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Research Article

Distribution of fungi in Thane Cyclone affected areas in Marakkanam, Tamil Nadu, India

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Abstract

The present study was confined to the diversity of fungi in Thane cyclone affected areas in Marakkanam , comprising of 1. Anumanthaikuppam (S1), 2. Vasavan kuppam(S2),3. Koonukedu(S3) ,Manjakuppam(S4) and Kilputhupattu (S5). Water, sediment, and natural substrates of marine organisms were collected to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. The water and sediment sample were collected separately and analysed for temperature, pH, dissolved oxygen, biological oxygen demand, chemical oxygen demand, salinity and total dissolved solids on water. A total of 40 fungal species were isolated and enumerated by plating, techniques. Among these,141 species were represented in (S1), 20 in (S2), 20 in (S3) ,18 (S4) and 22 in S5. . In this study, 30 species of fungi were recovered from sediment samples whereas water samples yielded 27 species and natural substrates with 14 species. Among the Hyphomycetes, *Aspergillus* was the common genus followed by *Penicillium* and *Cladosporium*. The physico-chemical parameters of water and sediment in all stations were analysed and correlated with fungal diversity and frequency of occurrence of fungi were also analysed.

Keywords: Thane cyclone ; Water; sediment; natural substrates of marine organisms; Hyphomycetes; *Aspergillus;* fungal diversity; frequency of occurrence.

Introduction

Biological diversity refers the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystem and ecological complexes of which they are part. Biodiversity encompasses all life forms, ecosystems and ecological processes and acknowledges the hierarchy at genetic, taxon and ecosystem level.

The essential ingredients of biodiversity are phenotypic flexibility genetic variation within populations and ecotypic variations (Ananthakrishnan, 1997). Microbial biodiversity can be viewed from a variety of perspectives, including physiological diversity, interaspecific genetic diversity and phylogenetic diversity of

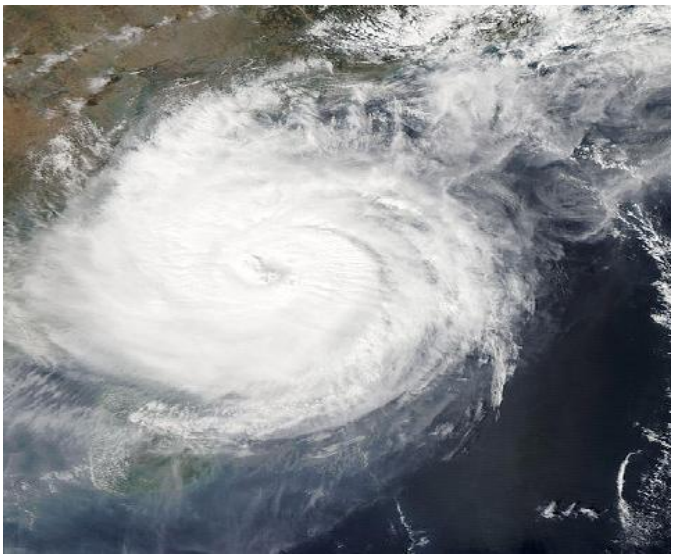
species and higher taxa (Delong, 1997). Microbial diversity represents the largest untapped reservoir of biodiversity for potential discovery of new biotechnological products, including new pharmaceuticals, new enzymes, new special chemicals or new organisms that carryout novel process (Jensen and Fenical, 1994).

Diversity most simply can be expressed as species richness, that is the number of species (Magurran, 1988). However, since richness increase in direct relation to number of individuals, area and variety of habitats sampled. Ecological variation over the temporal and spatial dimensions of the sample may augment diversity because of the increased number of areas, habitats or seasons included. Hyde (1990c) recognized the different in the common species at study sites, a core group of fungi occurring in the mangrove ecosystem. The Majority of the species *Dactylospora haliotrepha*, *Leptosphaeria avicenniae* were also reported from Brunei and other tropical mangroves (Hyde 1989a). Alias *et al*., (1995) reported that more than 60 fungal species can be recorded as common to mangrove ecosystems of the West Indo Pacific region.

