Fungal diversity in Rhizospere and Rhizoplane soil of *Avicennia officinalis* from Netravathi river, Mangalore, Karnataka

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Abstract

Avicennia officinalis is a species of mangrove plants, also known as Indian mangrove. The young tree forms a low, dense bushy crown. When it matures, it forms a columnar tree up to 15m and may grow up to 30m. The microorganisms are known to form a healthy growth in all provided environment. This work was undertaken to study the fungal diversity in Rhizoplane and Rhizospere soil of Avicennia officinalis from netravathi river region. Rhizoplane region is the external surface of roots together with closely adhering soil particles and debris and Rhizospere region is the arrow region of soil that is directly influenced by root secretions and associated soil microorganisms. The work was carried out by inoculating Rhizoplane and Rhizospere soil of A. officinalis to different growth medias and incubated the cultured plates and then they are identified. From this work total 19 fungal species belonging to different genera were isolated and identified, among which Ascomycota were dominating with 16 species, Zygomycota with 2 species and one species of Oomycetes. The Ascomycota genera have shown high percentage of occurrence i.e. frequently occurring (5-10%) and also higher diversity in Simpson's index.

Key words: *Avicennia officinalis, Indian mangrove, Rhizoplane soil, Rhizospere soil, Ascomycota, Zygomycota, Oomycetes, Simpson's index.*

Introduction:

A mangrove is a shrub or small tree that grows in costal saline or blackish water. The term is also used for tropical coastal vegetation consisting of such species. Mangrove occur worldwide in the tropics and sub tropics, mainly between latitudes 25°N and 25°S. The total mangrove forest area of the world in 2000 was 137800 sq km, spanning 118 countries and territories. Mangroves are salt-tolerant trees, also called halophytes, and are adapted to life in harsh costal conditions. They contain a complex salt filtration system and complex root system to cope with salt water immersion and wave action. They are adapted to low oxygen (anoxic) conditions of waterlogged mud. The mangrove biome, or mangal, is a distinct saline woodland or shrub land habitat characterised by depositional coastal environments, where fine sediments (often with high organic content) collect in areas protected from high-energy wave action. The saline conditions tolerated by various mangrove species range from brackish water, through pure sea water (3-4%), to water concentrated by evaporation to over twice the salinity of ocean sea water (up to 9%).

The unique ecosystem found in the intricate mesh of mangrove roots offers a quiet marine region for young organisms. In areas where roots are permanently submerged, the organisms they host include algae, barnacles, oysters, sponges and bryozoans, which all require a hard surface for anchoring while they filter feed. Shrimps and mud lobsters use the muddy bottoms as their home. Mangrove crabs munch on the mangrove leaves, adding nutrients to the mangal muds for other bottom feeders. In at least some cases, export of carbon fixed in mangroves is important in coastal food webs. Mangrove forest can decay into peat deposits because of fungal and bacterial processes as well as by the action of termites. It becomes peat in good geochemical, sedimentary and tectonic conditions. The nature of these deposits depends on the environment and the type of mangrove involved. In Puerto Rico the Red (*Rhizophora mangle*), White (*Laguncularia racemosa*) and Black (*Avicennia germinans*) mangroves occupy different ecological niches and slightly different chemical compositions so the carbon content varies between the speciesas well between the different tissues of the plant eg: leaf litter vs root.

Although microbial diversity is one of the difficult areas of biodiversity research, extensive exploration is required for understanding the biogeography, community assembly and ecological processes which will for isolating and identifying new and potential micro organisms having high specificity for recalcitrant compounds. The present review highlights on the diversity study of potential bacteria, fungi and actinomyetes in mangrove environments. Mangroves are among the major sources of terrestrial organic matter to oceans and harbour a wide microbial diversity. The red sea gray mangroves (*Avicennia marina*) Ascomycota was the dominant phylum (76-85%), while basidiomycotawas less abundant (14-24%). Marine ascomycetes, basidiomycetes and dueteromycetes occur on submerged parts of roots (proproots, pneumatophores), stem and branches. High content of tannin do not protect mangrove plants from decomposition by marine fungi and wood boring animals (shipworms, isopods). Submerged bark and wood of mangroves are deteriorated by higher marine fungi. Growth of marine and terrestrial fungi overlaps at the high tide line. Some fungi, chiefly host specific ones, have a limited distribution, while omnivorous species are found in mangroves throughout the tropic and sub-tropic. Most mangrove fungi are warm water species.

