

SOIL MICROBIAL POPULATION NUMBERS IN SACRED GROVE FOREST OF MEGHALAYA, NORTHEAST INDIA

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Abstract - Microbial population numbers were estimated at two different soil depths in Mawphlang sacred grove forest of Meghalaya. In general, the number of fungi and bacteria were higher in the surface layer (0-10 cm) than in the deep layer of soil (10-20 cm). A total number of 48 and 18 species of fungi were isolated from soil depths of surface and deep layer respectively. Eighteen species of fungi isolated were distributed to all depths. *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Cladosporium herbarum*, *Fusarium moniliforme*, *Penicillium funiculosum*, *Trichoderma harzianum*, *T. viride* and white sterile species were the dominant fungi in both the soil depths.

INTRODUCTION

At the time when ecological degradation and deforestation have been taking place at an alarming rate through out the globe in Northeast India there are hundreds of natural vegetation scattered throughout the region are preserved almost in a pristine condition such pockets are commonly referred to as sacred groves. These sacred groves are completely undisturbed from human interference due to religious beliefs. In Meghalaya these are set aside for religious purposes and are managed by the religious Head or Priest. One of the facts that make sacred groves such important factors in conservation is that the behavior enjoined in regard to them protected not just the trees, but every possible element of the habitat and as such they are very rich in biodiversity. Their importance in biodiversity conservation has long been recognized (Khiewtam and Ramakrishnan, 1993; Ramakrishnan, 1998). They offer very important information on their genetic resources on site, within the natural or original ecosystems in which they occur, their ecological status and the level of interaction between life forms (Me. Neely, 1994; UNEP, 1995 and Edwards *et al.* 1998). In the hills of Meghalaya, sacred groves reflect and epitomize a tradition, lifestyle and belief which are

intrinsically linked to nature and its conservation (Myers, 1988; Shiva, 1992). Since there is minimal exploitation of these groves, they are a home to a number of flora and fauna. Therefore they are the best habitats for the reproduction of species and serve as propagates required for colonization of wastelands and fallow land.

Little is known about the importance of the soil microbial communities for the sustained functioning of terrestrial ecosystem (Beare *et al.*, 1995; Moore *et al.*, 1995 and Pankhurst *et al.*, 1996). Studies on microbial diversity are important in order to understand the microbial ecology in soil and other ecosystems (Atlas, 1984). A number of studies on soil microorganisms have been done related to changes in community structure of microorganisms in agricultural soil, tropical, subtropical humid forest and temperate forest soils (Giller *et al.*, 1997). Microbiological studies in soil of sacred grove forest are still in its infancy and no comprehensive study has so far been made to explore and conserve the microbial diversity as a whole. In particular our knowledge of soil microbiology in sacred grove forest of Meghalaya is still meager. Therefore, such studies are needed to explore not only new fungal and bacterial diversity but also to gather knowledge on their substrate relationship, cultural and physiology, seasonal

variations, quantitative estimations etc. Understanding of the seasonal variations in fungal and bacterial population has a paramount importance due to its relevance in biodiversity (Kennedy and Smith 1995) and in regulating population of other organisms and ecosystem processes (Reid, 1994). Therefore the aim of the study was to investigate the diversity of fungi and bacteria in relation to physico-chemical characteristic. This information may be of use to present day and future microbiologist who would be interested in the microbial diversity of this region.

MATERIALS AND METHODS

Description of Study site

The study was carried out at Mawphlang sacred grove, 25 km southwest of Shillong (25° 34' N and 91° 56' E at an altitude of 1842 m) in Meghalaya, northeast India. The grove is a thick forest patch covered an area of about 75 hectares and surrounded by a degraded rocky landscape.

