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# Physico-Chemcial Parameters of Puducherry Soil and the Associated Mycoflora

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**ABSTRACT**

*The rhizosphere region is rich with the plenty of microorganisms. It may be either pathogenic or non-pathogenic to the plants and animals. Fungi were the ubiquitous organisms present in all types of habitats (Ainsworth et al., 1995).They play a vital role in conversion of waste and degradation (Diana 1994).. There were the previous researchers so far explained more than million fungal species on earth (**Barnett et al.,** 1972). The physcio-chemical parameter was also analyzed. The soil samples were serially diluted and inocualted on the SDA plates. The different species belongs to various groups of fungi such as Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures. A total of -- taxa belonging to -- genera were isolated, these include one species of Acomycetes, one species of Coelomycetes five species of Zygomycetes and remaining species were Deuteromycetes. Penicillium and species of Aspergillus were also recorded from samples. Basidiomycetes were also identified. The macro nutrients such as N, P and K content were not rich in the samples. There is no proper record so far regarding the fungal in these soil areas along with the soil nutrients. The present study is mainly focused on the mycoflora present in the rhizosphere regions of three different soil of puducherry region based on the macro and micronutrients present in the soil.*

**SUMMARY :** Studies on the prevalence of soil mycoflora in three rural villages of Ariankuppam, Nonankuppam and Veerampattinam coastal village of Pondicherry were carried out by serial dilution method on June 2014. Composition and concentration of fungal spores considerably varied from these villages. Ariankuppam harbored maximum number of species its very high when compared to two other areas. It has predominant fungus in soil due to agriculture land other two areas are saline and river-bed soils In qualitative analysis, out of the total species recorded, *Aspergillus* was found with the highest frequency and had eight members *i.e A. candidus, A.fumigatus, A. niger, A. flavus, A. flavipes, A. nidulans, A. ochraceous, A. terreus and A. versicolor. Penicillin* is the second most CFUs followed by *Aspergillus*.

**Introduction:**

Fungi play very important role in various biogeochemical cycles (George 1989, Migahed 2003) and are responsible for the cycling of organic compounds. Soil microorganisms mainly influence the soil ecosystems by contributing to plant nutrition (Filion et al., 1999, Meyer, G. H., Prince, H. E. and Raymer,W. J. 1983) plant health (Ellis et al., 1985)and soil structure and soil fertility (Molin et al., 1997).

## METHODOLOGY

### Study site and location

### Study sites

Pondicherry region is situated on the Coromandel Coast between $11^0$ 46' and $12^0$30' N latitudes and $79^0$36' and $79^0$53' E longitudes. The region is bounded on the north, south and west by Marakkanam, Cuddalore and by Villupuram districts of Tamilnadu, and on the east by Bay of Bengal. It covers an area of 29377 ha, according to village revenue records and consists of 179 villages. The present study was carried out in three different localities of rural villages; Ariyankuppam(S1),Nonankuppam(S2) and Veerampattinam(S3) coastal areas on May 2014. The villages are adjacent and previously agriculture was in practice. Recently the agriculture was overthrown and buildings were raised.

**Methods for collection of soil samples**

The soil samples were collected from three different localities of rural villages; Ariyankuppam(S1),Nonankuppam(S2) and Veerampattinam(S3) coastal areas on May 2014. The samples were collected 3 inches below the soil surface using the sterile spatula and carefully collected in the containers. The soil samples were collected randomly from the each place within the radius of 1 km. The sealed containers were brought to the Microbiology Laboratory, Botany Department, KMCPGS, Puducherry for further investigation.

**Determination of physicochemical properties of soil samples**

The pH values, electrical conductivity, soil moisture, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed (Table 1).

**Data collection and analysis:**

Soil physic-chemical properties The physico-chemical properties of experimental soil: texture, pH, organic carbon content, total nitrogen content, available phosphorus content and available potassium content, were estimated by combined glass electrode pH meter method, Walkley and Black's rapid titration method, modified macro Kjeldahl method, Olsen's method and flame photometer method, respectively (Jackson, 1973).

**Table 1:**

| Sample | pH | EC | Lime | Soil texture | Macronutrient | | | Micronutrient | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | N | P | K | Cu | Zn | Mn | Fe |
| **S1** | 8.1 | 1.3 | N | S | 71.83 L | 0.16 VL | 134.4 L | 1.1 0 L | 1.9 M | 3.2 M | 23.0 M |
| **S2** | 7.4 | 0.17 | N | S | 64.90 L | 19.57 L | 8.97 L | 1.2 0 L | 1.3 L | 121 VH | 21.3 M |
| **S3** | 7.8 | 1.10 | N | S | 120.65 L | 16.87 L | 33.45 L | 0.96 L | 1.6 M | 3.6 M | 24.5 M |

S 1 - Ariyankuppam, S 2 - Nonankuppam and S 3 - Veerampattinam

**Preparation of samples**

Dispensed one gram of organic sample in 10 ml of distilled water, mixed well by Vortexing and transferred one ml of suspension to another test tube to make $10^{-5}$ dilution. Dilution procedure was continued up to $10^{-6}$.