Lingan et al (1999) mentioned the presence of higher root colonization of VAM fungi that is 65 spores/100 g soils of *Avicennia officinalis*. The VAM species identified are *Glomusfasciculatum* and *G. Aggregatum*.

Qurshiet al (2004) identified fifty seven species of fungi belonging to 23 genera were isolated and identified from the rhizospere and rhizoplane of 65 plant species, belonging to 58 genera and 19 families from Sindh and Baluchistan (Pakisthan). A greater number of fungi were isolated from the rhizospere then the rhizoplane. In the rhizospere, *Fusarium solani* and *Aspergillus* sp were dominant followed by *Drehsleraaustraliensis*. In the rhizoplane, *F. Solani* was also dominant.

Bhattacharya et al (2012) studied the antagonistic relation among the different microbial forms in the Vallapattanam and Pappinishery mangrove soils. From these mangrove samples a total of 28 bacteria, 22 actinomycetes and 3 fungal forms were isolated. Based on colony morphology and the arrangement of conidia, conidiophores 3 species identified as *Trichoderma* sp, *Penicillium* sp and *Aspergillus flavus.*

Khalil et al (2013) studied distribution of fungi in mangrove soil were the mycobiota composition of the mangrove soil located in coastal area at red sea inEgypt was investigated, 24 soil sample were collected. 15 fungal species belonging to 9 genera were identified. Results showed that most of the genera belonged to Ascomycota with few belonging to Deuteromycota and Zygomycota. The frequent species were identified as *Aspergillus* sp, *Cladosporium*sp, *Alternaria*sp, *Penicilliums*p,*Rhizopus*sp, *Absidia*sp, *Acremonium*sp, and *Trichoderma*sp.

Sarma and Hyde (2001) reviewed frequently occurring fungi in mangroves. They mentioned different equation for calculation of percentage of similarity, species diversity and percentage of occurrence. Also they mentioned very frequent and frequently occurring fungi in different areas with number of samples. Factors discussed with suggestion for methodology also mentioned the protocol for sample collection for different mangrove species.

GlinaandKal et al (2011) assessed *Aspergillus* sp were found to be dominant among the various fungal species isolated from the mangrove ecosystem.

Materials and Methodology:

Study area:

The Netravati River or Netravathi Nadi has its origins at Bangrabalige valley, Yelaneeru Ghat in Kudremukh in Chikkamagaluru district of Karnataka, India. This river flows through the famous pilgrimage place Dharmasthala and is considered as one of the Holy rivers of India. It merges with the Kumaradhara River at Uppinangadi before flowing to the Arabian Sea, south of Mangalore city. This river is the main source of water to Bantwal and Mangalore. Thokkotu- Netravathi River in Karnataka state was the study area. The coastal Karnataka is divided into 3 districts- Dakshina Kannada, Udupi and Uttarakannada. Dakshinakannada and Udupi seaboard lies between 12◦27’ North latitude and 75◦35’ and 75◦49’ East longitude. It is about 177 kms in lengths, about 80 kms at its widest part. From North to South, it is a narrow strip of territory and from east to West it is a broken low plateau, which spreads from the Western Ghats to the Arabian Sea. The major part of its length lies along the sea board. The area is intersected by many coast parallel rivers and streams and presents varied and most picturesque scenery. The length of the coastline is almost straight, but broken at places with numerous splits by rivers, creeks and sandy ridges and bays. The principle river of Dakshinakannada district is Netravati river and the estuarine complex is Netravati- Gurupura estuary.

