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Research Article

Diversity of marine lignicolous and non- lignicolous fungi along the south east coast in Tamil Nadu, India

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Abstract

The present study was confirmed to the marine and mangrove eco- system in Tiruvarur, Pudukkottai and Ramanathapuram districts, Tamil Nadu. Sediment samples, and natural and decaying marine and mangrove plant substrates were collected to isolate and enumerate fungal species by plating and baiting techniques. Totally, 52 species of fungi were isolated by plating and baiting techniques, identified, enumerated and arranged according to the classification of Hendrick.B (1992) of which out of 52 species, 40 species non lignicolous and 12 ligniclous fungi were identified. Among the fungal isolates, species of *Aspergillus* (15) were seem to be dominant members of this marine and mangrove eco-system followed by *Penicillium* with 5 species, 4 species with *Rhizopus*, and 4 species with *Curvularia*. The species richness and diversity of fungi at 11 sampling stations were determined by Simpson and Shannon indices. Simpson and Shannon indices were highest at S5 represented by 0.9889 (Simpson), and Shannon was 6.6204 at S3 The Shannon evenness was highest at S4 (0.9427) while it was least at S6 with 0.0867.

Keywords: Mangrove fungi- Physico-Chemical parameters ; Species diversity;– Frequency of occurrence; Seasonal variation;– Mangrove vegetation.

Introduction

Marine fungi have the ability to grow at certain seawater concentrations (Johnson and Sparrow, 1961 and Tubaki, 1969). It has been shown that marine fungi cannot be defined strictly on a physiological basis where as, a broad ecological definition names that the marine fungi of obligate types are those that grow and sporulate exclusively in a marine and estuarine habitat. Facultative form are those from fresh water or terrestrial milieus able to grow in the marine environment (Kohlmeyer, 1974).The higher marine mycota or manglicolous fungi on submerged parts of mangroves include 42 species, and are the fourth largest ecological group after the wood, salt-marsh,- and algae – inhabiting species. These mangrove fungi are almost exclusively saprobes and belong to the family of Ascomycetes, Deuteromycetes, and Basidiomycetes. The majorities of manglicolous

marine fungi are omnivorous and occur mostly on dead cellulosic substrates all around the tropics. (Kohlmeyer and Kohlmeyer, 1979). The present investigation was therefore initiated to study about the distribution of fungi in coastal region of Thiruvarur Pudukkottai and Ramanathapuram districts

Materials and Methods

Study area

The study area comprises a stretch of 16 kilometers in the coastal region of Thiruvarur, Pudukkottai and Ramanathapuram districts which were selected for present study. Totally 11 sampling stations were selected based on the richness of natural substrates availability. . The 11 sampling stations are as follows;Muthupettai (S1), Iyampattinam (S2),Kumarapattinam (S3),Gopalapattinam (S4), R.Pudupattinam (S5), Arasangaripattinam (S6) , Muthukuda (S7), Sethadimunai (S8),Sundrapandiapattinam (S9),Pasipattinam (S10), Therthandathanam (S11)

Isolation of fungi sediment samples by plating technique

Sediment sample

One gram of the sediment weighed and then dissolved with 99 ml of sterile seawater and then subjected to dilution series as done for water samples. 0.1 ml of the samples was directly inoculated onto medium containing plates and incubated in the incubation chamber at 28ºC for further observation. In this technique, 10^{-2} to 10^{-5} dilutions were prepared and taken into account for plating . Control plates were also maintained**.** Sterilization of glass wares, preparation of media were carried out as per the methods described by Booth 1971

Isolation of mycoflora by membrane filtration techniqe

Through nitrocellulose membrane filter disc of 0.45 μm pore size (Sartorius) 100 ml of the sediment mixed sterile sea water samples were filtered using membrane filtration unit. Then the discs were transferred aseptically into agar plates (Corn meal agar and Czapek dox agar) and incubated in room temperature at 28°C with appropriate control plates for further observation (Vrijmoed, 2000).