Floristic composition of the sacred grove

The forest is undisturbed subtropical, humid and broad leaved and represents a relic climax vegetation. The dominant tree species are *Quercus griffithi*, *Q. dealbata*, *Q. glauca*, *Schima khasiana*, *Taxus baccata*, *Rhododendron arboreum*, *Myrica esculenta*, *M. nagi*, *Pyrus pashia*, *Castanopsis kurzii*, *Prunus undulata*, *Corylopsis himalayana*, *Engelhardtia spicata*, *Ficus nerifolia*, *Camellia caduca*, *Exbucklandia populnea*, *Litsea* spp. The under-storey vegetation comprised of shrubs, weeds and herbs. This layer was composed of the species such as *Symplocos chinensis*, *Berberis wallichiana*, *Mohonia pycnophylla*, *Lyonia ovalifolia*, *Agapetes obovata*, *Sarcandra glabra*, *Daphne bholua*, *Sarcococca saligna*, *Elaeagnus conferta*, *Osbeckia stellata*, *Eupatorium adenophorum*, *Lantana camara*, *Eurya japonica* while the herbaceous component were dominated by species like *Corylopsis himalayana*, *Manglietia insignis*, *Centella asiatica*, *Hypocharis* spp, *Taraxacum*, *Pieris ovalifolia*, *Sonchus asper*, *Houtuynia cordata*, *Cyperus rotundus*.

Climate

The climate has typical monsoonal character. The

year can be subdivided into a cold winter (November -February), spring (March - April) and rainy season (May - October). Mean air temperatures in the winter, spring and rainy seasons are 12.5, 17.5 and 20.5° C respectively. The annual rainfall averages 2500mm, a major portion of which occurs during the rainy season.

Collection of soil samples

Soil samples were collected in sterilized polythene bags, using a sterilized soil digger, at monthly interval from two depths viz. 0-10 and 10 -20 cm randomly from five points within the grove. All aseptic measures were taken during collection of samples to avoid contamination. Before processing, the samples of each depth were mixed separately to make it a bulk composite sample.

Enumeration of fungal and bacterial Population

Serial dilution plate method (Parkinson *et al*, 1971) was followed for the isolation of fungal and bacterial populations. One gram of soil was taken in a 250 mL conical flask containing 100 mL of sterilized distilled water to give a 1:100 dilutions. It was then diluted to 10^{-3} and 10^{-4} respectively for fungal and bacterial counts. Bacteria were enumerated using nutrient agar (Difco manual, 1953) and fungi on rose Bengal agar (Martin, 1950). In each case 0.5 mL of the soil suspension was spread onto plates containing 20 ml of the solidified medium and incubated at $25 \pm 1^\circ \text{C}$ for 5 days for fungi and at $30 \pm 1^\circ \text{C}$ for 24 hours for bacteria. Three replicate were maintained for each set. The Colony Forming Unit per gram soil was calculated on the dry weight basis.

$$\text{CFU / g } D_w = \frac{\text{Number of colony form} \times \text{dilution factor} \times \text{inoculums}}{\text{Dry-weight of soil (g)}}$$

Where, D_w = Dry weight of the soil (g)

The fungal species were identified on the basis of their morphological and reproductive structures, consulting monographs by Oilman (1957), Subramanian (1971), Domsch *et al.* (1980), and Ellis and Ellis (1985). The relative abundance (%) was calculated using the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{Total number of the colonies of individual species}}{\text{Total number of colonies of all species}} \times 100$$

Analysis of soil physicochemical properties

The soil moisture content was determined by drying log fresh soil in a hot air oven at 150° C for 24 h. The pH was observed on an electric digital pH meter in a 1: 5 (w:v) soil-water suspension. For N, P and K, the samples were air-drier and sieved (0.2mm). Total N was estimated by the Indophenol blue method described by Alien (1974). The molybdenum blue method of Jackson (1967) was followed to determine the available soil P. Exchangeable K was extracted from the soil in an ammonium acetate solution (pH=7) and was measured with a digital flame photometer (Systronic - 121, India).

RESULTS

The soils in general are derived from the parent rock material which is composed of mainly gneiss, schist and granite. The soil of this grove is sandy loam in texture. The ranges for different soil properties are: pH, 4.5 - 5.6; total nitrogen, 0.43 - 0.96 %, available P, 0.01 - 0.07 %, exchangeable K, 0.01 - 0.05 %. The soil moisture content ranged from 30 - 72 %.