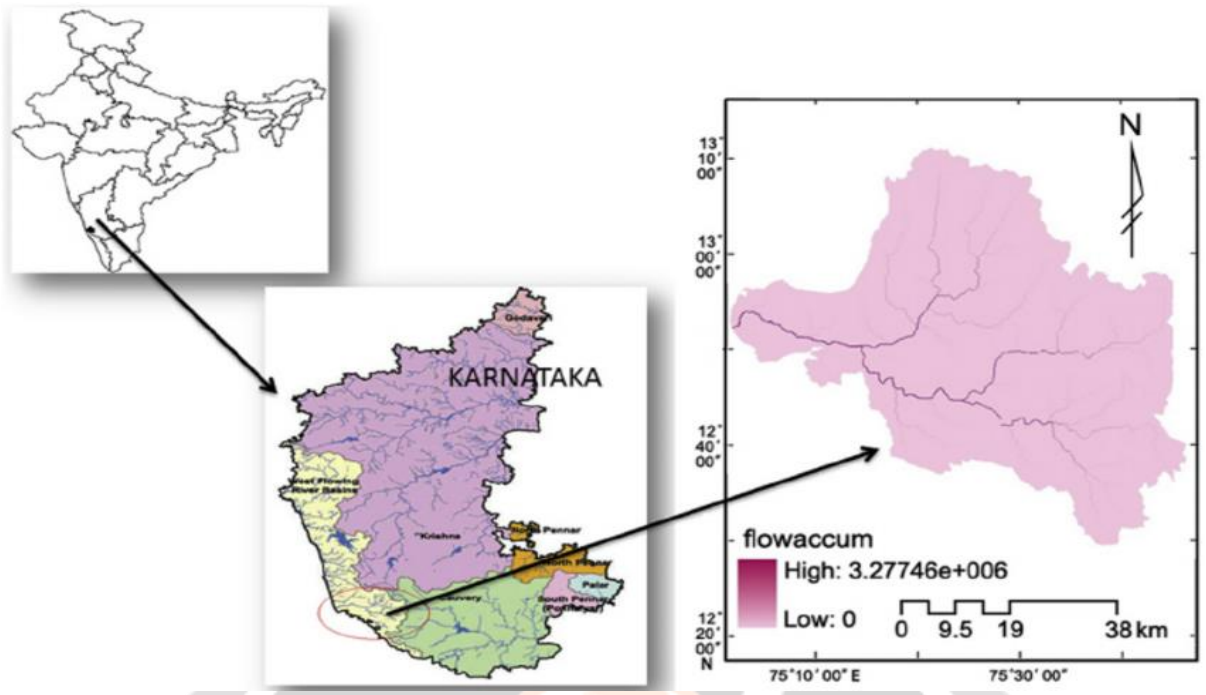


Fig 1: Map showing Netravathi river basin.

Fig 2: A view of study area near Netravathi river.

Methadology:

The aim was to isolate fungus from rhizospere and rhizoplane of mangrove plant (*Avicennia officinalis).* Totally five medias were selected for the growth of fungus from rhizospere and rhizoplane. The following culture media were selected for the isolation

1. Potato dextrose agar media(PDA):

COMPOSITION	1000 ml	250 ml
Agar	20 g	5 g
Potato	200 g	50 g
Dextrose	20 g	5g
Distilled water	1000 ml	250ml

2. Malt extract agar media(MEA):

COMPOSITION	1000 ml	250 ml
Agar	20 g	5 g
Potato	200 g	50 g
Dextrose	20 g	5g
Distilled water	1000 ml	250ml

3. Rose Bengal Agar (RBA) media:

COMPOSITION	1000ml	250ml
Dextrose	15g	3.75g
Magnesium sulphate	0.1g	0.125g
Rose Bengal	0.05g	0.012g
Agar	15g	3.75g
Distilled water	1000ml	250ml

4. Czapek-dox agar media (CAM):

COMPOSITION	1000ml	250ml
Sucrose	30g	7.5g
Sodium nitrate	2g	0.5g
Di potassium phosphate	1g	0.25g
Magnesium sulphate	0.5g	0.125g
Potassium chloride	0.5g	0.125g
Agar	15g	3.75g
Distilled water	1000ml	250ml

5. Esabouraud Dextrose agar (EDA) media:

COMPOSTION	1000ml	250ml
Dextrose/glucose	40g	10g

Peptone	10g	2.5g
Agar	15g	3.75g
Distilled water	1000ml	250ml

Determination of soil temperature:

The temperature of soil was determined using thermometer. Inserted into depth of 5cm for 5 min, after which the temperature reading is obtained.

Determination of soil and water pH:

pH of water and soil is determined by using pH strip; the strip is dipped into water sample and soil sample. Then the strip is compared with standard colour given in the pH strip and value is obtained.

Collection of sample:

Young and healthy plants were carefully taken from a depth of 15cm and root samples with adhering soil were collected in polythene bags. Rhizoplane soil is collected from the surface.

Isolation methods:

1. **Blotter method:**

   Sterile dry filter papers are cut into circular shape and wetted by sterile distilled water. Excess water has to be removed and placed in sterile petriplates. 1cm long lateral and tap root are inoculated into petriplates and incubated for 5-7 days at 25°C.