Isolation for fungi from natural substrates employing plating technique

Wood substrates

The naturally occurring different wood substrates such as, Driftwood, and intertidal woods found in the sandy beach were collected 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 with different sizes and again washed with sterile seawater and allowed to drain for 1 hour to remove excess surface waters. (Vrijmoed, 2000). 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 such as, sabourard's dextrose agar, corn meal agar and czapek dox agar. The plates were as usually incubated at 28-30^{o}C for 4-5 days and observed the occurrence of fungal colonies **.**

Direct observation

Isolation of fungi from natural substrates by Baiting technique

The collected specimens of the wood samples were used for the isolation of mycoflora using sterile polythene bags. All these individual specimens were kept in sterile polythene bags and aerosal was created inside the bags by spraying with sterile seawater. The bags were tightly covered and kept under illumination and subsequent transferred to dark conditions during 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 2x and 4x magnification. The fungal spores observed on the natural substrates (baits) along with hyphae were picked up using sterile fine forces or sharp Nichrome wire mounted on needle holder then these were transferred to agar containing plates to ensure with the germination of the spores and development on the agar media employed .

Identification of fungi

The identification of fungal taxa is based on illustrated Genera of imperfect fungi (Barnett, 1965), Hyphomycetes (Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hphomycetes (Ellis, 1971,1976),Marine mycology(Jan Kohlmeyer and Erika Kohlmeyer,1979),Micro fungi on land plants; (Ellis and Ellis 1985) Micro fungi on Miscellaneous substrate ; An introduction hand book (Ellis and Ellis, 1988) and A manual of soil fungi (Joseph. C. Gilman, 1959, 1998).

Enumeration of fungi

The distribution of fungal taxa was listed out and the nomenclature followed is based on the fungi: fifth kingdom: (Hawoksworth, 1995). Each taxon is briefly described by its bionomical followed by morphology (diagnostic features).

Quantitative analysis

Frrquency of Ocuureence of fungi

The physico- chemical data obtained were correlated with the fungal diversity and seasonal distribution of fungi (Summer and Pre-Monsoon). The frequency of occurrence of fungi in the five sampling stations was calculated (in percentage) and represented in the following frequency grouping per sample.

Most frequent	-	13-15%	(3+)
Frequent	-	10-12%	(++)
Rare	-	1-3%	(+)

Diversity Indices of fungi

The diversity of fungi in the marine sediment and wood samples of 11 sampling stations were assessed on the diversity indices,

$$\text{Simpson index } D' = \frac{1}{\sum (pi)^2},$$

and

$$\text{Shannon index, } H' = -\sum (p_i \ln p_i),$$

Where *pi* is the proportion of individuals that species *i* contributes to the total (Mugurran, 1988). The Shannon Evenness, J′, was expressed by:

$$J' = \frac{H'}{H'_{\max}}$$

Where *H′* mark is the maximum value of diversity for the number of species present (Picolou, 1975).

Physico – chemical analyses of water samples

Temperature (Venugopalan and Paulpondian, 1989)

The temperature of the sample in natural condition before sampling was measured using thermometers. The thermometer was inserted into depth from which samples were collected and the mercury levels was noted. Then the meter was cleaned with distilled water, wiped to remove moisture.

Results and Discussion

Ramanathapuram districts which were presented and discussed under two sections , viz., Enumeration of taxa, and Ecology of fungi

Enumeration of taxa

The various fungi isolated by plating and baiting techniques, in the entire study area enumerated with their morphological characteristics and ecological descriptions. The system of classification was based on "**The Fungi – Fifth kingdom, (Eds) Hendrick.B, (1992)** and followed for the arrangement of genera under their respective orders and families (Table: 1 and Figure.1). The genera and species within each family are arranged in alphabetical sequences

Ecology of fungi

Species diversity of fungi in the marine system

During the study period, a total of 52 fungal species were enumerated from 11 sampling stations by plating and baiting techniques and also direct observation techniques. Among these, 33 species were represented inS1, 21 in S2, 19 in S3, 23 in S4 and 14 in S5, 20 in S6, 30 in S7, 25 in S8, 20 in S9, 18 in S10,and 16 in S11 (Table.1 and Figure.2).

