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A STUDY ON ENVIRONMENTAL QUALITY AND DIVERSITY OF MICROBES IN THE MANAKUDY MANGROVES

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ABSTRACT

Mangrove biodiversity have received considerable attention in recent years since research has increased the understanding of the values, functions and attributes of mangrove ecosystems. The present study was carried out to determine the physico-chemical characteristics of water in mangrove ecosystem of Manakudy, South west coast of India, for periods of one year (2014 to 2015). The study revealed that the physico-chemical parameters like temperature, transparency, pH, total dissolved solids, salinity, dissolved oxygen, carbonate, bicarbonate and nutrients such as nitrite, nitrate, phosphate and chlorides exhibited considerable seasonal and spatial variations. The diversity and load of microorganisms, cyanobacteria, bacteria and fungi associated with the mangrove water were determined by using the standard method. One year study revealed that 12 species of bacteria, 20 fungi and 30 species of cyanobacteria were observed from the water stream. Among bacteria, *Pseudomona*, *Aeromonas* and *Vibrio* with two species and others with single each were recorded. *Aspergillus* was dominant (6 species) among the fungi followed by *Penicillium* with three. Cyanobacteria were a dominant group, recorded 30 species. *Ocillatoria*, *Microcystis* and *Spirulina* with each three species were the dominant genus and followed by *Phormidium* and *Lyngbya* with two species other with single one each. More hardness and alkalinity with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria. The study exemplified the fact that the Manakudy mangrove ecosystem is in a good state of health.

Key Words: Mangrove, Biodiversity, Cyanobacteria, Bacteria, Fungi..

INTRODUCTION

Mangrove-forest is a vegetation community formed by a variety of mangrove vegetation species growing in the intertidal areas and estuary mouths between land and sea. Mangroves can provide critical habitat for a diverse marine and terrestrial flora and fauna. Mangrove forest beaches have an abundant nutrition and a good ecological environment for the marine creatures. Mangrove forests are one of the most productive and biodiversity wetlands on the earth. Thereby, this condition has formed the mangrove ecosystem. The large area mangrove forest is very important to protect the ecological balance of sea and coastline. Healthy mangrove forests are a key to a healthy marine ecology. Yet, these unique coastal tropical forests are among the most threatened habitats in the world (Ramamurthy, 2010).

Wetlands are vital to the health of environment due to their immense ecological and socioeconomic value. India has $7.6x10^{-4}$ km^2 of wetland comprising estuaries, bays, lagoons, brackish waters, lakes and saltpans. The various tidal zones, with characteristic substratum sustain a variety of specialized habitats of productive and complex nature. Mangroves, influenced by marine as well as fresh water are the most predominant and ecologically fragile habitats of coastal wetlands. About 8 % of India’s coastline mangroves have occupied admeasuring 3760 km^2 areas. These habitats have been to harbour varieties of biota of ecological and commercial importance. However, being easily accessible, mangrove ecosystems from the country have been over-exploited in the recent past leaving them in the most degraded stage (Ramamurthy *et al*., 2012).

The mangrove forest at Manakudy is located on the southern extremity of Indian Peninsula (Lat $8^0$2'N Long $77^0$30'E) along the south west coast of India, about 10 km from Kanyakumari. Manakudy estuary which has an area of about 150 ha is situated about 8 kilometers northwest of Cape Comorin in Kanyakumari District. It is the confluence of river pazhayar, which has its origin from the Western Ghats. The Manakudy mangroves is abound with varied habitats that include shallow open waters, sandy beaches, muddy flats, mangrove forest, river delta and sea grass. Mangroves are a significant ecosystem in the estuary with a luxuriant growth on the mud flats. The litters on the mangrove floor undergo humification and mineralisation and the nutrients are leached into the mangrove water due to surface run-off adding to the productivity of the estuary. There is luxuriant growth of mangroves on the mud flats of Manakudy mangroves.

