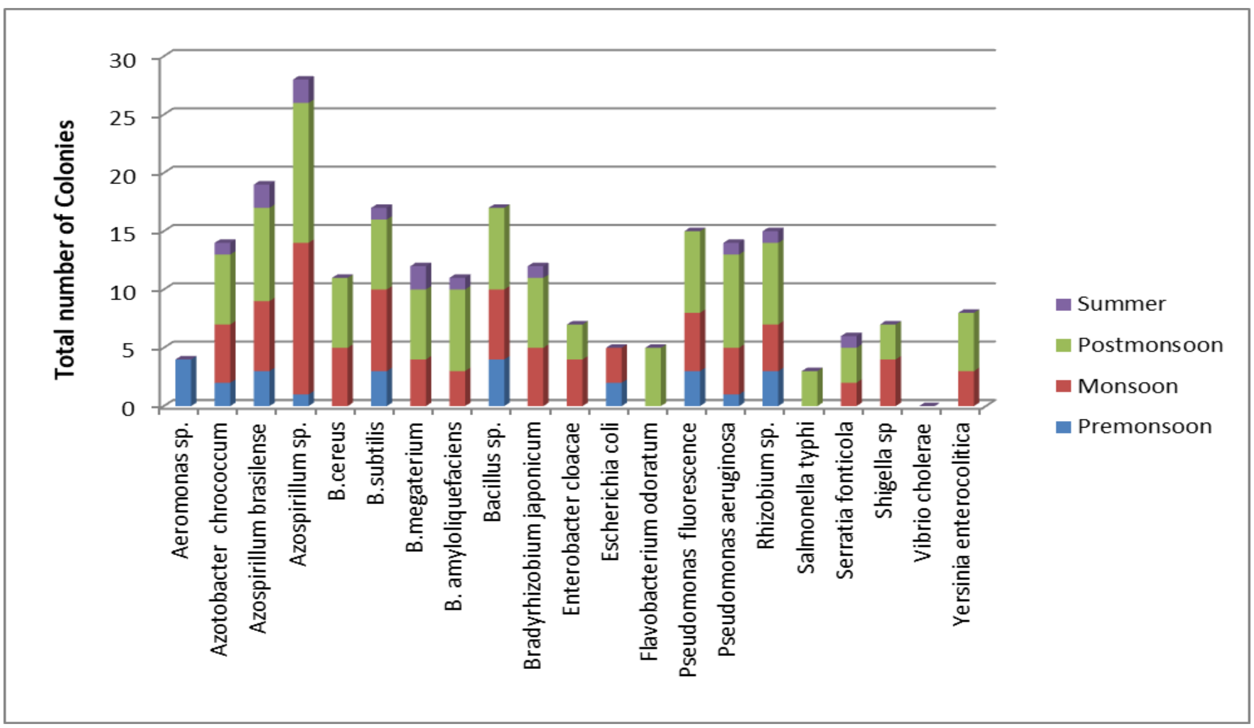


Fig.7 Bacteria of paddy fields in Needamangalam region of Tiruvarur district during different seasons



Phyla and Class analysis

A total of three bacterial phyla were discovered in different seasons such as premonsoon, monsoon, post monsoon and summer. Proteobacteria (96.45%) is the most abundant phylum found in all five sites of Tiruvarur district. Bacillota (2.2%), and Bacteroidetes (1.3%) are the next two abundant phyla found in the rice field soil. A total of four bacterial classes were observed in rice field soil. They are Gammaproteobacteria, Alphaproteobacteria, Bacilli and Flavobacteriia. Gammaproteobacteria (82.5%) was the most abundant class, while Flavobacteria(1.66%) was the least abundant in all seasons of Tiruvarur rice field soil. Next to Gammaproteobacteria, Alphaproteobacteria (13.25%) and Bacilli (4.24%) ranked as the second and third most abundant classes in rice field soil, respectively.

Order and Family analysis

There are eight orders found in Tiruvarur rice field soil observed in all seasons. They are Aeromonadales, Pseudomonadales, Rhodospirillales Bacillales, Hyphomicrobiales, Enterobacterales, Flavobacteriales, Vibrionales. Among them, Pseudomonodales (43.9%) was the most abundant order whereas Flavobacteriales (1.69%) was the least abundant in all seasons of Tiruvarur rice field soil. Next to Pseudomonodales, Bacillales (28.3%), Rhodospirillales (4.6%) and Hyphomicrobiales(4.39%) ranked as the second, third and fourth most abundant orders in rice field soil, respectively. In total, ten different bacterial families were found in the Tiruvarur rice field during the year. Aermonadaceae, Azospirillaceae, Bacillaceae, Nitrobacteraceae, Enterobacteriaceae, Pseudomonadaceae, Flavobacteriaceae, Rhizobiaceae, Vibrionaceae and Yersiniaceae were the eight abundant families found in the rice fields of Tiruvarur. Pseudomonadaceae (42.3% of the total) was the most abundant family while Yersiniaceae (1.33%) was the least abundant in all seasons of Tiruvarur rice field soil.