Thane cyclone

Very Severe Cyclonic Storm Thane named by Burma (IMD designation: *BOB 05*, JTWC designation: 06B, also known as Cyclone Thane) was the strongest tropical cyclone of 2011 within the North Indian Ocean. Thane initially developed as a tropical disturbance within the monsoon trough to the west of Indonesia. Over the next couple of days the disturbance gradually developed further while moving towards the northwest, and was declared a Depression during December 25, before being declared Cyclonic Storm Thane during the next day. As it was named, Thane started to turn towards the west under the influence of a subtropical ridge of high pressure before its development slowed down during December 27, as a strong outflow and marginally favourable sea surface temperatures fought with persistent vertical wind shear. After its development had slowed down during December 27, Thane became a Very Severe Cyclonic Storm during December 28, before as it approached the Indian states of Tamil Nadu and Andhra Pradesh, it weakened slightly. Thane then made landfall early on December 30, on the north Tamil Nadu coast between Cuddalore and Pondicherry and rapidly weakened into a depression.

Figure.1 Thane cyclone



Objectives of this study

Based on the necessary basic information obtained on marine fungi and marine ecosystem, the present study has been undertaken in the proposed study area in Thane cyclone affected areas in marine ecosystem , a coastal deltaic habitat along the East coast of Bay of Bengal in Marakkanam, Tamil Nadu with the following objectives, Eco - taxonomical characterization of fungi, andBiodiversity of fungi.

Materials and Methods

Study area

Totally five (5) sampling stations were selected. The five sampling stations are: 1. Anumanthaikuppam (S1), 2. Vasavan kuppam(S2),3. Koonukedu(S3) Manjakuppam(S4) and Kilputhupattu (S5).

Isolation of fungi from water and sediment samples by plating technique

After sampling, within 24 hrs the water and sediment samples from each station were subjected to appropriate dilutions (10^{-2} to10^{-5}) and 0.1 ml of sample was aseptically transferred into the plates containing Potato dextrose agar/ Czapek dox agar/Corn meal agar/Rose Bengal agar with addition of mixture antibiotics, Tetracycline and Penicillin (Spread plate method) The plates were incubated at room temperature (28oC) for 4-5days. Control plates were also maintained. Sterilization of glasswares and preparations of media were carried out as per the method described by Booth (1971).

Isolation of fungi from natural substrates by plating technique

Wood substrates

The naturally occurring different wood substrates such as drift wood, and intertidal woods found in the crevices of rock along the banks of the estuary were collected (randomly in the study area) in sterile polythene bags and brought to the laboratory for further processing. In the laboratory the surface fouling organisms were gently scraped off and washed off by exposing under running tap water and the samples were again washed with sterile seawater. Then wood samples were cut into small pieces of different sizes and were again washed with sterile seawater and allowed to drain for 1 h. to remove excess surface waters. The samples were kept at 4˚C for further use (Kohlmeyer and Kohlmeyer, 1979). The wood samples were placed aseptically on surface of the agar media in the petriplates. The plates were incubated at 28^{o}C for 4-5 days and observed for the occurrence of fungal colonies.

Isolation of fungi from natural substrates by Baiting technique

The collected specimens of the substrates of plants were also used for the isolation of mycoflora. Typical marine and mangrove fungi were isolated using Baiting technique. Wood samples were collected from the study area and studied for isolation of marine fungi. All these individual specimens were kept in sterile polythene bag and aerosal was created inside the bags by spraying with sterile seawater. The bags were tightly covered and kept under illumination and subsequently transferred to dark conditions. This was carried out for the entire study periods to observe the colonization of fungi on these different natural substrates. All the plant baits were regularly observed under aseptic condition using stereoscopic Dissection Microscope under 2 X and 4 X magnifications. The fungal spores observed on the natural substrates (baits) along with hyphae were picked up using sharp Nichrome wire mounted on needle holder, then these were transferred to agar containing plates to ensure the germination and development of the spores (Sarma and Vittal, 2000).

Identification of fungi

The identification of fungal taxa was based on illustrated Genera of imperfect fungi (Barnett, 1965), Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Micro fungi on land plants (Ellis and Ellis, 1985) Micro fungi on Miscellaneous substrate (Ellis and Ellis, 1988), and Manual of soil fungi (Gilman, 1957, 1998).

Enumeration of Fungi

The distribution of fungal taxa was listed out and the nomenclature followed is based on the fungi: "**The Fifth Kingdom - Mycota (ed.) Kendrick. (1992)**. Each taxon is briefly described by its binomial followed by morphology (diagnostic features), frequency.

Quantitative analysis

The percentage of frequency of occurrence of fungi was determined based on the number of stations from which the particular fungi was isolated and the total number of fungal isolation.

$$\text{Frequency of occurrence} = \frac{\text{Number of sampling stations where the species occurred}}{\text{Total number of sampling stations studied}} \times 100$$

Physico – chemical analyses of water and sediment samples

The water and sediment sample were collected separately and analysed for temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), salinity, total dissolved solids (TDS) on water (Venugopalan and Paulpandian,1989; Aneja, 2001; APHA, 1998) .

