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Investigation of soil characters and *Azospirillum* isolated from paddy soils of Thanjavur district,East Coast of Tamilnadu, India

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ABSTRACT

Totally 30 different paddy field soils were collected from in and around Thanjavur District, Tamilnadu and their physico-chemical properties were analyzed. Among them, 11 samples were loamy soil, 11 samples were sandy loam and the rest of 8 samples were sandy clay loam. The pH (8.2-5.8) ,bulk density (1.65g / cm^3 - 1.00g/cm^3),water holding(61.85% - 10.86%),electrical conductivity (2.40 - 0.19), organic carbon (1.27% - 0.11%),total nitrogen (1.78% - 0.55%), phosphorus content (1.17% - 0.11%), potassium (1.85% - 1.14%) also available micronutrients like Zn (2.02% - 1.06%), Cu (3.78% - 1.27%), Fe (10.47% - 7.10%), Mn (5.95% - 2.66%), B (0.594% - 0.28%), available nitrogen(203.0kg/acre - 110.0kg/acre), phosphorous (9.10kg/acre - 3.85 kg/acre), potassium (340 kg/acre - 245 kg/acre) were in all sampling station.

Key words: Paddy field soil, *Azospirillum* spp, Population density.

INTRODUCTION

Soil microorganisms, like *Azospirillum* spp., *Azotobacter* sp. and *Enterobacter* sp. have shown to encourage plant growth, by promoting the outbreak of secondary roots. *Azospirillum* have been isolated from the rhizosphere and roots of a variety of plants including cereals and grasses. Inoculation with indigenous *Azospirillum* is an important procedure when studying their inherent capacity to benefit crops. In some cases, indigenous strains can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment.

Azospirillum species are commonly found in soils and in association with roots of plants namely rice, maize, wheat and legumes. Rhizosphere colonization by *Azospirillum* species has been shown to stimulate the growth of a variety of plant species. The success of the *Azospirillum*

plant interaction depends on the survival and persistence of these bacteria in soil and the effective colonization of the rhizosphere. Chemotaxis is one of the several properties which may contribute to survival, rhizosphere colonization and the initiation of mutualistic interactions by *Azospirillum* species (Lopez-de-victoria. 1989)[1].

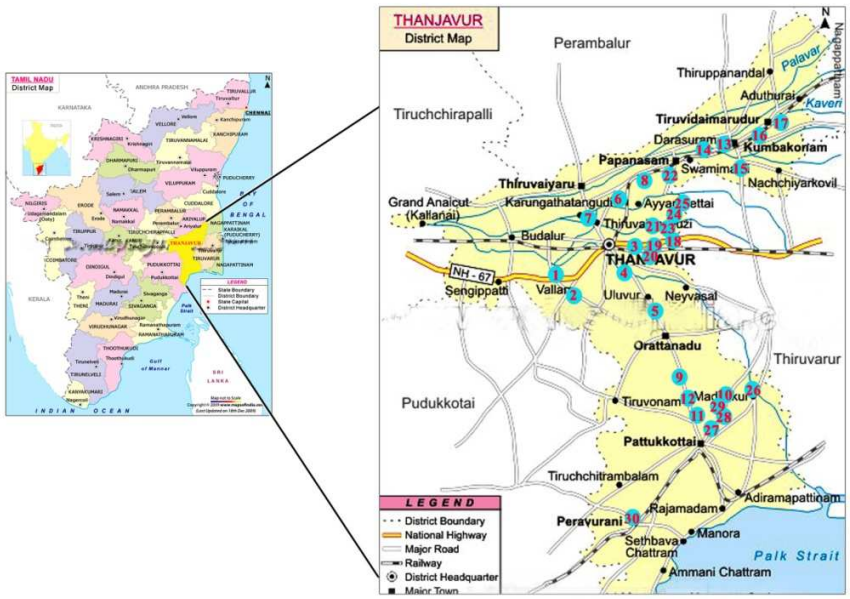
Apart from being a general plant colonizer (Bashan *et al.*, 2004)[2], *Azospirillum* is remarkably versatile. *Azospirillum* is not only able to fix atmospheric N (Dobereiner and Day 1976)[3], but also to mineralize nutrients from the soil, to sequester, Fe, to survive to marsh environmental conditions, and can help plant minimize the negative effects of abiotic stresses. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes and soil biodiversity. An understanding of microbial diversity perspectives in agricultural context, is important and useful to arrive of measures that can act as indicators of soil quality and plant productivity.

