

Original Research Article

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## Microbial Profile of a Calcareous Soil of South Tamil Nadu, India

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### ABSTRACT

#### Keywords

Bacteria, Fungi,  
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Phosphobacteria

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A large number of total bacteria, fungi, actinomycetes, diazotrophs, *Azotobacter* and phosphobacteria were present in a black soil of calcareous nature during summer and cropping periods. They were higher in summer season even though the soil was totally dry with a moisture content of only 6.3 per cent. During the cropping period a higher population of fungi and actinomycetes were observed. The calcareous soils have pH values ranging from 8.1 to 8.7. Sorghum roots collected from five locations harboured *Azospirillum* and *A. brasilense* was predominant in Aruppukottai and Kovilpatti soil and *A. lipoferum* in Madurai, Kamudhi and Pudhur. They also exhibited VAM infection.

### Introduction

Calcareous soils are commonly observed in the central, western and southern states of India, including Maharashtra, western parts of Madhya Pradesh, Gujarat and some parts of Andhra Pradesh, Karnataka and Tamil Nadu. Black soils are found scattered in Tirunelveli, Virudhunagar, Tuticorin, parts of Madurai, Coimbatore, Dharmapuri, Vellore, Trichirapalli, Salem, Cuddalore and Villupuram districts of Tamil Nadu. Agriculture is mainly depends upon the North East Rainfall in southern Tamil Nadu. The farmers were mainly cultivated Sorghum, maize, cotton, ragi and rice in Tamil Nadu. So

the microbial community study is important for knowing the soil aggregation and fertility soil in this location. In general, the carbonate content is more in calcareous soil, which is not suitable for uptake of major and minor nutrients in the soil.

Soil and microflora are inseparable, interrelated and interdependent as the soil is the abode for the microbes which are responsible for the productivity and fertility. Black cotton soil is subjected to dry and hot weather during summer, leading to decline in moisture and desiccation. The mean annual rainfall at Aruppukottai where the study was conducted is about 700-1000 mm out of

which about 300-350 mm is recorded in a period of about three months in Oct-Dec when rainfed sorghum is raised. During the other months the soil therefore appears dry and friable after the cropping season with very low moisture in summer. The soil show very scanty weed population.

Indian farmers are highly dependent on rainfall and all activities of agriculture starting from sowing to post harvest are affected either directly or indirectly by weather especially by rainfall. In the vagaries of weather condition and make it us suitable condition for cultivation to utilize such type of soil for improving the agricultural activity without any yield reduction. Soil microbial profile is affected by biotic and abiotic factors such as soil texture affected by biotic and abiotic factors such as soil texture (de Vries *et al.*, 2012), pH (Lange *et al.*, 2014), temperature (Medeiros *et al.*, 2006), seasonality (Cao *et al.*, 2011) and vegetation (Epron *et al.*, 2011).

## Materials and Methods

The microbial profile of the soil was monitored at monthly intervals in the Aruppukottai soil. The soil samples were collected from the field no A<sub>2</sub> of Regional Research Station, Aruppukottai at monthly intervals up to one year. In this soil sample, the total bacteria, fungi, actinomycetes, diazotroph, *Azotobacter* and phosphobacteria were enumerated as per the standard plate count (Allen, 1953). Using nutrient agar for bacteria, Martin's Rose Bengal agar and Kenknight's agar for fungi and actinomycetes respectively. The diazotrophs, *Azotobacter* and phosphobacteria were enumerated in diazotroph medium, Medium No. 77 and Sperber's hydroxy apatite agar.

The sorghum roots and the rhizosphere soil samples were collected from the major sorghum growing area of South Tamil Nadu

*viz.*, Aruppukottai, Kamuthi, Pudhur, Kovilpatti and Madurai. Sorghum crop rhizosphere soil was examined for the presence of *Azospirillum*, VA mycorrhizal spores and the roots of sorghum plants were analyzed for VAM - mycorrhizal infection. Sorghum crop root samples were examined for the presence of *Azospirillum* and the VAM - mycorrhizal infection. The presence of *Azospirillum* in the subterranean roots of sorghum root sample was tested following the method of Dobereiner and Day (1976). The *Azospirillum* isolates were characterized by gram staining, utilization of carbon sources and biotin requirements.