Results and Discussion

The results of study in marine ecosystem comprising 1. Anumanthaikuppam (S1), 2. Vasavan kuppam(S2),3. Koonukedu(S3), Manjakuppam(S4) and Kilputhupattu (S5). in Marakkanam are presented and discussed under three sections, *viz*., Enumeration of taxa and Ecology of fungi.

Enumeration of taxa

The fungi belonging to different genera which were isolated by plating and baiting techniques were enumerated with morphological and ecological descriptions. The system of classification was based on "**The Fifth Kingdom – Mycota (ed.) Kendrick (1992)** for the arrangement of genera under their respective orders and families. The genera and species within each family are arranged in alphabetical order.

Ecology of fungi

Physico-chemical status of water and sediment samples with respect to fungal distribution

Hence, ten parameters *viz*. temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), salinity, and total dissolved solids (TDS),of water samples were observed and recorded (Table.2). The physico-chemical parameters of water samples in all stations, salinity, BOD, TDS, were 47, 1.06 mg, 0.10 respectively. These parameters influenced the occurrence of (14) fungi belonging to Zygomycotina and Deuteromycotina in the S1. Salinity, DO and total TDS organic were 45%, 0.48 mg, and 0.30 respectively in S2 These parameters were influenced the occurrence of 20 fungi which belonged to Zygomycotina, Ascomycotina and Deuteromycotina. The physico-chemical parameters of water and sediment in S3 stations during S3 as follows: DO (0.08mg), salinity (45), total dissolverd solids (0.2 mg), and during this 20 fungi belonging to Zygomycotina and Deuteromycotina were recovered.

Table 2 shows, the parameters of water and sediments in all five stations S4 The salinity, total dissolved solids, and DO were 43,0.10 mg, and 18.30 mg respectively in S4. The parameters influenced the occurrence of 18 fungi, belonging to Zygomycotina and Deuteromycotina. The physico-chemical parameters of water and sediment in S5 stations as follows: DO (18.16mg), salinity (45), total dissolverd solids (0.20 mg), and during this 22 fungi belonging to Zygomycotina and Deuteromycotina were recovered. Altogether 40 fungi belonging to genera comprising 32 Deuteromycotina, 2 Ascomycotina, 6 Zygomycotina were isolated.

By holding the above physico-chemical data observation were also made on the marine microorganisms. The terrestrial form of fungi in the estuarine water and sediments exhibited temperature tolerance and germination activity with increasing salinity where as, the species of marine fungi *Varicosporina ramulosa, Halosphaeria maritima, Didymosphaeria maritima* and *Pleospora aquatica* recorded from sea foams demonstrated their salinity tolerance in their habitats. This would suggest the effect of salinity and temperature on spore germination of terrestrial, fresh water and marine fungi. On the other hand the marine species were indicated high tolerance against salinity and temperature stress on that by earlier observations by

Byrne and Jones (1975). However, from the standpoint of fungal ecology, the most important single factor that governs the fungal occurrence is capacity of water to store/release oxygen. The oxygen content is directly influenced by temperature and the former has a direct bearing on the metabolism and growth of aquatic populations (Cooke and Rayner, 1984).

Species diversity of fungi in the marine ecosystem

During the four month study period, a total of 40 fungal species were enumerated from Four sampling stations S1, S2, S3, S4and S5 by plating and baiting techniques. Among these,14 species were represented in S1, 20 in S2, 20 in S3,18 in S4 and 22 in S5 (Tables 3,4). Maximum fungal diversity was observed in S5 with represented by 22 species and minimum of 14 species was isolated in S1.

In this study, 30 species of fungi were recovered from sediment samples whereas water samples yielded 27 species and 14 species were isolated from natural substrates. When the fungal species diversity was analyzed in relation to different classes, it has been observed that the maximum number of species recorded belonged to Hyphomycetes. This was followed by Zygomycetes and Ascomycetes . Among the Hyphomycetes, *Aspergillus* was the common genus represented by 13 species followed by with *Cladosporium* 4 species, 3 species with *Penicillium* and *Alternaria*. In addition to this C*ladosporium*, *Mucor, Rhizopus, Fusarium,* were the common genera found in this marine system.