Genera analysis

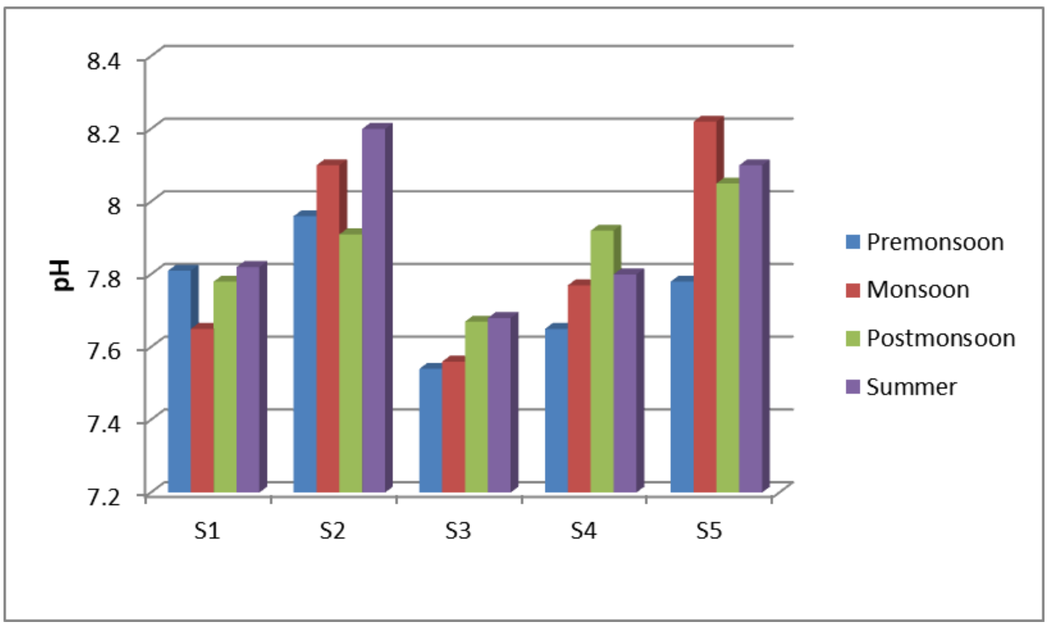
There are about 15 genus-level taxa found in various sites of the Tiruvarur rice field. Among the genera, *Pseudomonas* (27%), *Azotobacter (*15.6%), *and Azospirillum (*12.5%) appear to be the most abundant during all seasons of rice growth. The other abundant genera are in descending order as follows: *Bacillus* (10.8%), *Aeromonas* (5.2%),*Bradyrhizobium* (5.5%), *Flavobacterium* (4.5%), *Escheichia*(3.88%), *Rhizobium*

(3.66%), shigella(2.8%) , Enterobacter(2.66%) , *vibrio*(2.4%), *serratia*(1.32%), *salmonella*(0.9%), and *Yersinia* (0.33%).

Physiochemical Parameters

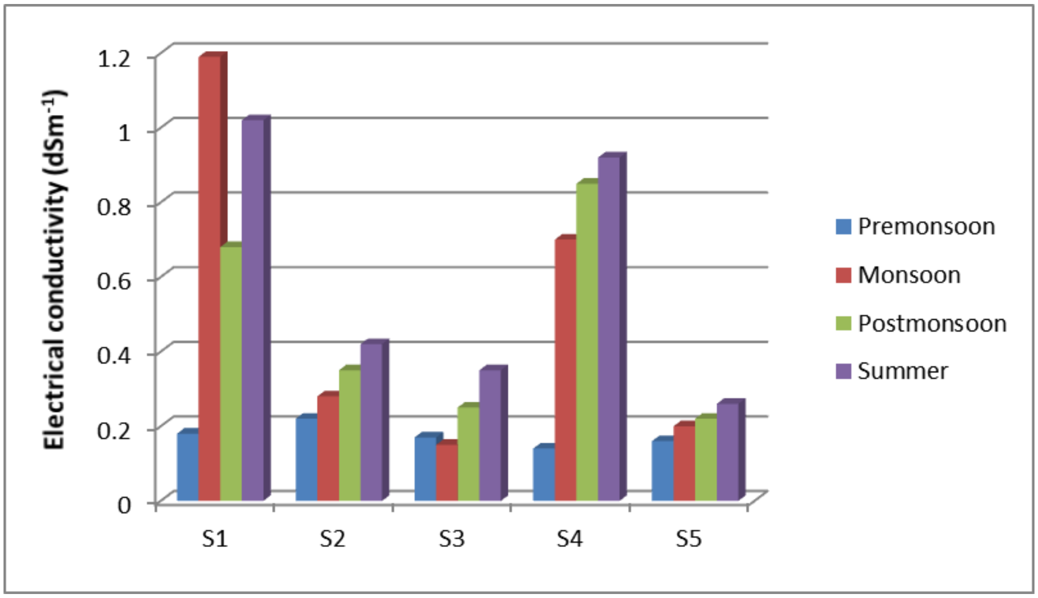
The Present study includes the observation of physiochemical factors with respect to the monsoon, premonsoon, postmonsoon and summer seasons. Physiochemical parameters such as p^H, electrical conductivity, organic carbon, nitrogen, phosphorus, potassium, iron, manganese, zinc, and copper are shown as graphs (Fig. 8–17).