Even though, the some of sediment samples in all sampling stations, the number of fungi common to all the sampling stations was 5 out of the total 52 fungal species recorded..Maximum fungal diversity was observed in S1 Muthupettai (33 species) and Muthukuda (30 speceis).

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 (7 genera; 37 species. This was followed by Ascomycetes (9 genera, 9 species) Mucorceae (3 genera; 5 species).

Among the Hyphomycetes, *Aspergillus* was the common genus represented by 15 species followed by 5 species with *Penicillium and Curvularia* with 4 species. In addition to this C*ladosporium*, *Neurospora crassa*, *Fusarium semitectum* were the common genera found in this marine system.

Out of the total 52 species isolated only 12 were of typical marine lignicolous fungi *Leptosphaeria* sp.1 and 2, *Lulworhtia grandispora, Verruculina enalia, Dactylospora* sp., *Trematosphaeria lineolatispora, Pleospora triglochinicola, Clavatospora bulbosa, Cirrenalia tropicals, Massaria* sp.1, *Massaria* sp.2 and *Trimmastoma* sp. isolated from wood samples while remaining 40 species were of from marine derived fungi migrated from terrestrial souecs. It is to be noted that the marine fungi enumerated in this study were isolated exclusively from the wood samples by direct microscopic examination.

Occurrence of fungi in the marine sediment

Employing all the above said techniques, from the marine sediment samples, totally 40 fungi were isolated. Among these, 6 belng to Zycomycotina, 9 belongs Ascomycotina and 22 were belong to Deutromycotina . In sediments samples also the genus *Aspergillus* was found to be dominant represented with 16 species, followed by *Penicillium* (5 species) and *Curvularia* (4species).

Distribution of fungi in relation to woody substrates:

The fungi in the marine system was studied by plating and baiting techniques. Totally, 25 species of fungi belong to different groups were enumerated from the natural decaying wood substrates attempted with direct plating technique . In this, *Aspergillus* was found to be more predominant fungi, *A.flavus, A.fumigatus, A.luchuensis, A.terreus, A.nidulans,* followed by *Penicillium* sp. Where as,12species of fungi were isolated by baiting technique. Of which, 1 (*Clavatospora bulbosa*) were from woody samplecollected from S7 – S11 and remaining marine fungi such as *Leptosphaeria sp.1 and 2, Lulworhtia grandispora, Verruculina enalia, Dactylospora sp, Trematosphaeria lineolatispora, Plospora triglochinicola, Clavatospora bulbosa, Cirrenalia tropicals, Massaria* sp.1, *Massaria sp* 2. *and Trimmastoma* sp were isolated from mangove wood samples (Avicennia marina) collected from muthupettai

The fact that the mangrove vegetation play on important role in the distribution of fungi in the