**Spread plate methods**

Nutrient agar plates were prepared and 0.1 ml of suspension was pipetted from each dilution on the agar surface. The L rod was dipped in 95% alcohol which was taken in the beaker. The glass rod was removed from the beaker and the bent position was sterilised in the Bunsen burner flame. The rod was cooled for 10-15 sec. and softly touched on the agar and spread the suspension on the agar surface. The procedure was repeatedly carried out to prepare up to $10^{-6}$ and then the plates were incubated in an inverted position at $25^0$c for 24 to 48 hrs.

**Enumeration of colonies**

The method, Most Probable Number (MPN), was used for the enumeration of cultured colonies. The different colonies in the plate were counted manually.

**Identification of organisms**

After the growth of microbial colonies in the spread plates the various colonies were differentiated by colony morphology. Then the colonies are streaked onto the different agar slants by taking a loop full of culture. From those slants a single colony was inoculated into the sterile broths and incubated for 4 to 6 hrs. These were used for further experiment.

**Isolation of soil mycoflora**

The soil micro fungi were enumerated by two methods, namely, Soil dilution, (Waksman, 1927), and Soil plate method (Warcup, 1950) on different media such as potato dextrose agar and Sabourard Dextrose Agar at pH 6.5. All the Petri dishes were incubated at room temperature 27 ± 3°C for a period of 4 – 7 days and then examined. The first set of observations were made at the end of two days to make sure that the fast growing flocculent types such as Rhizopus, Mucor and Trichoderma, etc., has grown excessively to interfere with observations of other species. Second observation was made when these had come to an advanced stage to enable identification. Finally, the slow growing organisms has to be sub-cultured in different media for the purpose of further growth to save them from being overrun by the more aggressive types. The number of colonies per plate in 1 g of soil was calculated.

**Identification**

Identification of the organisms was made by microscopic analysis using taxonomic guides, standard procedures and relevant literature (Kenneth et al., 1976; Kenneth et al., 1985; and Ellis, 1971). While presenting the data two terms, viz; periodicity of occurrence and 'percent contribution and statistical analysis were used. The percent contribution of each isolate was calculated by using the following formula:

Total no. of CFU of an individual species
X 100 Total no. of CFU of all species

Table 2:

| Location | Soil pH | Temperature | Humidity |
|---|---|---|---|
| Ariankuppam | 8.1 | $34^0$C | 50% |
| Nonankuppam | 7.4 | $33.5\ ^0$C | 49% |
| Veerampattinam | 7.8 | $34\ ^0$C | 46% |
| | | | |

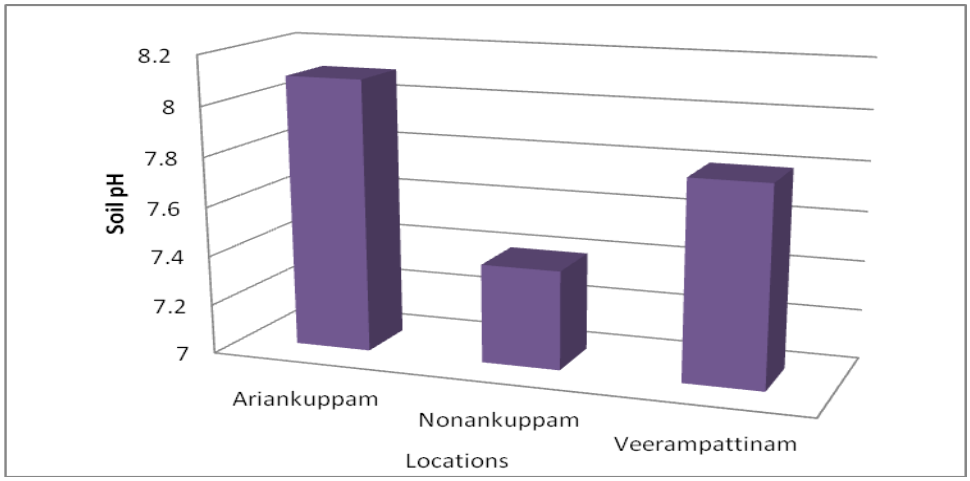
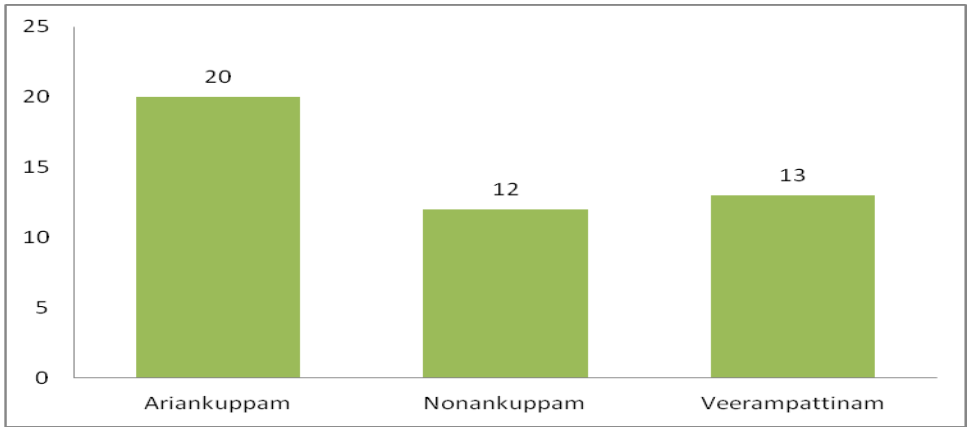