Microorganisms can be defined as life forms that cannot be seen with the unaided eye. This broad definition encompasses an extensive and diverse assemblage of organisms, which exhibit widely different morphological, ecological and physiological characteristics. They are represented by groups like viruses, bacteria, fungi, diatoms, algae, protozoans etc; of these the first three groups require not only microscopic examination but also in many cases growth in pure culture is essential for identification and hence, they can be strictly treated as microorganisms. As cyanobacteria are a unicellular and autotrophic photosynthesis and they doing not take part either in biogeochemical cycles or biological interactions usually the bacteria and fungi are considered important microbial groups. The microbial diversity encompasses vast number of species and the number of species of these groups known to science is only a tip of the iceberg. The total number of known species of bacteria (including Cyanobacteria) in the world is only 4000 whereas; the estimated species number is 3,000,000. A scanty of 0.1 percent alone has been described. In the case of fungi (including yeasts forming fungi, slime moulds, oomycetes) the described species were 70,000 but the estimated number is 1,500,000. Only 5 percent of the species were described (Groombridge 1992). Considerable difficulty arises in the estimation of those, which remain undescribed. This may be because they are unculturable by the conventional methods and lack of suitable methods of culture of all groups or because we have not explored enough. There are evidences for the occurrences of large numbers of bacterial species, which are 'unculturable' forms. In recent years blue green algae have been drawing tremendous attention because of their ability to treat biomedicine and improve disease quality. Taking the above facts into consideration, a survey was undertaken in mangrove environs to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria.

MATERIALS AND METHODS

The water was collected from Manakudy mangroves situated at Kanyakumari district, Tamil Nadu, India. A sampling programme consisting of a series of monthly water quality and microbial survey was conducted for one year (2014 - 2015). Population of bacteria and fungi were identified and isolated from the water samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey (1984). Fungi were identified by using standard manuals (Gillman 1957; Ellis 1971). Water samples were collected in duplicate from the same place in pre-sterilized bottles. For cyanobacterial survey, 5 places were selected along the water stream. Samples were collected from the places along with water in polythene bags.

Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary 1959). Physico-chemical characteristics of mangrove water were done according to the standard methods (APHA 1981). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

Atmospheric temperature varied between 30.9 and 32.1°C in post monsoon and pre monsoon respectively. The minimum (28.5°C) was recorded during monsoon season in November and the maximum (34.2°C) was observed during summer in May. Surface water temperature ranged from 28.3 to 31.3°C in post monsoon and pre monsoon respectively. The minimum (27.4°C) was recorded during monsoon season in November while the maximum (33.4°C) was during summer in May. The environmental parameters showed variations in different seasons in the study region depending on the topography. Salinity showed the highest values (29.1 ppt) in summer nearer to the coastal environment associated with low phosphorus (0.901 mg/l) concentrations. The lowest value of salinity (23.8 ppt) was noticed in monsoon seasons, accompanying high phosphorus (1.655 mg/l) concentration due to the freshwater zone of this aquatic environment. Low DO (5.1 mg/l) values in summer season may be due to the stagnant not conditions of the water with increasing waste load in the mangrove environment. This in turn enhances the concentrations of ammonia (7.3 mg/l) and nitrite (5.7 mg/l) at these monsoon periods. High nitrate (17 mg/l), inorganic phosphorus (1.091 mg/l) and organic phosphorus (0.620 mg/l) concentration observed in the monsoon periods indicates the impact of terrestrial runoff.

Estuarine mangrove waters in general have relatively low stocks of inorganic phosphorus and nitrogen (Alongi *et al* 1992). In some cases, the degree of human impact seems to control nutrient profiles (Nedwell 1975), while in others the degree of upland influence and the hydrology of the system appear to be of greater importance (Boto & Wellington 1988; Ovalle *et al* 1990). In the present study, the ecosystem was found to be nutrient rich and the ratios of N: P (9: 1) as well as TN: TP (7: 1) were low. The water pH, temperature and salinity fluctuations in the Manakudy mangrove are consistent with seasonal cycles. However, the influence of the Manakudy mangrove on hydrographic conditions was observed at the sampling stations. The spatial and temporal differences in physicochemical variations indicate the diversity of habitats that exist within this lagoon. Monsoon season and post monsoon have a lower temperature and salinity than the pre monsoon.