MATERIALS AND METHODS

Description of the study area

The present study focused on the area in and around Thanjavur district (Fig.1). The study area is situated in Tamilnadu state (Lat. 11˚10′– 11˚ 30′ N and Long. 78˚15′ – 78˚30′ E) with the significant features of evergreen forests and also it was a less explored ecosystem for the investigation of *Azospirillum* population.

Fig. 1 Map showing the Study Stations



S.No.	Sampling place	Texture
1.	Vallam	SL
2.	Kurungulam	L
3.	Punnainallur	SL
4.	Nanjikottai	SL
5.	Soorakkottai	L
6.	Pudupattinam	SL
7.	Vellamperambur	SCL
8.	Rajagiri	SCL
9.	Pinnaiyur	L
10.	Vadacherri	L
11.	Amblapettu	L
12.	Ulur	L
13.	Darasuram	SCL
14.	Thiruvalanjuli	SCL
14.	Sakkottai	SCL
16.	Narasingampettai	SCL
17.	Kanjanur	SCL
18.	Saliyamangalam	SL
19.	Poondi	SL
20.	Nallavaniyankudikadu	SL
21.	Raramuthirakottai	Sl
22.	Thirupuvanam	SL
23.	Ukkaadai	SCL
24.	Neikkunnam	SL
25.	Aruthavapuram	SL
26.	Madhukkur	L
27.	Alathur	L
28.	Sendankadu	L
29.	Thittakudi	L
30.	Peravurani	L

Collection of soil samples (Bashan and Wolowelsky, 1987)[4]

For the enumeration of *Azospirillum*, soil samples were collected by aseptic manner at a depth of 5-10 cm according to the V - shaped method, at thirty different locations in and around the

Thanjavur district. From each site, five samples were collected and pooled together and considered as one sample. The soil samples were brought to the laboratory and kept in the refrigerator for further process.

Analysis of physico – chemical parameters of the soil

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)[5], Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973)[5].

Nutrient analysis

Organic carbon (OC) content was determined by adopting chromic acid wet digestion method as standard procedure of Walkley and Black (1934)[6], available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956)[7] and available phosphorus by Brayl method (Bray and Kutz, 1945)[8]. Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer (Standfold and English, 1949)[9], calcium (Neutral 1 N NH_4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by versenate method (Jackson, 1973)[5]. Available micronutrients such as Zn, Cu, and Mn, were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer model 2280 atomic absorption spectrophotometer (Lindsay and Norvell, 1978)[10]. Other nutrients such as Fe, and available Boron were analysed following methods of Bernes (1959)[11]; Muthuvel and Udayasoorian (1999)[12].

Isolation of *Azospirillum*

From the collected soil samples, 1 g was taken and serially diluted using sterile distilled water upto 10^{-8} dilutions. One ml of diluted sample from 10^{-6} to 10^{-8} dilutions was taken, and 0.1ml of aliquot was inoculated in test tube containing Nfb (Nitrogen free bromothymol) semisolid media. All the tubes were incubated at 32°C for 48 h and observed the growth by the formation of pellicles. The pellicles were streaked on Nfb solid media and incubated at 32°C for 24 h.