The sorghum crop rhizosphere soil was examined for the presence of VA mycorrhizal spores by wet sieving and decanting method of Gerdemann and Nicolson (1963). The roots of sorghum plants were analysed for VAM - mycorrhizal infection by clearing and staining method of Phillips and Hayman (1970). The mycorrhizal infection was calculated using the following formula expressed in percentage.

$$\text{Root infection (\%)} = \frac{\text{Number of root segments infected}}{\text{Number of root segment examined}} \times 100$$

## Results and Discussion

The microbial profile of the calcareous soil of Aruppukottai over a period of a year was assessed and presented in Table 1 and illustrated in Fig.1. In the present study the total bacteria, fungi, actinomycetes, diazotrophs, *Azotobacter* and phosphobacteria were present in large numbers in the calcareous black cotton soil even during summer when the soil was dry and the moisture was low i.e., 6.3 per cent. The soil moisture ranged from 5.4 to 24.2 per cent. A maximum of  $79 \times 10^6$  cfu g<sup>-1</sup> bacterial population was observed in July with the lowest of  $4 \times 10^6$  cfu g<sup>-1</sup> in March. The population increased during the cropping

period and declined after harvest. The soil microbial profile is altered by season, soil organic matter (Mellado-Vázquez *et al.*, 2016), plant root exudates, soil carbon dynamics (Chen *et al.*, 2016), soil type, soil fertility, soil pH, soil moisture and soil temperature (Voroney and Heck 2015). Plant root exudates attract some microorganism mainly gram negative bacteria because which is one of the important carbon source for bacteria (Mellado-Vázquez *et al.*, 2019).

The microbial profile is also affected by ecological functions like decomposition, accumulation of soil organic matter and nutrient cycling (Liang *et al.*, 2017). Hence, it is essential to study about the relationship between dynamics of microbial profile and environmental functions.

The soil exhibited a lesser population of fungi when compared to other organisms. During the cropping period actinomycetes and fungal population increased. The fungi were relatively higher during the cropping period Sep-Dec but declined afterwards. The lowest fungal population of  $2 \times 10^4$  cfu g<sup>-1</sup> was observed in March month and the highest population of  $22 \times 10^4$  cfu g<sup>-1</sup> in November i.e., during the cropping period. The rhizosphere soil fungal population contribute to the soil aggregation through fungal hyphae and promotes the soil fertility status (Duchicela *et al.*, 2013). The fungal population is always inversely proportional to the bacteria population (Siciliano *et al.*, 2014)

In the present study the actinomycetes population was higher after the receipt of the rainfall. A similar finding was also reported by Tsao *et al.*, (1960). The type of microflora varies with the soil and season. The microflora of soils of Tamil Nadu has been studied in the past by Ramasami (1966) who reported the bacterial population in the surface soil varied from as low as 20,000 to as

high as 16 lakh cfu per gram of dry soil. Fungi and actinomycetes population recorded 15935 and 57780 cfu per gram of soil. Indira Raja and Raj (1979) observed bacteria, fungi and actinomycetes populations in black wet land soil and black garden land soil. The bacteria, fungi and actinomycetes population were higher in the black garden land soil than in wet land soil.

The diazotrophs recorded a higher population in February with  $254 \times 10^4$  cfu g<sup>-1</sup> but declined until October with a subsequent increase when the cropping period commenced. Diazotroph bacteria can fix the atmospheric nitrogen and provides the plant. But the diazotrophic bacterial population might be suppressed by the application of high dose of nitrogenous fertilizers (Yeoh *et al.*, 2016).

The result on the enumeration of *Azotobacter* showed that they were generally higher in April – June during the summer period. Subsequently there was a decline followed by an increase in September, October during the cropping period. There was a marked increase in the population of these organisms after December when the harvest was over. The higher number of *Azotobacter* and phosphobacteria observed in the black cotton soil might be due to their capability to form cyst and spores. For the cyst formation in *Azotobacter* the calcium requirement is there which is readily available in black soil.

The cyst coat components are synthesized when sufficient quantities of Ca ions are present in soil (Neeru Narula *et al.*, 1998). Such cyst formation and polysaccharide production might have aided the bacterium to survive in the soil at moisture stress besides favouring soil aggregate formation which improves infiltration rate and fertility of soil (Sivapriya and Pasupuleti Reddy Priya 2017).