Fig.8 p^H Value of different regions of Tiruvarur with respect to monsoon periods



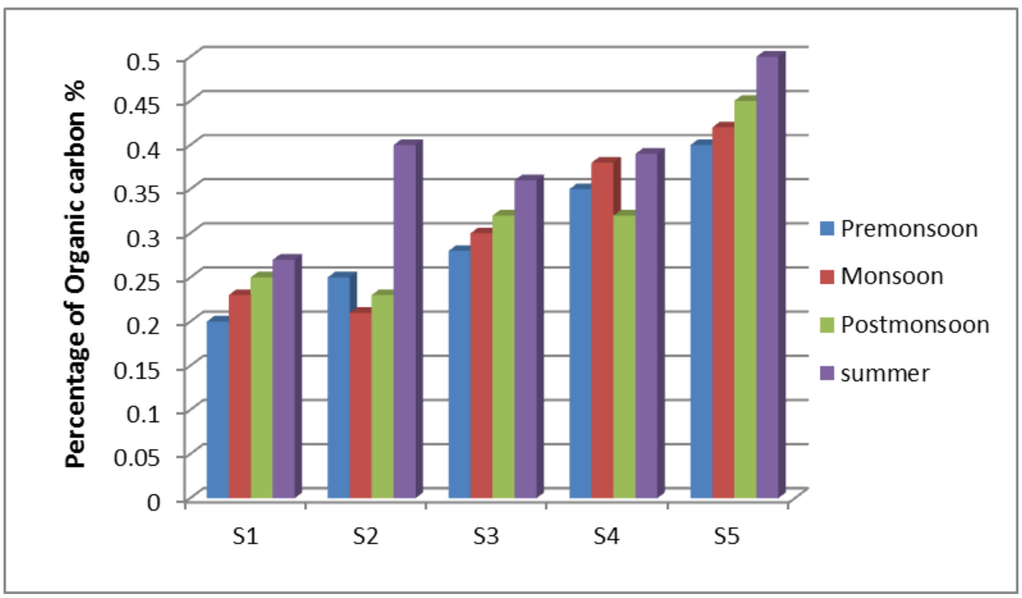
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.9 Electrical conductivity (dSm^{-1})Value of different regions of Tiruvarur with respect to monsoon periods



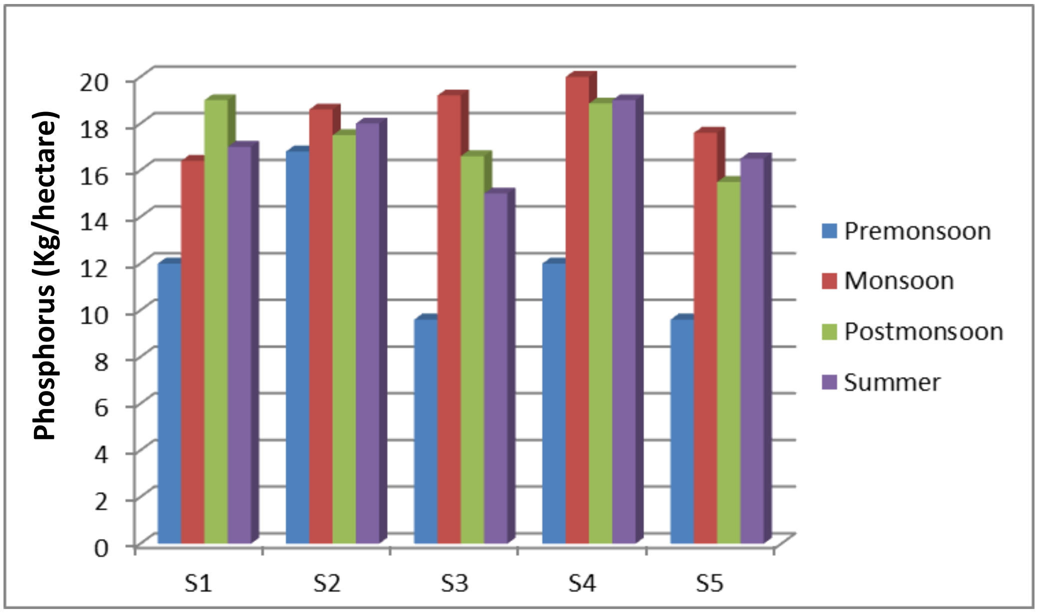
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.10 Percentage of Organic carbon in soil during different seasons in Tiruvarur district