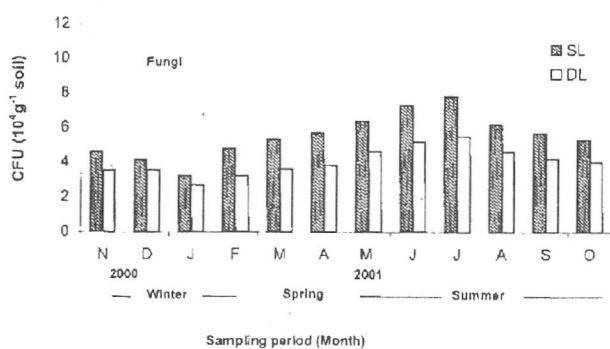


Fig.1. Colony forming units (CFU) of fungi in soil of Sacred groove forest. (SL - Surface layer, 0-10 cm; DL - Deep layer, 10-20 cm)

In general, the number of fungi and bacteria were higher in the surface layer (0-10 cm) than in the deep layer of soil (10-20 cm). Quantitatively the bacterial community was more numerous than the fungal

community. The numbers of fungal propagules per gram dry soil were maxima in surface layer (0-10 cm) and minimum in deep layer (10 - 20 cm) depth (Fig.1 and 2). In depth wise studies fungal population was always high in surface layer and decreased along with soil depth. Maximum numbers of fungi were recorded in July, thereafter their numbers decreased subsequently in both the depths. Average number of fungi and bacteria was high in summer months and low in winter months in both the soil depths. The total number of bacteria in both the soil depths showed a steady increase up to September and then a sharp decline in October. Qualitatively, similar fungal species were isolated and no marked difference in the species composition was noticed. Altogether 48 and 18 species were isolated from the soil layer of 0 -10 cm and 10 -20 cm depths respectively (Table 1). *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Cladosporium herbarum*, *Fusarium moniliforme*, *Penicillium fellutanum*, *Trichoderma harzianum*, *T. viride* and white sterile species were the dominant fungi in both the soil depths. Moreover, fungi like *Curvularia lunata*, *Fusarium moniliforme*, *Mucor hiemalis*, *Penicillium chrysogenum*, *P. funiculosum* and *Phoma humicola* were also of common occurrence to both the soil depths.

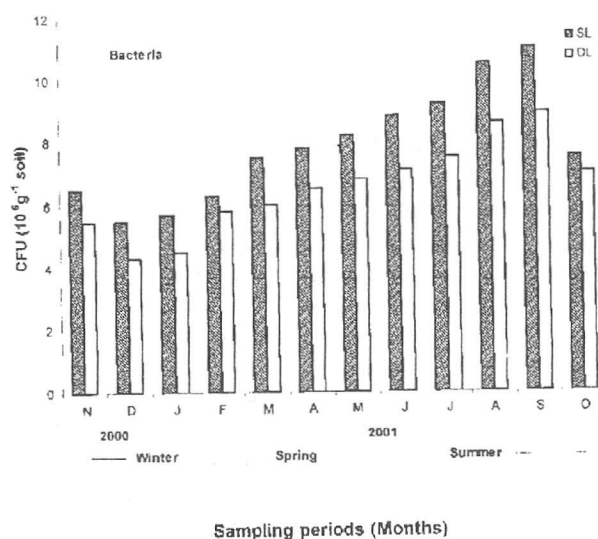


Fig.2. Colony forming units (CFU) of bacteria in soil of Sacred groove forest. (SL - Surface layer, 0-10 cm; DL - Deep layer, 10-20 cm)

Table 1. Fungi isolated from soils of sacred grove forest at two different depths.