Morphologically divergent *Azospirillum* colonies (white, yellow and pink) were picked from the plates and streaked on basal minimal salt agar medium and incubated at 32°C for 24 h. After attained sufficient growth, all the isolates were preserved in a refrigerator for further investigation. The stock cultures were sub cultured in fresh nutrient agar slants once in a month and maintained at refrigerated condition. The isolates were also streaked separately on Basal Minimal Salt and potato agar media separately and incubated at 32ºC for 48 h.

Enumeration of *Azospirillum* isolates

For enumeration of *Azospirillum* cells, Nfb solid medium was used. After 48 h of incubation the colonies in the NFB plates were counted by using Quebec colony counter.

Population density is expressed in terms of colony forming unit (CFU) per gram of soil with dilution factor.

$$\text{Number of cells / ml} = \frac{\text{Number of colonies}}{\text{Amount plated X dilution}}$$

Statistical analysis

Pearson's correlation co efficient analysis was made to assess the relationships between physico-chemical parameters and total population density of *Azospirillum* isolates. The data were computed and analysed using Statistical Package for Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere. Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival. Rhizospheric bacterial communities have an efficient system for uptake and catabolism of organic compounds present in root exudates (Barraquio *et al.*, 2000)[13].

The rhizosphere is the narrow zone of soil, surrounding the root that is under the immediate influence of the root system. This zone is rich in nutrients when compared with bulk soil, due to the accumulation of a variety of organic compounds released from roots by exudation, secretion and deposition (Curl and Truelove, 1986)[14]. Because, these organic compounds can be used as carbon and energy sources of microorganisms for their growth and activity particularly, intense in rhizosphere. In the present study, totally 30 *Azospirillum* isolates were isolated from 30 different paddy field soil samples (10^5 dilution) collected from in and around Thanjavur district. Cristyakova and Kalininskaya (1984)[15] reported that bacteria of the genus *Azospirillum* are widespread in the soils of various regions and usually occur in the rhizosphere of vascular plants. Their presence in the rhizosphere soil has been observed in the paddy field, where their cell numbers did not exceed $2x10^5$ cells / g of soil.

Physico-chemical properties of soil

The results of physico-chemical properties of soil samples from 30 different locations of Thanjavur District were summarized in Table 1.

Out of 30 soil samples, 11 samples were loamy soil, while 11 samples were sandy loam and 8 samples were sandy clay loam. The maximum (8.2) pH of the soil was recorded at both Narasingampettai and Kanjanur and minimum pH (5.8) was recorded at Raramuthirakkottai. The bulk density of the soil was maximum 1.65g / cm^3 recorded at Vallam and minimum was 1.00g/cm^3 recorded at Madukkur. The maximum (61.85%) water holding capacity (WHC) of the soil was recorded at Peravarani and minimum 10.86% was recorded at Pudupattinam. The electrical conductivity of soil was maximum (2.40) recorded in Raramuthirakkottai and minimum was (0.19) in Poondi and Ukkadai .

Table 1. Physico – chemical properties of soil samples from Thanjavur Dt.