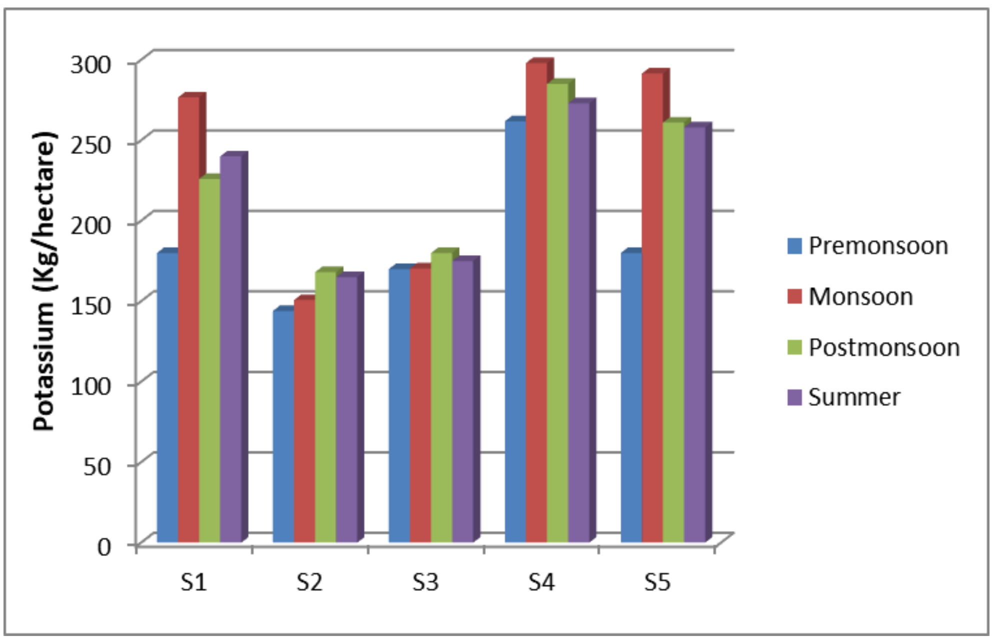
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.11 Phosphorus (Kg/hectare) in soil during different seasons in Tiruvarur district



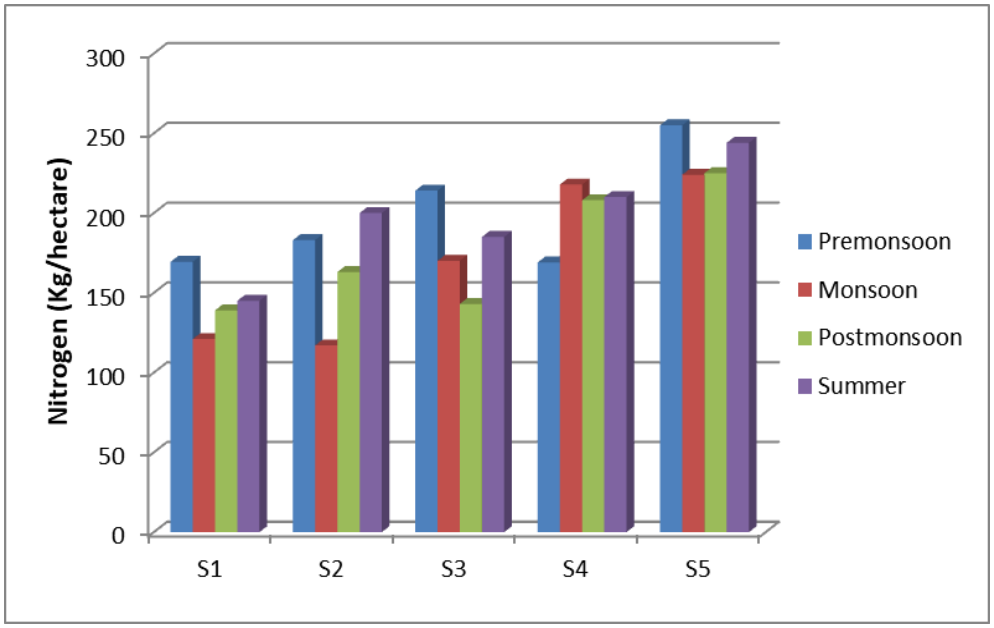
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.12 Potassium (Kg/hectare) in soil during different seasons in Tiruvarur district



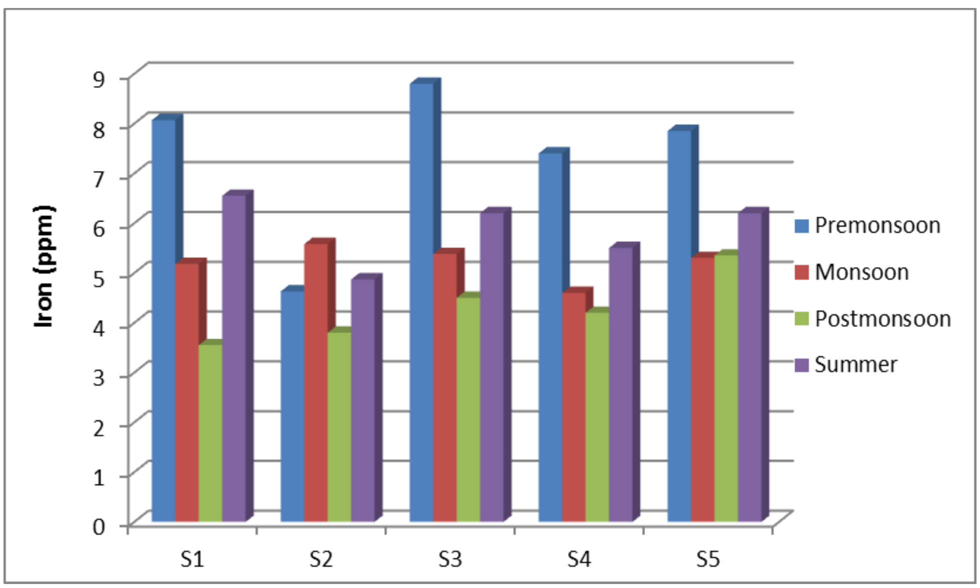
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.13. Nitrogen (Kg/hectare) in soil during different seasons in Tiruvarur district