Table.1 Total number of fungi isolated from all the sampling stations

Name of the fungi	***Sampling areas***										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
Abisidia sp.	-	-	+	+	-	-	+	+	+	+	+
Mucor sp.	+	-	-	+	-	-	+	-	-	+	-
Rhizopus nigricans	+	-	+	+	-	-	+	+	-	-	-
Rhizopus oryzaye	-	+	-	+	+	-	+	+	+	+	-
Rhizopus stolonifier	-	+	+	+	+	-	+	+	-	+	+
Neurospora crassa	-	+	-	+	-	-	+	+	+	+	-
E. nidulans	+	-	-	-	-	-	+	-	-	+	+
Dectylopora sp.	+	-	-	-	-	-	-	-	-	-	-
Massarina sp1	+	-	-	-	-	-	-	-	-	-	-
Massarina sp 2	-	+	-	-	-	-	+	-	-	-	-
Leptosphaeric sp1	+	-	-		-	-	-	-	-	-	-
Lephatasporia sp 2	+	-	-	-	-	-	-	-	-	-	-
Lulworthia sp.	+	-	-	-	-	-	-	-	-	-	-
Pleospora trichinicola	+	+	-	-	-	-	-	-	-	-	-
Trimmatostroma	+	-	-	-	-	-	-	-	-	-	-
T. lineolatspora	+	-	-	-	-	-	-	-	-	-	-
Veruculina enalina	+	-	+	-	-	-	-	-	-	-	-
Aspergillus clavatus	+	+	-	+	-	-	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	-	+	+	+	+	+
Aspergillus fumigatus	+	-	+	+	+	-	+	+	+	+	+
A.funiculoss	+	-	+	-	-	+	+	-	-	-	-
Aspergillus luchuensis	+	-	-	-	+	-	+	+	+	+	+
Aspergillus nidulans	+	-	-	-	+	-	+	+	+	-	-
Aspergillus niger	+	-	-	+	-	+	+	+	+	-	-
Aspergillus ochraceous	+	+	-	-	-	+		+	+	-	-
Aspergillus oryzae	-	+	+	+	-	+	+	+	+	-	-
Aspergillus quercinus	-	+	-	+	+	+		+	+	-	-
Aspergillus sulphureus	+	+	-		+	+		-	+	-	-

Aspergillus terreus	+	+	-	+	+	+	+	+	+	-	+
Aspergillus ustus	+	+	-	+	+	+	+	-	+	-	+
Asprgillus versicolor	-	+	+	+	+	-	+	+	+	+	+
Penicillium citrinum	+	+	+	+	-	+	+	+	+	-	-
Penicillium frequentans	-	-	_-	+	+	+	+	+	-	+	-
Penicillium funiculosum	-	-	-	+	-	+	+	+	+	+	+
Penicillium rubrum	-	-	-	-	+	+	-	+	+	+	+
P.jamthnellam	+	-	+	-	-	-	-	-	-	+	-
Verticillium sp	-	+	-	+	-	+	+	-	-	-	-
Cirrenalia tropicalis	+	-	-	-	-	-	+	-	-	-	-
Alternaria brasicola	-	-	+	-	+	+	-	+	+	-	-
Alternaria cinerariae	+	-	+	+	+	+	+	+	-		+
Cladosporium tennssimum	+	-	+	+		+	-	+	-	-	-
Cladosporia uredinicola	-	+	+	+	-	-	+	-	-	-	+
Curvularia andropopogonsis	-	-	+	+	-	+	-	-	-	-	+
Curvularia catnulata	+	-	+	-	-	+	-	+	-	-	-
Curvularia palmarrum	+	+	+	+	-	+	+	+	-	+	+
C. lunata	-	+	-	-	-	-	-	-	-		-
C.richardiae	-	+	-	-	-	-	-	-	-	-	-
Curvularia subulata	+	-	-	-	-	-	+	-	-	-	-
Drechslera sp	-	+-	+	-	-	-	+	-	-	-	-
Fusarium semitectetum	+	-	-	-	-	+	-	-	-	+	-
Ascochyta vulgaris	-	+	+	-	-	-	+	-	-	-	-
Total Number of species	**33**	**21**	**19**	**23**	**14**	**20**	**30**	**25**	**20**	**18**	**16**