The result on the enumeration of phosphobacteria showed that they were generally higher in April – June during the summer period. Subsequently there was a decline followed by an increase in September, October during the cropping period. There was a marked increase in the population of these organisms after December when the harvest was over. Phosphobacterial colony character of the presumption test is the presence of halo zone around the colony.

The phosphobacteria comprises of a variety of organisms primarily *Bacillus* spp. besides *Pseudomonas* and others. *Bacillus* spp. forms endospores readily and survive the hot condition. For the formation of endospores, Ca-dipicolinic acid complex is to be formed for which also soil Ca can help. Hence *Bacillus* can survive well in this type of soil (Setlow, 2013; Shiwei Wang *et al.*, 2015).

Phosphorus, a key element in crop production is very low in calcareous (black cotton) soil and its availability is still low due to fixation. Application of phosphatic fertilizers to the soils also faces the same fate of fixation owing to fixation by calcium in soils above neutrality and due to iron and aluminum in acid soils. These fixed phosphorus is rendered soluble only by the action of phosphobacteria through the production of organic acids (lactic, gluconic, isobutyric, propionic, fumaric and isovaleric), vitamins, growth hormones (Indole acetic acid - IAA and Gibberellic acid – GA3). These substances are secreted by the *Pseudomonas* and *Bacillus* (Ponmurugan and Gopi 2006).

Phosphorus solubilizing microbes includes bacteria (*Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kusneria*, *Paenibacillus*) *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Serratia*, *Bradyrhizobium*, *Salmonella*, *Sinomonas*, and *Thiobacillus* fungi (*Achrothcium*, *Alternaria*, *Arthrotrys*,

*Aspergillus*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Cunninghamella*, *Chaetomium*, *Fusarium*, *Glomus*, *Helminthosporium*, *Micromonospora*, *Mortierella*, *Myrothecium*, *Oidiodendron*, *Paecilomyces*, *Penicillium*, *Phoma*, *Pichia fermentans*, *Mucor*, *Candida*, *Populospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Saccharomyces*, *Schizosaccharomyces*, *Schwanniomyces*, *Sclerotium*, *Torula*, *Trichoderma*, and *Yarrowia* and actinomycetes (*Streptomyces*) (Kalayu, 2019).

Phosphobacteria produces organic acids and these acids decreasing the soil pH that can favor the dissolution of calcium bound phosphorous. These organic acids proliferate the plant root system ultimately improves the uptake of nutrients and water (Marasco *et al.*, 2013). The mode of action of phosphobacteria is in the soil from insoluble form of phosphate into soluble form through production of organic acids and thereby lowering the soil pH. This pH could be able to mineralize the organic form of phosphates into acid phosphates (Behera *et al.*, 2017).

The results on the survey of VAM spores in soil and colonization of VAM and *Azospirillum* in sorghum plants are presented in Table 2 and 3. The soil and sorghum roots were collected from the major sorghum growing area of South Tamil Nadu viz., Aruppukottai, Kamuthi, Pudhur, Kovilpatti and Madurai and the VAM spores in soil and *Azospirillum* and VAM root infection were assessed. All these soils exhibited VAM spores ranging from 2 to 9 / 100 g of soil.

The soil fertility index is mainly depends upon the presence of VAM spores and the VAM spore distribution is vary in different soil profile (Sanjoy Kumar *et al.*, 2011). Hence, the VAM spore count is essential to know about the fertility status of the soil.