Occurrence of fungi in the marine water

In this study, totally 27 species of fungi were isolated and enumerated from the water samples by dilution-plating technique (Table 5). Among the fungi isolated belonged to Zygomycetes to Ascomycetes and Deuteromycotina. Of all these, *Aspergillus* were found to be dominate genus followed by *Penicillium* and *Curulanata*. The above result was discussed with previous reports of Chandralata (1999) and Raghukumar and Raghukumar (1998) also reported adaptation and activity of terrestrial fungi under marine/ mangrove ecosystem as facultatives or indwellers or residents. Terrestrial fungi are common in mangrove water and mud (Chowdhery *et al*., 1982; Garg, 1983). Seawater, seafoam and beach soil of Arabian Gulf Coast, Saudi Arabia yielded terrestrial fungi, typical marine and freshwater fungi (Bokhary *et al*., 1992).

Occurrence of fungi in the marine sediment

By employing the plating technique, 30 fungi were isolated from the mangrove sediment samples. Among these, belonged to Zycomycotina, Ascomycotina and Deutromycotina . As like in the water samples, in sediments samples also the genus *Aspergillus* was also found to be dominant followed by *Cladosporium* (Table 5).With the above-presented results while, assessing the species diversity of fungi in the estuarine waters and sediments, the fungal genera, *Aspergillus, Penicillium, Curvularia, Alternaria, Cladosporium* and *Drechslera* were found to be dominant members of this system. This well agreed with the findings of Garg (1982), Rai and Chowdhery (1978), Raper and Fennell (1965) and Roth *et al*. (1964). According to their findings Aspergilli dominated over Mucorales and Penicillia in the mud of mangrove swamps of Sunderband. Nicot (1958) recorded the dominance of Aspergilli and Penicillia in the coastal soils of France. Further more, Raper and Fennell (1965) have also suggested that certain non-osmophilic species of *Aspergillus* may grow luxuriantly under halophytic conditions. Although terrestrial fungi are found in coastal environments frequently as part of the spore population, only species adapted to saline environments appear to be able complete their life cycles fully in coastal and marine environments (Jennings 1986).

Distribution of fungi in relation to marine vegetation and their substrates

The fungi in the marine system were studied by plating and baiting techniques at certain specific

Table.1 Details of physico-chemical parameters of water in four stations.

Parameters	S1	S2	S3	S4	S5
Temperature (° C)	30	28	30	32	30
pH	7.5	8.0	8.1	7.9	8.0
Dissolved oxygen (mg/l)	16.1	17.2	17.8	18.3	18.1
Biological oxygen demand (mg/l)	1.06	0.48	0.08	1.10	0.96
Chemical oxygen demand (mg/l)	0.09	0.10	0.18	0.23	0.25
Salinity (%)	47	45	45	43	42
Total dissolved solids (mg/l)	1.0	0.3	0.20	0.10	0.20

Table.2 Fungi isolated from all the five sampling stations during the study period

Name of the fungi	S1	S2	S3	S4	S5
Actinomucor sp.	-	-	+	-	+
Mucor sp.	-	+	-	-	-
Rhizopus oryzae	+	+	+	+	+
R. nigricans	+	+	+	+	+
R. stolonifer	-	-	-	-	
Saccharomyces sp. 1	-	-	-	-	-
Neurospora crassa	-	-	-	-	+
A. flavus	+	+	+	+	-
A. fumigatus	+	+	+	+	+
A. luchuensis	+	+	+	+	
A. niger	+	-	-	+	+
A. ochraceus	+	+	+	+	-
A. oryzae	+	+	+	+	+
A. quercinus	-	+	+	+	+
A. sulphureus	-	-	-	+	+
A. terreus	+	+	+	+	+
A.terricola	-	-	+	-	+
Pencillium citrinum	-	+	+	-	-
P. janthinellum	-	+	-	-	+
Penicillium sp.	+	-	-	-	+
Trichoderma viride	-	-	-	-	+
Verticillium luteo -album	-	-	+	-	+
A. alternata	-	+	-	-	+
A. citri	+	-	-	-	+
Cladosporium apicale	-	+	+	+	+
C. britannicum	-	+	+	-	-
C.cladosporideus	+	-	-	+	+
Cladosporium sp.	-	+	-	-	
Curvularia indica	-	-	+	+	-
C. lunata	-	-	-	+	-
C.richardiae	-	-	+	+	+
Drechslera sp	-	+	-	-	-
Periconia sp.	-	-	+	+	+
Fusarium moniliforme	+	+	-	-	-
F. oxysporum	-	+	+	-	+
F. semitectum	+	+	+	+	-
Fusarium sp.	-	-	-	-	+

(+) – Present; (–) - Absent

Table.3 List of Fungi isolated from various mangrove substrate samples collected in the study area.