Table.2 Frequency of fungi in all the sampling stations

Name of the fungi	Sampling areas										
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**
Abisidia sp.	-	-	+	+	-	-	+	+	+	+	+
Mucor sp.	+++	-	-	+	-	-	+	-	-	+	-
Rhizopus nigricans	+	-	+	++	-	-	+++	+	-	-	-
Rhizopus oryzaye	-	+	-	++	+	-	+++	++	+++	+	-
Rhizopus stolonifier	-	++	+++	+	+++	-	+	++	-	+++	+
Neurospora crassa	-	++	-	+	-	-	+	+	+	+++	-
E. nidulans	++	-	-	-	-	-	-	-	-	-	-
Dectylopora sp.	+	-	-	-	-	-	-	-	-	-	-
Massarina sp1	+	-	-		-	-	-	-	-	-	-
Massarina sp 2	+++	-	-	-	-	-	-	-	-	-	-
Leptosphaeric sp1	+	-	-	-	-	-	-	-	-	-	-
Lephatasporia sp 2	+	+	-	-	-	-	-	-	-	-	-
Lulworthia sp.	+	-	-	-	-	-	-	-	-	-	-
Pleospora trichinicola	+	-	-	-	-	-	-	-	-	-	-
Trimmatostroma	+++	-	+	-	-	-	-	-	-	-	-
T. lineolatspora	+	++	-	+	-	-	+++	+	+	+	+
Veruculina enalina	+	++	+	+	+++	-	+++	+	+	++	+++
Aspergillus clavatus	+	-	+	+	+	-	+++	+	+	++	+++
Aspergillus flavus	+++	-	-	-	+	-	+	+	+	+	+
Aspergillus fumigatus	+	-	-	-	+	-	++	+++	+	-	-
A.funiculoss	++	-	-	+	-	+	+	+++	+	-	-
Aspergillus luchuensis	+	+	-	-	-	+		+++	+++	-	-
Aspergillus nidulans	-	+++	+	+	-	++	+	+++	+++	-	-
Aspergillus niger	-	+	-	+	++++	++		+	+++	-	-
Aspergillus ochraceous	+	+	-		+	+		-	+++	-	-
Aspergillus oryzae	+	+	-	+	+	+	+	+	+	-	+