2. **Root impression method:**

   Root sample were rinsed in distilled water. Tap root and lateral roots are cut in to 1cm long pieces. The root segments are surface sterilised by immersing in 96% ethanol for 1 min, 1% sodium hypochlorite for 3 min and 96% ethanol for 30 sec. immediately after surface sterilisation the root segments are rinsed in sterile distilled water. The root segments are inoculated to respective agar plates which contain streptomycin (0.5g/250ml). Prepare 2 replica of each agar media and incubate at 28°C for 5-7 days.

3. **Isolation of fungi from rhizospere soil:**

   Root pieces with adhering soil should be collected and 1gm soil is weighed. Serially dilute the soil by adding distilled water. After dilution from each test tubes 0.1ml of sample is taken and inoculated into autoclaved, solidified media along with streptomycin (0.5g/250ml). These samples are inoculated into 5 different media as mentioned above. Prepare 2 replicas for each dilution. Plates should be incubated at 25-28C for 5-7 days under 12 hour light and dark condition.

4. **Isolation of fungi from rhizoplane soil:**

   1gm of rhizoplane soil is weighed. Serially dilute the soil by adding distilled water. After dilution from each test tubes 0.1ml of sample is taken and inoculated into autoclaved, solidified media along with streptomycin (0.5g/250ml). These samples are inoculated into 5 different media as mentioned above. Prepare 2 replicas for each dilution. Plates should be incubated at 25-28C for 5-7 days under 12 hour light and dark condition.

RESULTS AND DISCUSSIONS:

Rhizospere and Rhizoplane soil along with roots were inoculated in 5 different media's, 19 different fungal species were isolated and identified.

Sl no	Media	Root impression	Rhizospere soil	Rhizoplane soil
1	PDA media	*Mucor* sp	*Cladosporium* sp	*Rhizopus* sp
		Fusarium oxysporum	*Penicillium* sp	*Curvularia* sp
			Geotrichum candidum	*Aspergillus* sp
			Aspergillus sp	*Byssochlamys* sp
2	SA Media	*Chaetomella* sp	*Colletotrichum* sp	No colony
		Piggotia sp		
		Colletotrichum sp		
		Penicillium sp		
		Meliola sp		
3	CA Media	*Rhizopus* sp	*Paecilomyces* sp	*Penicilium* sp
		Paecilomyces sp		*Aspergillus* sp
				Cladosporium sp
4	MEA media	*Aspergillus* sp	*Cladosporium* sp	*Phytophthora* sp
		*Alternaria*sp	*Phytophthora*sp	*Aspergillus* sp
		Penicillium citrinum	*Cheatomella horrida*	
5	RBA media	No colony	*Pithomyces* sp	*Trichoderma* sp
			Penicillium sp	*Aspergillus* sp
			Paecilomyces sp	

Table 1: Fungi obtained by different isolation methods in each media.

The term "percentage occurrence" is used to denote the number of samples on which a paticular fungus was found as against the total number of samples (supporting sporulation) examined in each bimonthly collection and is calculated according to the formula outlined by Hyde (1986) and lones and Hyde (1988).
The diversity and the occurrence is calculated using different formula,

Percentage of occurrence of fungus = $\frac{Number\ of\ occurance\ of particular\ fungus}{Total\ number\ of\ sample\ examined} \times 100$

Frequency of occurrence of fungi can be tabulated using the following frequency groupings:

Very frequent ≥10% Frequent = 5 to 10%
Infrequent = 1-5% Rare≤ 1%

Species diversity is defined as the number of species and abundance of each species that live in a particular location.
Species diversity could be calculated using formula like,