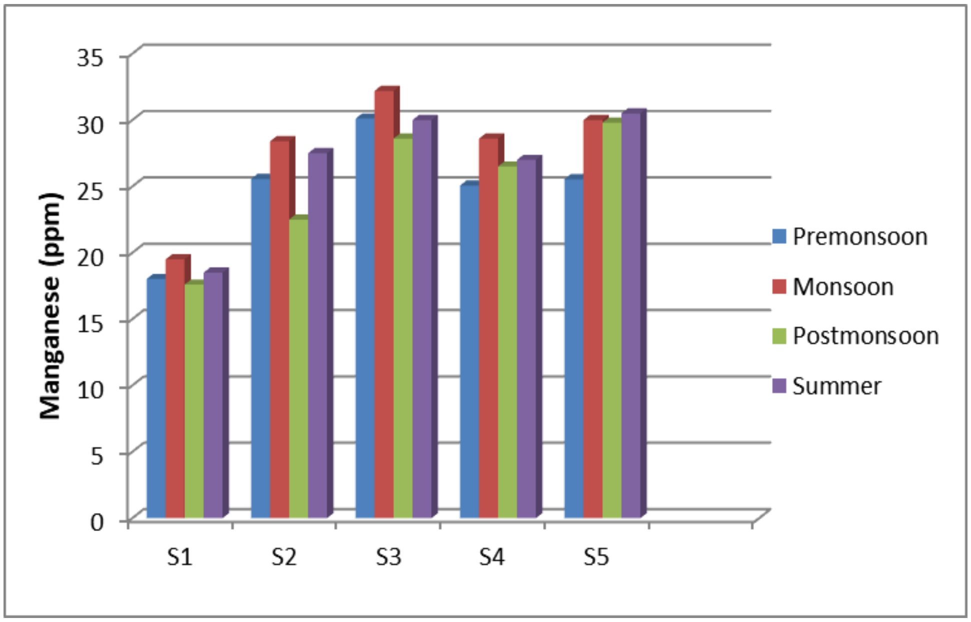
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.14. Iron (ppm) in soil during different seasons in Tiruvarur district



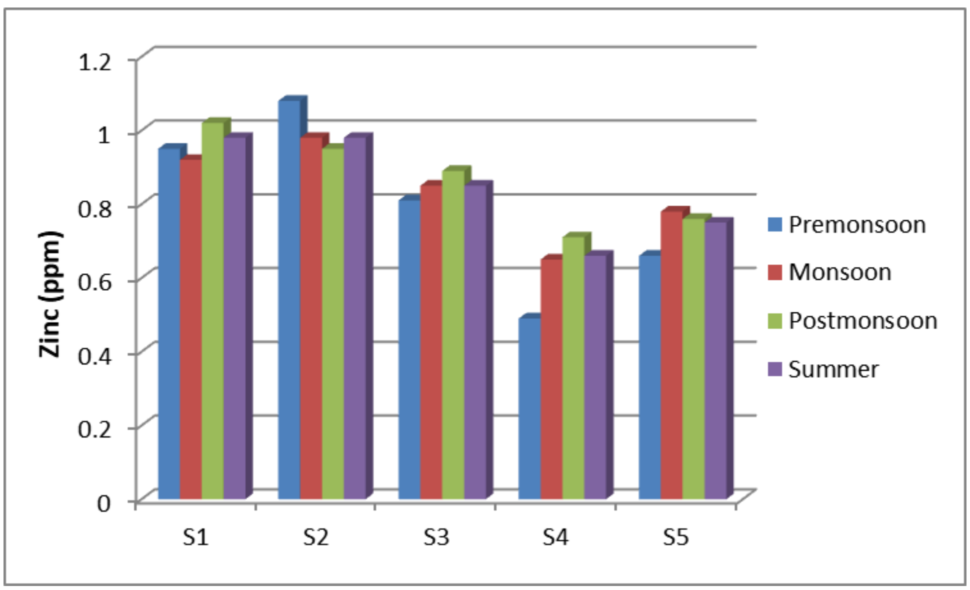
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.15 Manganese (ppm) in soil during different seasons in Tiruvarur district