**Table.1** Microbial profile\* of a calcareous soil of Aruppukottai

Sl. No.	Month*	Moisture %	Microbial population**					
			Total bacteria x 10 <sup>6</sup>	Fungi x 10 <sup>4</sup>	Actinomycetes x 10 <sup>4</sup>	Diazotrophs x 10 <sup>4</sup>	Phosphobacteria x 10 <sup>3</sup>	<i>Azotobacter</i> x 10 <sup>3</sup>
1.	April	11.8	60 (1.78)	17 (1.23)	<1 (0.00)	180 (2.26)	181 (2.26)	103 (2.01)
2.	May	11.6	16 (1.20)	5 (0.69)	37 (1.56)	20 (1.30)	37 (1.57)	32 (1.50)
3.	June	6.3	<1 (0.00)	16 (1.20)	<1 (0.00)	<1 (0.00)	88 (1.94)	60 (1.78)
4.	July	22.0	79 (1.89)	11 (1.04)	36 (1.55)	9 (0.95)	9 (0.95)	15 (1.18)
5.	Aug	5.4	8 (0.90)	9 (0.95)	33 (1.52)	<1 (0.00)	<1 (0.00)	6 (0.78)
6.	Sep	22.0	24 (1.40)	10 (1.00)	24 (1.38)	12 (1.08)	51 (1.71)	13 (1.11)
7.	Oct	22.5	12 (1.08)	10 (1.00)	25 (1.40)	5 (0.70)	18 (1.26)	39 (1.59)
8.	Nov	24.2	10(1.00)	22 (1.34)	30 (1.47)	42 (1.62)	11 (1.04)	36 (1.55)
9.	Dec	13.8	24 (1.36)	13 (1.09)	53 (1.72)	21 (1.32)	4 (0.60)	10 (1.00)
10.	Jan	12.1	32 (1.5)	5 (0.69)	27 (1.43)	105 (2.02)	64 (1.81)	43 (1.63)
11.	Feb	12.4	6 (0.78)	11 (1.04)	32 (1.51)	254 (2.41)	46 (1.66)	49 (1.69)
12.	Mar	6.8	4 (0.60)	2 (0.30)	34 (1.53)	53 (1.72)	84 (1.92)	10 (1.00)

\*\* cfu g<sup>-1</sup> on oven dry basis; Figures in parentheses are log values

**Table.2** Survey of VAM spores in soil, root colonization of VAM and *Azospirillum* in sorghum

Sl. No.	Location	Soil type	VAM spore (No/100 g of soil)	VAM root infection (%)	<i>Azospirillum</i>
1.	Aruppukottai	Black cotton soil	9	30	+
2.	Pudhur	Black cotton soil	5	31	+
3.	Kamuthi	Black cotton soil	9	71	+
4.	Kovilpatti	Black cotton soil	6	42	+
5.	Madurai	Loamy soil	2	75	+

+ Present

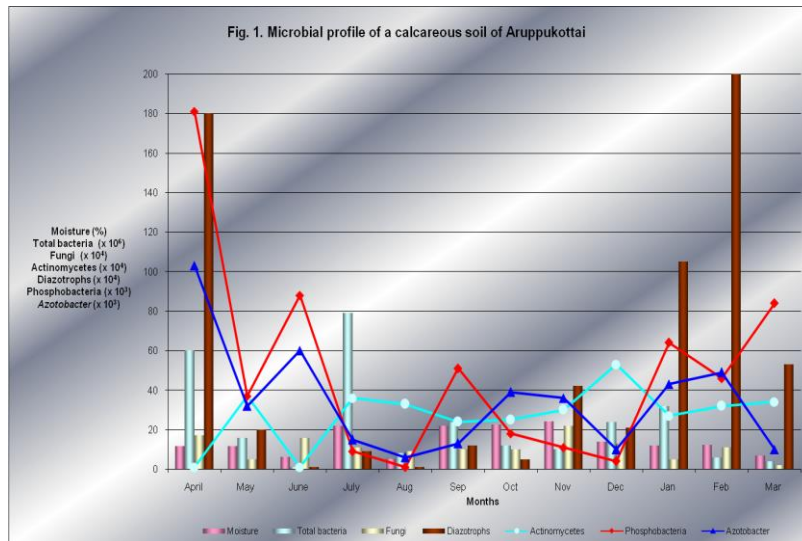
**Table.3** Utilization of different carbon sources and biotin requirement of *Azospirillum*

Sl. No.	<i>Azospirillum</i> isolate location	Carbon sources						Biotin requirement
		Malate	Glucose	Fructose	Sucrose	Mannitol	$\alpha$ KGA	
1.	Aruppukottai	+	-	-	-	-	-	No
2.	Kovilpatti	+	-	-	-	-	-	No
3.	Pudhur	+	+	+	-	+	+	Yes
4.	Kamuthi	+	+	+	-	+	+	Yes
5.	Madurai	+	+	+	-	+	+	Yes