S. No.	Sampling place	Texture	pH	Bulk Density (g/cm^3)	Water Holding Capacity (%)	Electrical Conductivity (dSm^{-1})	Organic Carbon %
1	Vallam	SL	7.2	1.650	27.396	0.70	0.91
2	Kurungulam	L	6.7	1.510	41.110	0.6	0.80
3	Punnainallur	SL	7.2	1.270	56.675	0.66	0.88
4	Nanjikottai	SL	7.4	1.158	53.078	0.74	0.96
5	Soorakkottai	L	7.7	1.082	43.671	0.88	0.97
6	Pudupattinam	SL	7.8	1.157	10.865	0.67	0.90
7	Vellamperambur	SCL	6.6	1.350	25.122	0.71	0.91
8	Rajagiri	SCL	7.5	1.470	28.466	0.62	0.11
9	Pinnaiyur	L	7.3	1.460	30.655	0.79	0.82
10	Vadacherri	L	7.1	1.420	21.534	0.69	0.85
11	Ambalapattu	L	7.3	1.175	50.321	0.63	1.27
12	Ulur	L	7.8	1.610	18.050	0.70	0.85
13	Darasuram	SCL	7.5	1.320	37.619	0.69	0.96
14	Thiruvalanjuli	SCL	7.5	1.315	23.407	0.72	0.88
14	Sakkottai	SCL	7.8	1.320	30.754	0.71	0.91
16	Narasingampettai	SCL	8.2	1.420	31.885	0.79	0.82
17	Kanjanur	SCL	8.2	1.310	22.300	1.10	0.87
18	Saliyamangalam	SL	8.1	1.250	31.309	0.89	0.97
19	Poondi	SL	6.9	1.310	24.201	0.19	0.58
20	Nallavanniyankudikadu	SL	7.1	1.301	15.545	0.80	0.95
21	Raramuthirakottai	SL	5.8	1.151	31.307	2.40	0.92
22	Thirupuvanam	SL	6.1	1.136	22.510	0.38	0.55
23	Ukkaadai	SCL	7.5	1.372	32.654	0.19	0.85
24	Neikkunnam	SL	7.5	1.136	35.682	0.23	0.75
25	Arunthavapuram,	SL	7.7	1.110	25.654	0.19	0.66
26	Madhukkur,	L	7.0	1.000	25.357	0.25	0.78
27	Alathur,	L	7.0	1.136	24.357	0.35	0.72
28	Sendankadu,	L	7.8	1.510	26.341	0.30	0.85
29	Thittakudi,	L	7.5	1.495	26.010	0.30	0.95
30	Peravurani	L	7.4	1.372	61.851	0.30	0.85

L = Loamy; SL = Sandy Loam; SCL = Sand Clay Loam

Nutrients

The organic carbon content of soil maximum as 1.27% recorded at Ambalapattu, and minimum was 0.11% at Rajagiri (Table 1). The total nitrogen maximum as 1.78% were recorded at Alathur soil and minimum was 0.55% at Poondi. The maximum (1.17%) phosphorus content of the soil was observed at Sakkottai and minimum (0.11%) was at Sendankadu soil. The maximum (1.85%) potassium of the soil was observed in Madhukkur soil and minimum (1.14%) in Vellamperambur soil.

The available micronutrients like Zn of the soil were recorded maximum as 2.02% at Kanjanur 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 Raramuthirakkottai 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 the B (0.594%) was recorded in Soorakottai and Nallavanniyankudikadu soils and minimum 0.28% was in Thiruvalanjuli soil (Table 2).

Table 2. Micronutrients of soil sample from Thanjavur Dt.