Aspergillus quercinus	+	+	-	+	+	+	+	-	+	-	+
Aspergillus sulphureus	-	+++	+	+	+	-	+	+	+	+	+
Aspergillus terreus	+	+++	+	+++	-	+	+++	+	+	-	-

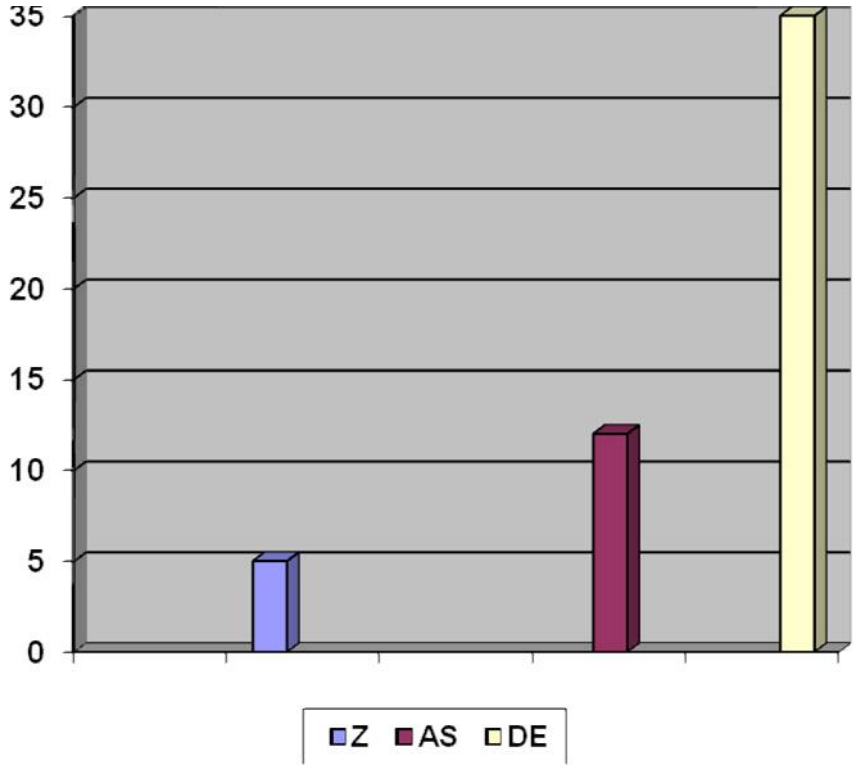
Aspergillus ustus	-	-	_-	+++	+	++	+++	++	-	+	-
Asprgillus versicolor	-	-	-	+++	-	++	+++	++	+++	+++	+++
Penicillium citrinum	-	-	-	-	+	+	-	+	+++	+++	+++
Penicillium frequentans	+	-	+	-	-	-	-	-	-	-	-
Penicillium funiculosum	+	-	-	-	-	-	+	-	-	-	-
Penicillium rubrum	-	-	+	-	+	+	-	++	+	-	-
P.jamthnellam	+	-	++	+	+	++	+	++	-		+
Verticillium sp	+	-	++	+		++	-	++	-	-	-
Cirrenalia tropicalis	-	-	++	+	-	+	-	-	-	-	+
Alternaria brasicola	+	-	+	-	-	+	-	+	-	-	-
Alternaria cinerariae	+	+++	+	+	-	+++	+	+	-	+	++
Cladosporium tennssimum	-	+++	-	-	-	-	-	-	-		-
Cladosporia uredinicola	-	+++	-	-	-	-	-	-	-	-	-
Curvularia andropopogonsis	-	+	-	-	-	-	+	-	-	-	-
Curvularia catnulata	+	-	-	-	-	-	+	-	-	+++	+
Curvularia palmarrum	+	-	+	-	-	+	+	-	-	-	-
C. lunata	+	-	+	-	-	-	-	-	-	+	-
C.richardiae	-	+	-	+++	-	+	+++	-	-	-	-
Curvularia subulata	-	+	+	+	-	-	+++	-	-	-	+++
Drechslera sp	+++	-	-	-	-	-	+	-	-	-	-
Fusarium semitectetum	-	+-	+	-	-	-	+	-	-	-	-
Ascochyta vulgaris	+	-	-	-	-	+	-	-	-	+	-
Total Number of species	**33**	**21**	**19**	**23**	**14**	**20**	**30**	**25**	**20**	**18**	**16**