Sl.No.	fungal species	Depths (cm)	
		0-10	10-20
1.	<i>Absidia glauca</i> Hagem	+	-
2.	<i>Absidia spinosa</i> Lendner	+	-
3.	<i>Alternaria alternata</i> (Fr.) Keissler	+	-
4.	<i>Alternaria humicola</i> Oudemans	+	-
5.	<i>Aspergillus candidus</i> Link	+	-
6.	<i>Aspergillus clavatus</i> Desmazieres	+	+
7.	<i>Aspergillus flavus</i> Link: Fries	+	+
8.	<i>Aspergillus fumigatus</i> Fresenius	+	-
9.	<i>Aspergillus nidulans</i> (Eidam) Winter	+	-
10.	<i>Aspergillus niger</i> van Tiegham	+	+
11.	<i>Cephalosporium coremioides</i> Raillo	+	-
12.	<i>Cladosporium cladosporioides</i> (Fres) de vries	+	-
13.	<i>Cladosporium herbarum</i> (Pers.) Link	+	+
14.	<i>Cunninghamella echinulata</i> Thaxter	+	-
15.	<i>Curvularia lunata</i> (Walker) Boedijn	+	+
16.	<i>Curvularia pallescens</i> Boedijn	+	-
17.	<i>Fusarium moniliforme</i> Sheldon	+	+
18.	<i>Fusarium oxysporum</i> Schlecht em. Sny & Hans.	+	-
19.	<i>Fusarium solani</i> (Mart.) Sacc.	+	-
20.	<i>Geotrichum candidum</i> Link	+	+
21.	<i>Humicola grisea</i> Traaen	+	-
22.	<i>Monilia sitophylla</i> (Montagne) Saccardo	+	-
23.	<i>Mortierella minutissima</i> V. Tiegh	+	-
24.	<i>Mucor hiemalis</i> Wehmer	+	+
25.	<i>Mucor plumbeus</i> Bonord	+	-
26.	<i>Mucor racemosus</i> Fres	+	-
27.	<i>Nigrospora spaerica</i> (Sacc) Mason	+	-
28.	<i>Paecilomyces vrioti</i> Bain	+	+
29.	<i>Penicillium brevicompactum</i> Dierckx	+	-
30.	<i>Penicillium chrysogenum</i> Thorn	+	+
31.	<i>Penicillium citrinum</i> Thorn	+	+
32.	<i>Penicillium fellutanum</i> Biourge	+	-
33.	<i>Penicillium funiculosum</i> Thorn	+	+
34.	<i>Phoma glomerata</i> (Corda) Wollenweber & Hochapfel	+	-
35.	<i>Phoma humicola</i> Oilman & Abbot	+	+
36.	<i>Pythium intermedium</i> De Barry	+	+
37.	<i>Rhizopus nigricans</i> Ehrenberg	+	+
38.	<i>Rhizopus oryzae</i> Went & Prinsen	+	-
39.	<i>Torula herbarum</i> Pers ex Gray	+	-
40.	<i>Trichoderma harzianum</i> Rifai	+	+
41.	<i>Trichoderma koningii</i> Oudem	+	-
42.	<i>Trichoderma lignorum</i> (Tode) Harz	+	-
43.	<i>Trichoderma viride</i> (Pers) Gray	+	+
44.	<i>Verticillium albo-atrum</i> Reinke & Berthold	+	-
45.	<i>Verticillium chlamydosporum</i> Goddard	+	-
46.	Yellow sterile mycellia	+	-
47.	White sterile mycelia	+	+
48.	<i>Zygorhynchus</i> sp.	+	-

While fungi like *Absidia glauca*, *Alternaria alternata*, *Aspergillus candidus*, *A. fumigatus*, *Cladosporium cladosporioides* and *Fusarium solani* were common restricted only to surface layer depth (0-10 cm) and fungi like *Absidia spinosa*, *Aspergillus nidulans*, *Manilla sitophylla* and *Mortierella minutissima* were isolated frequently in deep layer depth (10-20 cm). Whereas fungi like *Alternaria humicola*, *Curvularia pallescens* and *Humicola grisea* were isolated occasionally in deep layer depth (10-20 cm). In the present study, however, no attempt has been made to classify bacteria isolated on dilution plates.

DISCUSSION

Decrease in fungal population along soil depth confirms the finding of Tiwari *et al.*, (1987) and Shukla and Mishra (1992). Surface layer of soil is usually provided with high organic matter content which in presence of adequate moisture supply is acted upon by microorganisms to decompose the complex organic residues into simpler forms, hence the species of microorganisms are higher on surface layer of the soil (Acea and Carballas, 1985, Jha. *et al.*, 1992). Generally, fungi found in deeper layer are slow growing due to unavailability of mineral nutrients and compaction of soil along depth (Dkhar and Mishra 1992). The Colony forming unit of fungi and bacteria in both the soil depths showed a significant positive correlation with nutrients (N and P), soil moistures and pH ($p < 0.05$). The improvement of moisture levels, moderation of temperature and higher relative humidity favoured the maximum build up of the microbial counts in summer (rainy) months. The peak of colony forming unit of fungi in July is due to high concentration of nitrogen, phosphorous and potassium when compared to other months (Berg *et al.*, 1998; Coyne, 1999). A gradual depletion in nutrient (N, P and K) coupled with less favourable climatic conditions was the reason for a reduction in the population numbers of fungi and bacteria during winter months (Berg *et al.*, 1998). The investigation provides further proof that climatic regime, moistures, pH, seasonal variation of the year and type of vegetation were the most important factors affecting the composition and distribution of microorganisms in soil (Gorres *et al.*, 1998 and Zeller *et al.*, 2001).

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