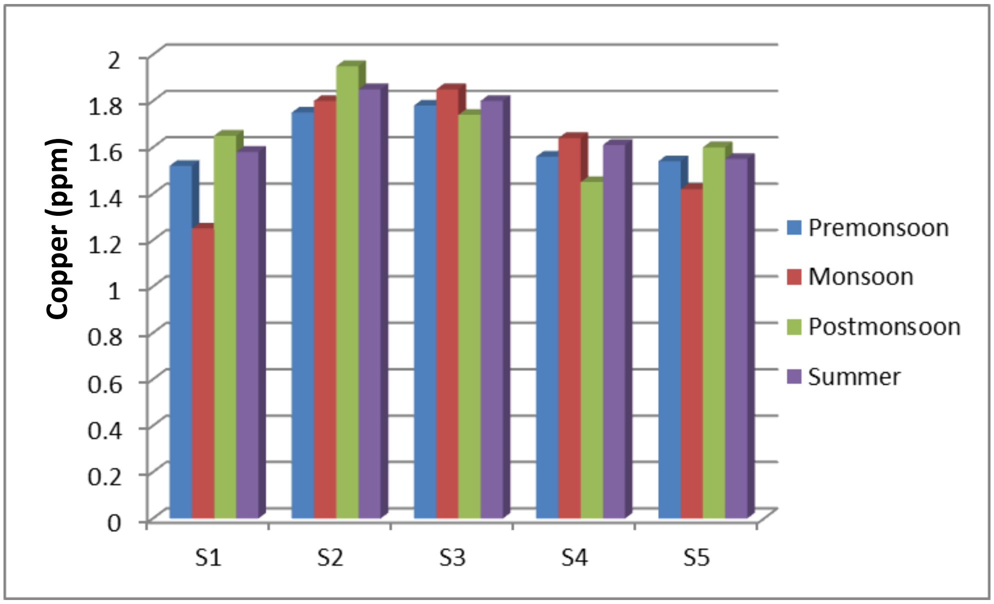
S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.16. Zinc (ppm) in soil during different seasons in Tiruvarur district



S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Fig.17. Copper (ppm) in soil during different seasons in Tiruvarur district



S1- Thiruthuraipoondi site , S2- Mannargudi site , S3- Tiruvarur site, S4- Nannilam site, S5- Needamangalam site.

Table3: Pearson correlation coefficient of physiochemical parameters and bacterial population density of five different soil samples in Tiruvarur district

	PH	*EC*	*OC*	*AP*	*AK*	*AN*	*AI*	*AM*	*AZ*	*AC*	*PD*
PH	1										
EC	-0.3	1									
OC	0.28	-0.48	1								
AP	0.22	0.44	-0.44	1							
AK	-0.03*	0.51	0.5	-0.02*	1						
AN	0.37	-0.5	0.99	-0.36	0.47	1					
AI	-0.46	-0.19	0.44	-0.93	0.27	0.35	1				
AM	0.04*	-0.82	0.68	-0.28	-0.15	0.68	0.23	1			
AZ	0.11	-0.06	-0.78	0.09	-0.76	-0.76	-0.32	-0.48	1		
AC	-0.001	-0.56	-0.3	0.25	-0.87	-0.26	-0.41	0.45	0.42	1	
PD	0.77	-0.51	0.74	-0.38	0.22	0.77	0.18	0.33	-0.25	-0.28	1

OC-Organic Carbon, AP- Available Phosphorus, AK- Available Potassium, AN – Available Nitrogen, AI- Available iron, AM – Available Manganese, AZ- Available Zinc, AC- Available Copper, PD- Population Density

*Strongly presented correlation significance at the level ($p < 0.05\%$)

All our sampling sites in Tiruvarur, such as Tiruthuraipoondi, Mannargudi, Tiruvarur, Nannilam, and Needamangalam, showed a p^H value above 7. Among them, the Nannilam and Mannargudi sites possess a high p^H value above 7.2 comparable to all other sites in Tiruvarur district. (Fig.8). The electrical conductivity in the Thiruthuraipoondi and Nannilam sites was high (0.8–1.2 dsm-1), but it was low at the Tiruvarur site (0.15 dsm-1). In every seasons examined, Nannilam and Needamangalam sites have a higher percentage of organic carbon, while Mannargudi and Thiruthuraipoondi sites have a lower percentage.Tiruvarur and Needamangalam have the lowest mass of phosphorus during the premonsoon season, whereas Nannilam has the highest mass during the monsoon season. Nannilam and Needamangalam sites have high potassium levels during the monsoon but Mannargudi sites have lower levels during the premonsoon season. Nannilam and Needamangalam sites have high potassium levels during the monsoon, but Mannargudi and tiruvarur sites have lower levels during the premonsoon season. Needamangalam has a higher mass of nitrogen than Thiruthuraipoondi which has a lower mass of nitrogen throughout the seasons studied. The levels of iron and manganese are higher in the Tiruvarur rice field during the monsoon season and lower in Mannargudi and Thiruthuraipoondi during the postmonsoon season. In a similar way, copper and zinc concentrations are higher in the mannargudi rice field in almost all seasons studied. Further the Pearson correlation coefficient of physiochemical parameters and population density of bacterial Isolates are performed as shown in Table1 [23]

Discussion

Although rice soil microbiota was well studied already but only limited studies reported the bacterial community present in rice rhizosphere. Especially, no reports were found in rice bowl of Tamilnadu, Tiruvarur. The distribution of bacteria is greatly influenced by environmental parameters such as soil pH, moisture, temperature, organic carbon, and nitrogen [24**].** In this study, we have collected soil samples of rice and their bacterial profile was recorded through classical culturing technique. Our studies showed the abundances of Phylum Proteobacteria followed by Bacillota and Bacteroidetes in rice soil microbiota and are consistent with previous reports [25]. Proteobacteria are a phylum of Gram-negative bacteria and are related to a wide range of functions involved in carbon, nitrogen, and sulphur cycling [26]. Their relative richness increases with high organic carbon availability in soils as aligned with findings of Fierer *et al.*,2012 .[27]. Bacteroidetes phylum have been found to function as degraders of polymeric organic matter.