+++ - Most Frequent, ++ - Frequent , + - Rare , - -No

Table.3 Species richness, diversity and evenness of fungi Recovered from 11 sampling stations

Sampling stations	Species richness special recovered	Simpson (D)	Shannon (H1)	Shannon Evenness(J)
S1	33	0.9997	0.4335	0.1035
S2	21	0.9889	0.8161	0.1902
S3	19	0.9995	6.6204	0.4713
S4	23	0.9800	4.1010	0.9427
S5	14	0.9889	0.9404	0.2213
S6	20	0.9998	0.3756	0.0867
S7	30	0.9989	0.7612	0.1772
S8	25	0.9995	0.6139	0.1369
S9	20	0.9800	0.0019	0.0003
S10	18	0.9989	0.8459	0.1991
S11	16	0.9990	0.5890	0.1355.

Figure.1 Total number of fungi and their division - wise



Z- Zygomycotina
AS – Ascomycotina
DE - Deuteromycotina

Figure. 2 Total number of fungi isolated from all sampling stations

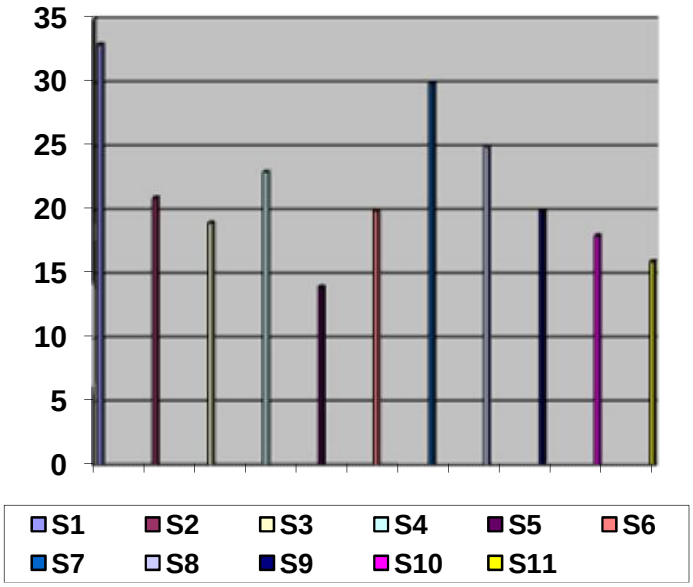
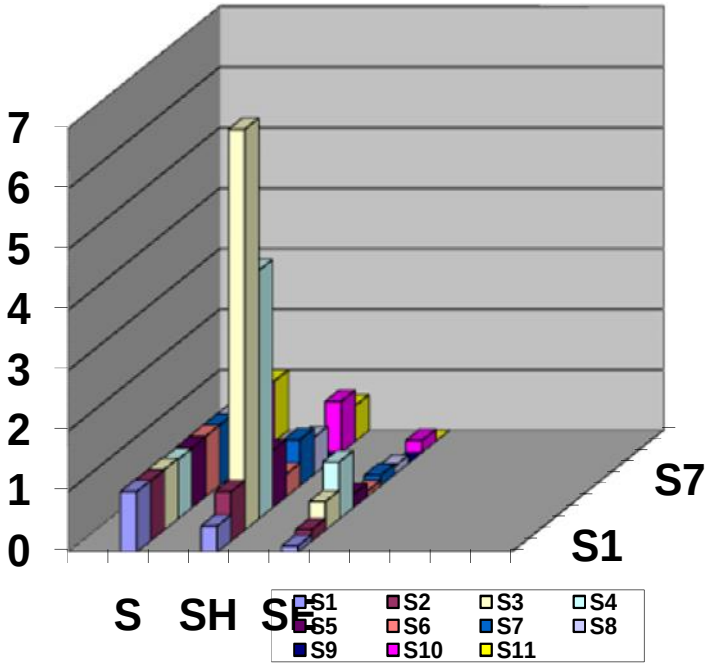


Figure.3 Species richness, diversity and evenness of fungi Recovered from 11 sampling stations



aquatic system, since they contribute to the leaf litter which harbor mycoflora was pointed out by Cunnell (1956). The fungal diversity of proproots, seedlings and wood of *Rhizophora apicualta* and wood and pneumatophores of *Avicennia* sp. were investigated by Sarma and Vittal (2000). Fungi occur on drift wood, intertidal wood, manalia rope and other lignocellolic substrates in marine and estuarine environments were reported by Johnson and Sparrow,(1961),Hughes (1974), Kohlmeyer and Kohlmeyer(1979).

Isolation of fungi from sediment mixed with water attempted with membrane filtration technique

As similar to dilution-plating technique fungi were also isolated by membrane filtration technique. Totally 20 species of fungal flora were isolated and enumerated .Of which, Zygomycotina represented with 3 species, followed by Ascomycotina (1 species) and Deuteromycotina represented with 14 species (Table.3 and Figure.5). Among the isolated fungi, *Aspergillus* was occurred predominantly represented with 10 species followed by *Penicillium* (3 species).

Frequency of occurrence of fungi

The fungal frequency of occurrence in the five sampling stations was calculated (in percentage) and it was represented in the following frequency groupings per sample.

The fungus *Aspergillus niger occurred* frequently and C*ladosporium*, *Neurospora crassa*, *Fusarium semitectum* were rarely occurred in the system. In Deuteromycotina *Aspergillus*, *Penicillium*, most frequently occurred in this system (Table.2).

Species richness, diversity and evenness of fungi:

When analyzing the diversity of fungi, the maximum diversity of fungi were observed in paddy field (S1) it represented with 33 species and minimum at S4with 14 species.

The species richness and diversity of fungi at 11 sampling stations were determined by Simpson and Shannon indices. Simpson and Shannon indices were highest at S5 represented by 0.9889 (Simpson), and Shannon was 6.6204 at S3 The Shannon evenness was highest at S4 (0.9427) while it was least at S6 with 0.0867 (Table.3, Figure.3).

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