+ Growth  
- No growth

**Table.4** Determination of carbonate content in soil

Sl. No.	Location	Soil type	Carbonate content (%)
1.	Aruppukottai	Black cotton soil	2.5
2.	Kovilpatti	Black cotton soil	1.0
3.	Madurai	Loamy soil	0.7



Aruppukottai soil and Kamudhi soil showed a higher number of VAM spores with 9 per 100g. The VAM root infection was higher with 71 percent in Kamudhi location but low with 30 percent in Aruppukottai sample. With a comparatively low VAM spore count with 2 numbers per 100g in Madurai soil the root infection was higher with 75 percent indicating the potentiality of Madurai VAM. Based on the spore morphology the

mycorrhiza was identified as *Glomus* sp. The VAM root infection is varying from plant to plant (Sanjoy Kumar *et al.*, 2011). VAM fungi are ubiquitous in nature (Hayman, 1982). VAM fungi increases the nutrient availability, drought tolerance, controls soil borne pathogen, heavy metals tolerance and production growth hormones (Priyadarshini *et al.*, 2017). VAM fungi can able to produce sugars and proteins (hydrophobins, glomalin,

mucilages that can help to increase the soil aggregation (Owen *et al.*, 2015).

Based on the growth in Nfb, 5 *Azospirillum* isolates were purified with repeated transfer on N free malate broth. They were characterized based on the morphology, Gram reaction, utilization of carbon sources and biotin requirements and the results are presented in Table 3.

Pudhur, Madurai and Kamudhi isolates utilized glucose, fructose, malate, and mannitol under nitrogen free condition. Sucrose was not utilized by any of these isolates. These isolates required biotin. Hence they are identified as *A. lipoferum*. Arppukottai and Kovilpatti isolates were able to grow in the absence of biotin. They were auxotrophic for biotin. Hence they are tentatively identified as *A. brasilense*.

Nitrogen is the major plant nutrient. The nitrogen content in Indian soil is very low. Application of chemical fertilizer is also lost by the rainfall or leaching. The nitrogen content in the soil is improved by application of *Azospirillum* or enrichment of the natural microflora. Application of *Azospirillum* could be able to produce phytohormones, defense against pathogens, tolerance to abiotic stress, increase the rate of mineral and water uptake (Josiane Fukami *et al.*, 2018).

The results on the carbonate content of soils collected from different locations are presented in Table 4. Carbonate content was higher in Aruppukottai black cotton soil with 2.5 per cent followed by Kovilpatti black cotton soil with 1 per cent. The loamy soil of Madurai recorded 0.5 per cent of carbonate. High carbonate content with high pH affects the availability of macro and micro nutrients. In this condition, Phosphorus solubilizing microorganism play an vital role in phosphorus uptake from unavailable form of

phosphorus to available form of phosphorus through organic acid and enzyme (phosphatases) (Awasthi *et al.*, 2011).

In conclusion the lack of microbial proliferation in soil is often termed 'microbiotasis'. By contrast, this also supports a relatively constant biomass, which responds little to the input at fresh substrates. In the present study the soil was dry for most part of the year and there were higher number of microorganisms and there was not lack of organisms and no microbiotasis. The higher number of *Azotobacter* and phosphobacteria observed in the black cotton soil might be due to their capability to form cyst and spores. For the cyst formation in *Azotobacter* the calcium requirement is there which is readily available in black soil. The cyst coat components are synthesized when sufficient quantities of Ca ions are present in soil. Soil fertility defined as organic matter, chloride and nitrogen content was important that could be associated with richness of bacteria and fungi. The soil fertility status is depends upon the soil microflora and fauna. The natural soil microflora promotes the plant growth through the action of phytohormone, organic acid production, nutrient cycling, plant protection against pest and diseases, tolerance to biotic and abiotic stress, environmental friendly and cost saving technology.

The appearance of the soil and hotness prevailing in Southern Tamil Nadu during summer gives an impression that this soil might not have any microbiological activity particularly in summer. In the present study it was observed that in reality a lot of organisms survive in this soil particularly bacteria including *Azotobacter*, phosphobacteria, diazotrophs and actinomycetes and also fungi. The survival in higher numbers in black cotton soil might be due to the adaptability of the microbes to higher temperature and a low moisture status. Whereas the natural and

applied microflora affected by the application of chemical fertilizers and pesticides.

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