S. No.	Sampling places	Total Nutrients (%)			Available Micro Nutrients (%)				
		N	P	K	Zn	Cu	Fe	Mn	B
1	Vallam	1.10	0.154	1.55	1.29	2.82	10.47	4.29	0.544
2	Kurungulam	0.92	0.136	1.22	1.21	2.86	8.74	5.12	0.516
3	Punnainallur	0.97	0.152	1.82	1.26	2.84	9.26	5.64	0.527
4	Nanjikottai	1.22	0.175	1.45	1.29	1.86	8.54	4.71	0.498
5	Soorakkottai	1.28	0.146	1.42	1.74	2.74	9.28	4.24	0.624
6	Pudupattinam	1.18	1.126	1.28	1.28	3.26	8.24	5.21	0.586
7	Vellamperambur	0.94	0.124	1.14	1.06	3.27	8.24	4.86	0.462
8	Rajagiri	0.86	0.144	1.24	1.34	3.14	8.26	4.24	0.456
9	Pinnaiyur	0.84	0.139	1.29	1.29	2.40	8.10	4.86	0.594
10	Vadacherri	0.89	0.142	1.45	1.51	2.87	8.54	4.86	0.612
11	Ambalapattu	1.72	0.154	1.56	1.76	1.94	8.27	3.87	0.519
12	Ulur	0.96	0.152	1.29	1.28	2.27	8.88	4.95	0.563
13	Darasuram	1.24	0.135	1.28	1.26	2.84	9.26	5.64	0.527
14	Thiruvalanjuli	0.98	0.271	1.46	1.24	1.94	8.96	4.66	0.286
15	Sakkottai	1.17	1.174	1.49	1.36	1.74	7.27	4.84	0.572
16	Narasingampettai	0.92	0.138	1.54	1.52	1.98	9.24	5.65	0.548
17	Kanjanur	0.98	0.114	1.76	2.02	2.48	7.44	4.78	0.533
18	Saliyamangalam	0.87	0.124	1.29	1.86	3.17	8.38	2.66	0.564
19	Poondi	0.55	0.132	1.30	1.75	3.10	8.40	3.65	0.530
20	Nallavanniyankudikadu	1.14	0.138	1.59	1.56	2.96	8.82	4.17	0.594
21	Raramuthirakottai	1.07	0.142	1.72	1.48	1.27	7.40	5.42	0.582
22	Thirupuvanam	0.65	1.112	1.50	1.32	2.53	8.55	4.55	0.458
23	Ukkaadai	0.75	0.125	1.32	1.55	2.80	9.10	3.55	0.410
24	Neikkunnam	1.10	0.165	1.44	1.42	3.10	7.58	3.78	0.420
25	Arunthavapuram	0.97	0.170	1.32	1.78	3.00	8.55	3.88	0.510
26	Madhukkur	1.38	0.155	1.85	1.38	2.98	7.95	3.25	0.555
27	Alathur	1.78	0.135	1.63	1.96	2.65	8.10	4.65	0.538
28	Sendankadu	0.97	0.112	1.35	1.68	1.55	7.98	3.10	0.432
29	Thittakudi	0.99	0.185	1.24	1.47	3.78	7.10	3.98	0.425
30	Peravurani	1.11	0.138	1.48	1.35	3.10	9.20	5.95	0.590

N = Nitrogen; P = Phosphorus; K = Potassium; Zn = Zinc; Cu = Copper; Fe = Iron; Mn = Manganese; B = Boron

The maximum (203.0kg/acre) available nitrogen was observed at Ambalapattu and minimum (110.0kg/acre) was recorded at Poondi. The phosphorous of soil was maximum (9.10kg/acre) in Peravurani soil and minimum (3.85 kg/acre) was in Punnainallur and Darasuram soil. The maximum potassium (340 kg/acre) content of the soil was recorded in Alathur soil and minimum (245 kg/acre) was in Nanjikottai soil (Table 3).

Table 3. Macronutrients of soil sample from Thanjavur Dt.

S. No.	Sampling places	Available Nutrients (Kg/acre)		
		N	P	K
1	Vallam	127.4	9.0	265
2	Kurungulam	119.0	6.0	285
3	Punnainallur	130.2	3.85	295
4	Nanjikottai	144.2	4.51	245
5	Soorakkottai	134.4	8.5	270
6	Pudupattinam	131.6	4.5	295
7	Vellamperambur	128.8	4.5	315
8	Rajagiri	113.4	4.75	315
9	Pinnaiyur	116.2	6.0	275
10	Vadacherri	120.4	6.5	280
11	Ambalapattu	203.0	5.25	295
12	Ulur	128.8	5.75	310
13	Darasuram	130.2	3.85	295
14	Thiruvalanjuli	124.6	5.25	295
15	Sakkottai	128.8	5.75	310
16	Narasingampettai	124.6	6.0	255
17	Kanjanur	130.2	5.35	265
18	Saliyamangalam	112.0	4.5	270
19	Poondi	110.0	5.50	300
20	Nallavanniyankudikadu	126.0	8.5	315
21	Raramuthirakottai	134.7	4.75	310
22	Thirupuvanam	125.2	6.7	288
23	Ukkaadai	140.3	5.83	320
24	Neikkunnam	132.5	4.75	310
25	Arunthavapuram	129.7	6.38	288
26	Madhukkur	140.8	8.55	278
27	Alathur	123.5	4.80	340
28	Sendankadu	135.2	8.30	250
29	Thittakudi	128.5	7.50	325
30	Peravurani	138.7	9.10	312