Simpsons index= D = ΣPi^2,

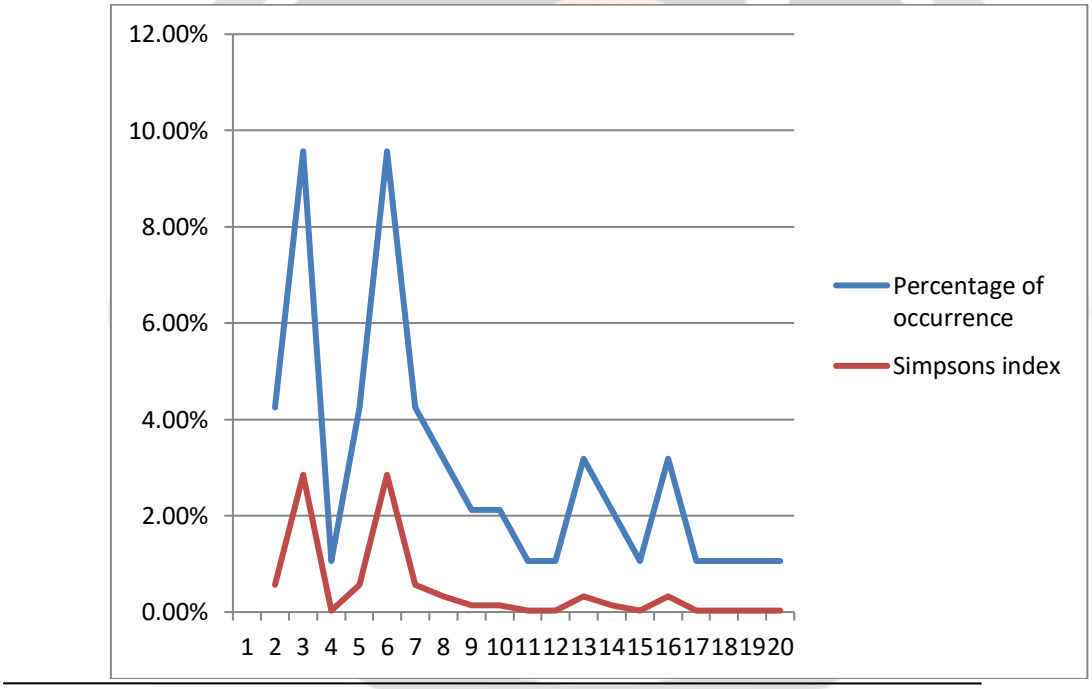
Pi= $\frac{ni}{N}$

Where N= Total number of individuals
ni= Number of individual species

Sl.no	Name of the fungi	Division	Number of fungi present	Percentage of occurrence	Simpsons index
1.	*Cladosporium* sp	Ascomycota	4	4.25%	0.0056
2.	*Penicillium* sp	Ascomycota	9	9.57%	0.0285

3.	*Fusarium oxysporum*	Ascomycota	1	1.06%	0.0003
4.	*Geotrichum candidum*	Ascomycota	4	4.25%	0.0056
5.	*Aspergillus* sp	Ascomycota	9	9.57%	0.0285
6.	*Paecilomyces* sp	Ascomycota	4	4.25%	0.0056
7.	*Rhizopus* sp	Zygomycota	3	3.19%	0.0032
8.	Colletotrichum sp	Ascomycota	2	2.12%	0.0014
9.	*Chaetomella horrida*	Ascomycota	2	2.12%	0.0014
10.	*Piggotia* sp	Ascomycota	1	1.06%	0.0003
11.	*Alternaria* sp	Ascomycota	1	1.06%	0.0003
12.	*Phytopthora cinnamomi*	Oomycota	3	3.19%	0.0032
13.	*Pithomyces* sp	Ascomycota	2	2.12%	0.0014
14.	*Meliola* sp	Ascomycota	1	1.06%	0.0003
15.	*Penicillium citrinum*	Ascomycota	3	3.19%	0.0032
16.	*Curvularia* sp	Ascomycota	1	1.06%	0.0003
17.	*Byssochlamys* sp	Ascomycota	1	1.06%	0.0003
18.	*Mucor* sp	Zygomycota	1	1.06%	0.0003
19.	*Trichoderma* sp	Ascomycota	1	1.06%	0.0003
			53		

Table 2: Percentage of occurrence and Simpsons index of different fungal species.



Graph 1: Graph representing percentage of occurrence and Simpson's index

D is a measure of dominance, so as D increases, diversity (in the sense of evenness) decreases. Thus, Simpson's index is usually reported as is compliment 1-D. since D takes on values between 0-1. From the collected data the D-value is more for *Penicillium* sp and *Aspergillus* sp. From the frequency grouping we can conclude that Penicillium sp and Aspergillus sp are frequently occurring (5-10%) with percentage of occurrence 9.5% and rest of the species belong to infrequently occurring with percentage range of 1-5%.

CONCLUSION:

It is concluded that from the present work total 19 fungal species belonging to different genera were isolated and identified. Among which, Ascomycota were dominating with 16 species, Zygomycota with 2 species and one species of Oomycetes. The Ascomycota genera has shown high percentage of occurrence i.e. frequently occurring (5-10%) and also higher diversity in Simpson's index.

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