In the present study, the bacterial population isolated from five different sites of rice field in Tiruvarur district with respect to four different seasons (Premonsoon, Monsoon,Postmonsoon and Summer) were recorded. The isolated bacterial species in rice field soil include Aeromonas sp., *Flavobacterium odoratum, Bacillus subtilis, Serratia fonticola, Pseudomonas fluorescence, Bacillus sp., Escherichia coli, Rhizobium sp., Vibrio cholera, Bacillus cereus, Enterobacter cloacae, Azospirillum sp., Azotobacter chrococcum, Azospirillum brasilense, B.amyloliquefaciens, B.megaterium, Bradyrhizobium japonicum, Salmonella typhi, Yersinia enterocolitica, Shigella sp*.The maximum bacterial colonies were screened (230) from needamangalam rice field clay soil whereas minimum colonies were observed (138) from tiruvarur rice field soil. In the rice rhizosphere, *Azotobacter* species, free-living nitrogen-fixing bacteria, have been used as biofertilizers to improve the productivity of non-leguminous crops including rice, due to their various plant growth-promoting traits. *Azospirillum* belong to rhodospirillales possess the ability to synthesize phytohormones, especially indole-3-acetic acid and gibberellins, and helps to promote proliferation of roots [28].

The Pearson correlation coefficient of physicochemical parameters (temperature, pH, organic carbon, organic matter, salinity, available nitrogen, phosphorus, potassium, zinc, copper, iron, manganese and sodium, calcium, magnesium, potassium) and population density of bacteria were analyzed using SPSS Software. The sign put in front of the coefficient value indicates the direction of the relationship. Relationship values can be between -1 and +1, with +1 signifying an absolutely perfect linear relationship, 0 signifying no linear relationship, and -1 signifying an entirely inverse relationship between the coordinates. The physicochemical parameters were positively correlated at the p 0.05% level of significance whereas the bacteria population density was negatively correlated with some physiochemical characteristics.

Conclusion

Soil microbiota study of rice illustrates a total of 827 bacterial colonies observed among 21 species in all seasons of Tiruvarur district. Among them, the Needamangalam clay soil possessed the highest number of colonies (230), and the lowest (138) was observed in the Tiruvarur soil. These distinct colonies may correspond to specific functional roles to be performed under various physiochemical conditions. The taxonomic analysis of the rice soil fungal community reported the abundance of Proteobacteria, Bacillota, Bacteroidetes at the phylum level; Gammaproteobacteria, Alphaproteobacteria and Bacilli at the class level; Pseudomonadales, Rhodospirillales and Hyphomicrobiales at the order level; and Azospirillaceae, Pseudomonadaceae, and Bacillaceae at the family level taxon. Moreover, the microbiota of rice soil is largely populated by bacteria belonged to genera such as *Pseudomonas* , *Azotobacter, Azospirillum and Bacillus.*

This study also addressed the physiochemical parameters such as p^H, electrical conductivity, organic carbon, nitrogen, phosphorus, potassium, iron, manganese, zinc, and copper in the seasons such as premonsoon, monsoon, postmonsoon and summer across all sites of the Tiruvarur district. It clearly indicates that the soil collected at monsoon and postmonsoon seasons showed maximum bacterial propagules compared to premonsoon and summer with few exceptions as studied by Kalaivani *et al.* [30]. In addition, more number of bacterial colonies observed during post monsoon season when compared to monsoon and premonsoon seasons.

Different agricultural plants have responded favourably to co-inoculation with *Azotobacter* and *Rhizobium* in laboratory, greenhouse, and outdoor environments. *Azotobacter* is able to produce growth hormones like auxins and gibberellins and thus enhancing root growth, it in turn could make available more root area to rhizobia for infection. This would result in increased nodulation, nitrogen fixation and ultimately crop yield improvement [28& 29].Here in our study both *Azotobacter* and *Rhizobia* naturally present in rhizospheric soil, Tiruvarur district . In addition to being useful in improving plant growth and yield attributes, co-inoculation of *Azotobacter* and *Azospirillum* have also been found to alleviate the adverse effect of salinity stress on some plants. The discovery of different bacterial taxa during different seasons of the year in rice-growing regions may stimulate the development of novel strategies such as incorporating populations of useful bacteria into agroecosystems, which could be used to improve plant growth and development..

Acknowledgement

The authors greatly thank Principal, Secretary and Correspondent, A.V.V.M Sri Pushpam College(Autonomous), Poondi, Thanjavur and The Director, Indian Biotrack Research Institute, Thanjavur for providing necessary laboratory facilities .

References

[1]. Ding LJ, Cui HL, Nie SA, Long XE, Duan GL, Zhu YG. Microbiomes inhabiting rice roots and rhizosphere. FEMS Microbiol Ecol.(2019);95(50).

[2]. Rathna Priya, T., Eliazer Nelson, A.R.L., Ravichandran, K. et al. Nutritional and functional properties of coloured rice varieties of South India: a review. J. Ethn. Food 2019 ;6(11)

[3]. Ricepedia, CGIAR, IRRI, Philippines 2018. Cultivated rice species. http://ricepedia.org/rice-as-a-plant/rice-species/cultivated-rice-species.