N = Nitrogen; P = Phosphorus; K = Potassium

Azospirillum spp. are members of the α - subclass of proteobacteria. Azosprilla have worldwide distribution and occur in large numbers (upto10^{-7}/g) in rhizosphere soils and associated with the roots, stems and leaves of a large variety of plants. Members of the genus *Azospirillum* fix nitrogen under microaerophilic conditions, and are frequently associated with root and rhizosphere, these are known as associated diazotrophs. Sen (1929)[16] made one of the earliest suggestions that the nitrogen content of cereal crops could be met by the activity of associated nitrogen fixing bacteria namely *Azospirillum*. This organism came into focus with the work of Dobereiner from Brazil (Dobereiner *et al*., 1976)[17], followed closely by reports from India

(Lakshmi-Kumari *et al.*, 1976[18]; Kavimandan *et al.*, 1978[19]; Tilak and Murthy, 1981[20]). After establishing in the rhizosphere azospirilla, usually but not always, promote the growth of plants (Okon, 1985[21]; Tilak and Subba Rao, 1987[22]; Bashan and Holguin, 1997[23]). Despite their N_2 fixing capability, the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake (Dewan and Subba Rao, 1979[24]; Okon and Kapulnik, 1986[25]; Fallik *et al.*, 1994[26]). Azospirilla proliferated in the rhizosphere of numerous plant species and the genus *Azospirillum* now contains seven species *A. brasilense* (Tarrand *et al.*, 1978[27]), *A. lipoferum* (Tarrand *et al.*, 1978[27]), *A. amazonense* (Magalhaes *et al.*, 1983[28]), *A. halopraferens* (Reinhold *et al.*, 1987[29]), *A. irakense* (Khammas and Kaiser, 1991[30]), *A. dobereinerae* and *A. largimobile* (Eckert *et al.*, 2001[31]).