**Table 2:**

| S. No. | Fungal species | S 1 | S 2 | S 3 | % frequency | F. Class |
|---|---|---|---|---|---|---|
| **Zygomycotina** | | | | | | |
| 1 | *Mucor racemosus* | + | + | + | 100 | C |
| *2* | *Rhizopus oryzae* | + | + | + | 100 | C |
| *3* | *Rhizopus stolonifer* | + | - | + | 0.67 | F |
| *4* | *Absidia glauca* | + | - | - | 0.33 | R |
| **Basidiomycota** | | | | | | |
| *5* | *Torula herbarum* | + | + | - | 0.67 | F |
| *6* | *Pseudotorula* | + | - | - | 0.33 | R |
| **Heterokontophyta** | | | | | | |
| *7* | *Pythium sp.* | - | + | + | 0.67 | F |
| **Ascomycota** | | | | | | |
| *8* | *Aspergillus candidus* | + | + | + | 100 | C |
| *9* | *Aspergillus flavus* | + | + | + | 100 | C |
| *10* | *Aspergillus fumigatus* | + | - | + | 0.67 | F |
| *11* | *Aspergillus nidulans* | - | - | + | 0.33 | R |
| *12* | *Aspergillus niger* | + | + | + | 100 | C |
| *13* | *Aspergillus sydowi* | - | - | + | 0.33 | R |
| *14* | *Aspergillus terreus* | + | + | - | 0.67 | F |
| *15* | *Aspergillus ustus* | - | + | - | 0.33 | R |
| *16* | *Botrytis sp.* | + | - | - | 0.33 | R |
| *17* | *Curvularia sp.* | - | - | + | 0.33 | R |
| *18* | *Fusarium sp.* | + | - | - | 0.33 | R |
| *19* | *Penicillium chrysogenum* | + | + | - | 0.67 | F |
| *20* | *Penicillium citrinum* | + | - | + | 0.67 | F |
| *21* | *Phoma leveillei* | + | - | - | 0.33 | R |
| *22* | *Trichoderma viride* | + | + | - | 0.67 | F |
| *23* | *Verticillium lecanii* | - | - | + | 0.33 | R |
| *24* | White sterile mycelia | + | + | - | 0.67 | F |
| *25* | Grey sterile mycelia | + | - | - | 0.33 | R |



The present study revealed that 45 fungal CFU's were isolated from the three different sites. 20 different species were isolated in the Ariankuppam soil belong to three different genera (S1), 12 species of 4 genera were identified in the Nonankuppam (S2) soil. The S3 veerampattinam soil consist of 13 different fungi of 4 different genera were also isolated. The enumeration showed that there are 5 frequently occurring fungi present in all the three soil samples belong to three different areas. The samples collected were neutral and alkaline supports the major growth of fungus. The S2 and S3 soil samples exhibited the growth of pythium sp. of Heterokontophyta genus.

The fungi include *Aspergillus* and *Penicillium spp*., are prevalent in the three samples they are ubiquitous as well as their predominance in coastal soils were early reported by researchers. (Domsch 1980) .These results were in accordance with our result which included *Absidia* spp,*Alternaria* spp, *Aspergillus* spp, *Paecilomyces* spp, *Penicillium* spp, and *Trichoderma* spp. Genus *Aspergillus* mainly *Aspergillusflavus* was the highest occurrence followed by *A. niger, and A. fumigatus* . *Aspergillus* genus has been cited as one of the fungi which are present in the atmosphere and soils of various areas (Kathiresan 2001; Larrondo 1989; Migahed 2003) *Aspergillus* showed the broadest spectrum range, it represented by five species of *Aspergillus flavus, A. versicolor, A. fumigatus, A. niger,* and *A. terreus*. Similar to this result, found that the *Aspergillus* spp. were the dominant fungi in mangrove ecosystem. Typically, reported that *Aspergillus* spp. were the most diverse genus isolated from a soil sample from mangrove soils of puducehrry region. The environmental saprophytic fungi were the most common in the coastal soil of Puducherry (Kathiresan 2001). It was worth mention that the total count of the genera or species in the twenty four soil sample did not always follow the number of case of isolation (Migahed 2003, Oliveira et al., 1993, Onions 1986 ).

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