Name of the fungi	**water**	**Sediment**	**Natural Substrates**
Actinomucor sp.	+	-	-
Mucor sp.	-	+	+
Rhizopus oryzae	+	+	+
R. nigricans	+	+	-
R. stolonifer	-	-	-
Saccharomyces sp. 1	+	-	-
Neurospora crassa	-	+	+
A. flavus	+	-	-
A. fumigatus	+	-	-
A. luchuensis	+	+	+
A. niger	+	+	+
A. ochraceus	+	-	-
A. oryzae	+	+	+
A. quercinus	+	+	-
A. sulphureus	+	+	+
A. terreus	+	+	+
A.terricola	+	+	-
Pencillium citrinum	+	+	-
P. janthinellum	+	-	+
Penicillium sp.	+	-	-
Trichoderma viride	+	+	+
Verticillium luteo -album	-	-	-
A. alternata	-	-	-
A. citri	+	+	+
Cladosporium apicale	+	+	+
C. britannicum	+	-	-
C.cladosporideus	+	-	-
Cladosporium sp.	+	+	-
Curvularia indica	-	-	-
C. lunata	+	-	-
C.richardiae	+	-	+
Drechslera sp	-	-	-
Periconia sp.	-	+	-
Fusarium moniliforme	+	-	-
F. oxysporum	-	+	-
F. semitectum	+	+	-
Fusarium sp.	+	+	-

(+) – Present; (–) – Absent

Table.4 Fungi isolated form natural substrates by plating

Name of the fungi
Mycota
Zygomycota
Mucor sp.
Ascomycotina
Neurospora crassa
Deuteromycotina
A. flavus
A. fumigatus
A. niger
A. oryzae
A. sulphureus
A. terreus
Verticillium louteo -album
Curvularia lunata

Table.5 Frequency of occurrence isolated fungi by plating/baiting/direct observation during the study period.

Name of the fungi	Frequency of occurrence (%)
Actinomucor sp.	25
Mucor sp.	50
Rhizopus oryzae	50
R. nigricans	75
R. stolonifer	50
Saccharomyces sp. 1	25
Neurospora crassa	25
A. flavus	100
A. fumigatus	50
A. luchuensis	100
A. niger	100
A. ochraceus	75
A. oryzae	100
A. quercinus	100
A. sulphureus	100
A. terreus	100
A.terricola	100
Pencillium citrinum	50
P. janthinellum	100
Penicillium sp.	25

Trichoderma viride	75
Verticillium luteo -album	25
A. alternata	50
A. citri	25
Cladosporium apicale	100
C. britannicum	50
C.cladosporideus	100
Cladosporium sp.	50
Curvularia indica	25
C. lunata	100
C.richardiae	25
Drechslera sp	50
Periconia sp.	25
Fusarium moniliforme	100
F. oxysporum	50
F. semitectum	100
Fusarium sp.	100

FO - Frequency of Ocurrence

sampling stations where, the plant vegetation was dense and varied.

Totally, 14 species of fungi belong to different groups were enumerated from the natural substrates wood, attempted with direct plating techniques. In this, 1 speices with Zygomycotina, 1 species with Ascomycotina and 12 speices with Deuteromycotina. *Aspergillus* was found to be more predominant fungi, *A. flavus, A. fumigatus, A. terreus,* followed by *Penicillium* sp. (Table 5,6;Figure 3).

Frequency of occurrence, density, abundance and relative frequency of fungi in all the five sampling stations

The fungal frequency of occurrence, in the five sampling stations was calculated (in percentage). Accordingly 21 species belonged to 100% of frequency of occurrence followed by 4 sp. with 75, 13 with 50, 13 with 25 observed in the marine eco-systems 9Table.7). It is well agreed with previous findings by Sarma *et al*. (2001), Aleem, 1980, Leong *et al*. (1991), Poonyth *et al*. (1999) and Hyde (1990a, 1991) have studied the fungal distribution in intertidal mangroves and provided information on (a) frequency of occurrence (b) vertical zonation (c) host and substratum specificity (d) succession and (e) seasonal occurrence. It is noted at here, that the percentage occurrence as an expression of the frequency of collections of fungi gives in indication of the more common fungi within the mangrove ecosystem (Hyde and Jones, 1988; Alias *et al*., 1995).

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