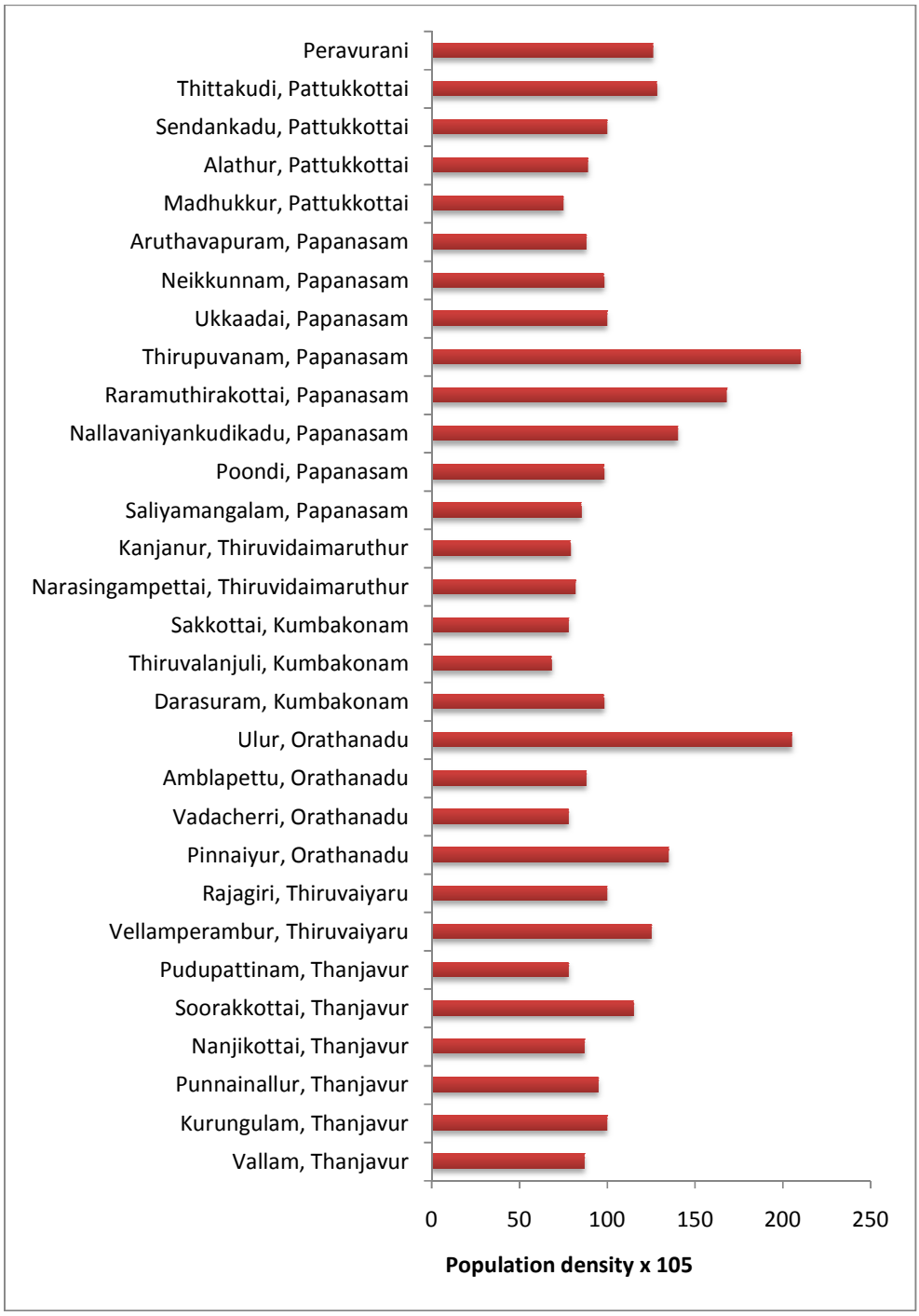
Table 4. Population density of *Azospirillum* isolates from different locations of Thanjavur Dt.

S. No.	Name of the Taluk	Sampling location	No. of Population X 10^{-5} dilution CFU/g of soil
1	Thanjavur	Vallam	87
2		Kurungulam	100
3		Punnainallur	95
4		Nanjikottai	87
5		Soorakkottai	115
6		Pudupattinam	78
7	Thiruvaiyaru	Vellamperambur	125
8		Rajagiri	100
9		Pinnaiyur	135
10	Orathanadu	Vadacherri	78
11		Ambalapattu	88
12		Ulur	205
13	Kumbakonam	Darasuram	98
14		Thiruvalanjuli	68
15		Sakkottai	78
16	Thiruvidaimaruthur	Narasingampettai	82
17		Kanjanur	79
18	Papanasam	Saliyamangalam	85
19		Poondi Papanasam	98
20		Nallavanniyankudikadu	140
21		Raramuthirakottai	168
22		Thirupuvanam	210
23		Ukkaadai	100
24		Neikkunnam	98
25		Arunthavapuram	88
26	Pattukottai	Madhukkur	75
27		Alathur	89
28		Sendankadu	100
29		Thittakudi	128
30	Peravurani	Peravurani	126

Diversity of *Azospirillum*

For the isolation of *Azospirillum* spp., Nfb semisolid medium was used. After 24 h incubation, the Nfb semi-solid medium showed white colored pellicle (Plate I).