[4]. Agricultural Statistics Division, Third advance estimates of production of food grains for 2016-17, Department of Agriculture, Cooperation and Farmers Welfare, India. https://agricoop.gov.in/Documents/Annual_rpt_201617.pdf

[5]. Kennedy G, Burlingame B, Nguyen VN. Nutritional contribution of rice and impact of biotechnology and biodiversity in rice-consuming countries.Proceedings of 20th session of International rice commission 2002 pp:59-69.

[6]. DeCorato,U.Soil microbiota manipulation and its role in suppressing soil-borne plant pathogens in organic farming systems under the light of microbiome-assisted strategies. Chem. Biol. Technol. Agric. 2020(7)17.

[7]. Kibble white MG, Ritz K, and Swift MJ. 2008. Soil health in agricultural systems. Philos Trans R Soc Biol Sci.2008 Feb27,363:685–701.

[8]. O'Donnell AG, Colvan SR, Malosso E..Twenty years of molecular analysis of bacterial communities in soils and what have we learned about function? In: Bardgett RD, Usher MB, Hopkins DW, editors. Biological diversity and function in soils. New York: Cambridge University Press 2005 pp:44–56.

[9]. Baskaran D, Pandiyan P, Selvam G, Annamalai P, Varatharajan A. Seasonal distribution of fungi and physiochemical properties of soil in different agricultural fields of Tiruvarur district, rice bowl of Tamil Nadu. Res. Jr. Agril. Sci. 2023, 14(2): 496-504.

[10]. Subbiah BV, Asija GL. A Rapid Procedure for the Estimation of Available Nitrogen in Soils. Current Science, 1956 Vol. 25, 259-260.

[11]. Olsen SR, Sommers LE. Phosphorus. In: Page, A.L., Ed., Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties, American Society of Agronomy, Soil Science Society of America, Madison, 1982, pp: 403-430.

[12]. Walkley AJ, Black IA. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci. 1937 Vol.37, pp:29-38.

[13]. Saravanakumar K, Kaviyarasan V. Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu. Afric. Jr. Plant Sciences 2010, 4(6): 190-196.

[14]. Bergey DH, Holt JG. Bergey’s Manual of Determinative Bacteriology. 9th Edition, Williams & Wilkins, Baltimore, Maryland 1994.

[15]. Coico R.Gram staining. Curr Protoc Microbiol. 2005 Appendix 3C. doi: 10.1002/9780471729259.mca03cs00.

[16]. Vracko, R., and J. C. Sherris Indole-spot test in bacteriology. Am. J. Clin. Pathol. 1963, 39:429–432.

[17]. Vaughn, R. H., N. B. Mitchell, and M. Levine. The VogesProskauer and methyl red reactions in the coli-aerogenes group. J. Am. Water Works Assoc.1939, 31:993–1001.

[18]. Koser, S. A. Correlation of citrate utilization by members of the colon-aerogenes group with other differential characteristics and with habitat. J. Bacteriol.1924, 9:59–77.

[19]. Taylor, W. I., and D. Achanzar. Catalase test as an aid to the identification of Enterobacteriaceae. J. Appl. Microbiol. 1972, 24:58–61.

[20]. Clarke H, Cowan ST. Biochemical methods for bacteriology. J. Gen. Microbiol.1952,6:187-197

[21]. Jurtshuk, P., Jr., and D. N. McQuitty. Use of a quantitative oxidase test for characterizing oxidative metabolism in bacteria. Appl. Environ. Microbiol.1976, 31:688–679.

[22]. Krumwiede, C. and L. Kohn. 1917. A triple sugar modification of the Russell Double Sugar medium. J. Med. Res.1917, 37:225.

[23]. Swedha A, Kanimozhi G and Panneerselvam A. Seasonal Distribution of Bacterial Diversity in Marine Soil of Kodiyakarai, Vedaranyam, Nagappattinam (DT.) Res. Jr. of Agril. Sci. 2022,13 (5):1560-1565.

[24]. Yu C, Lv DG, Qin SJ, Du GD, Liu GC. Microbial flora in Cerasus sachalinensis rhizosphere. Chinese. J. Appl. Ecol.,2007,18(10): 2277- 2281.

[25]. Zifcakova L, Vetrovsky T, Howe A, and Baldrian P. Microbial activity ˇ in forest soil reflects the changes in ecosystem properties between summer and winter. Environ. Microbiol. 2016, Vol.18,pp: 288–301.

[26]. Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., Stackebrandt, E. Introduction to the Proteobacteria. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (eds) The Prokaryotes. Springer, New York, NY (2006).

[27]. Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., & Knight, R.. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME journal,2012, 6(5),1007–1017.

[28]. Sumbul A, Ansari RA, Rizvi R, Mahmood I Azotobacter: A potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci 2020;27(12):3634-3640.

[29]. Verma J.P., Yadav J., Tiwari K.N., Jaiswal D.K. Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (Cicer arietinum L.) in India. Soil Biol. Bioch 2014;70:33–37.

[30]. Kalaivani R, Sukumaran V.Characterization of bacterial diversity in marine sediment at different seasons in karankadu, Tamilnadu, India. International Journal of Pure and Applied Zoology 2013,1(3):97-108.