Fig. 2. Population density of *Azospirillum* isolated from 30 different soils of Thanjavur District



Appearance of pellicle formation on Nfb semi-solid medium indicated successful isolation of *Azospirillum*. The pellicles were transferred into Nfb plates. After 48 h white, merged colonies were observed on the medium. Typical white or pink, often wrinkled colonies were picked out

and transferred into Nfb semi-solid medium. A total number of 30 morphologically distinct *Azospirillum* isolates were isolated and tabulated. For enumeration of population density, the number of colonies on the plates was counted in the range of 68 to 210 colonies. The highest population density was observed in sandy loamy soil at Thirubuvanam. The lowest population density was observed in sandy clay loamy soil at Thiruvalanjuli (Table 4; Fig.2).

Correlation coefficient analysis

The correlation coefficient analysis revealed that the significant positive correlation between total nitrogen and total potassium (r = 0.390; P<0.05) and total nitrogen and available nitrogen (r = 0.605; P<0.01), whereas the significant negative correlation was obtained between total potassium and copper (r = 0.372; P<0.05) and zinc and manganese (r = 0.465; P<0.01)(Table 5).

Table 5. Correlation coefficient values for various chemical parameters and total population density recorded from Thanjavur District (n = 30).

	TN	TP	TK	AZn	ACu	AFe	AMn	AB	AN	AP	AK	PD
TN	1											
TP	-.051	1										
TK	.390*	-.030	1									
Azn	.159	-.264	.191	1								
ACu	-.200	-.106	-.372*	-.037	1							
AFe	-.114	-.225	-.068	-.214	.113	1						
AMn	.081	.169	.157	-.465**	-.175	.211	1					
AB	.220	.043	.225	.240	.067	.145	.235	1				
AN	.605**	-.033	.279	.059	-.350	-.083	-.037	-.033	1			
AP	.019	-.083	.144	.080	.096	.232	-.226	.197	.042	1		
AK	.138	.087	-.120	-.132	.302	-.278	.137	-.149	-.027	-.181	1	
PD	-.275	.122	-.116	-.183	-.045	.012	.149	.099	-.079	.139	.273	1

TN – Total Nitrogen *AZn – Available Zinc* *AN - Available Nitrogen*
TP – Total Phosphorus *ACu-Available Copper* *AP – Available Phosphorus*
TK – Total Potassium *AFe – Available Iron* *AK – Available Potassium*
AMn – Available Manganese *PD – Population Density*
AB – Available Boron
**0.05% significant level **0.01% significant level*

The availability of selective media for isolating diazotrophs belonging to the genus *Azospirillum* and the case of its detection by characteristic features of sub-surface white pellicle formation in semi-solid agar medium has helped to isolate from the rhizosphere and root surface (Dobereiner *et al.*, 1976[17]; Hegazi *et al.*, 1979[32]; Baldani *et al.*, 1986[33]; Ladha *et al.*, 1987[34]; Sundaram *et al.*, 1988[35]). Several isolates were obtained on semi-solid nitrogen free media from roots of Kallar grass (Bilal and Malik, 1987[36]; Zafer *et al.*, 1987[37]; Malik *et al.*, 1991[38]). These isolates formed a fine sub-surface white pellicle in nitrogen-free malate medium within 24 h, which gradually moved to the surface (Krieg and Dobereiner, 1984[39]).

More meaningful results were obtained when *A. brasilense* and *A. lipoferum* were inoculated into the soil in *in vitro* condition to measure their population dynamics. In a comparison between the levels of *A. brasilense* in the rhizosphere and bulk soil of a heavy – textured tropical soil from Martinique (French West India), *A. brasilense* had a preference mainly for the macro-aggregates

of the soil where it sustained a high population level of over 10^6 cells g^{-1} of soil fraction and to a lesser extent for the fine clay particles. Yet, these numbers represented only 0.18% of the total bacterial counts of these fractions (Kabir *et al*., 1995[40]).

Soil microorganisms play an important role in soil biogeochemical processes which determine plant productivity, successful functioning of introduced microbial bioinoculants and their influence on soil health. Exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats (Hill, 2000[41]).

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