Bacteria isolated from the water and soils were identified based on colony morphology, Gram staining, and various biochemical characteristics. Totally 12 different bacteria were isolated from the mangrove environs (Table 2). Mangroves provide a unique ecological environment for diverse bacterial communities. The bacteria fill a number of niches and are fundamental to the functioning of these habitats. They are particularly important in controlling the chemical environment of the mangal. For example, sulfate-reducing bacteria Chandrika *et al* (1990) are the primary decomposers in anoxic mangrove sediments. These bacteria largely control iron, phosphorus, and sulfur dynamics and contribute to soil and vegetation patterns (Sherman *et al*., 1998 and Ramamurthy *et al*., 2009). Subsurface bacterial communities may sequester nutrients and hold them within nutrient-limited mangrove muds (Alongi *et al* 1993; Rivera-Monroy & Twilley 1996).

In addition to processing nutrients, mangrove bacteria may also help process industrial wastes. Iron-reducing bacteria are common in mangrove habitats in some mining areas (Panchanadikar, 1993). Eighteen bacterial isolates that metabolize waste drilling fluid have been collected from a mangrove swamp in Nigeria. Interestingly, four additional bacterial strains isolated from the same swamp depress growth rates of *Staphylococcus* and *Pseudomonas* species and could, therefore, decrease normal rates of organic decomposition (Benka-Coker and Olumagin, 1996).

Other mangrove bacteria are parasitic or pathogenic. Vibrios capable of parasitizing *Vibrio* sp. are common in an Australian mangrove habitat. Their abundance there (36.6 ml-1) is much higher than in nearby Great Barrier Reef habitats (9.5 ml^{-1}) Sutton and Besant (1994). Also in Australia, *Bacillus thuringiensis*, which shows insecticidal activity against mosquito larvae of *Anopheles maculatus*, *Aedes aegypti* and *Culex quinquefasciatus*, has been isolated from mangrove sediments (Lee *et al* 1990a; Lee & Seleena 1990). In the present studies the pathogenic bacteria were isolated from Manakudy mangrove water and soils. The isolated pathogenic bacteria were identified by various biochemical tests.

Fungi, from the mangrove sample were isolated based on serial dilution technique. Totally, twenty different species of fungi belonging to eleven genera were isolated from the mangrove samples (Table 3). Among the fungi recorded, *Aspergillus* was found to be dominant with six species followed by *Penicillium* three species. Mangals are home to a group of fungi called “manglicolous fungi.” These organisms are vitally important to nutrient cycling in these habitats (Hyde and Lee, 1995; Kohlmeyer *et al.*, 1995). They recognized 43 species of higher fungi, including 23 Ascomycetes, 17 Deuteromycetes, and 3 Basidiomycetes. Hyde (1990a) listed 120 species from 29 mangrove forests around the world. These included 87 Ascomycetes, 31 Deuteromycetes, and 2 Basidiomycetes.

Work in individual habitats has revealed surprisingly diverse fungal communities (Hyde 1990b; Hyde 1996). Chinnaraj (1993a) identified 63 species of higher fungi in mangrove samples from Andaman and Nicobar Islands alone. Similar samples from Lakshadweep Island yielded 32 species (Chinnaraj 1992) and 39 species were found in mangrove samples from the Maldives (Chinnaraj 1993a). Ravikumar & Vittal (1996) found 48 fungal species in decomposing *Rhizophora* debris in Pichavaram, south India. Table 3 lists some of the fungal species identified in these studies.

Altogether thirty species of cyanobacteria belonging to twenty-two genera were collected from the water stream (Table 4). Among the genera *Ocillatoria*, *Microcystis* and *Spirulina* with each three species and followed by *Phormidium* and *Lyngbya* with each two species were the dominant genus and other with single one each. Phytoplankton and benthic microalgal communities make important contributions to the functioning of mangrove environments. However, their contribution to total estuarine production is relatively small in most regions of Southeast Asia, Australia, Central America and tropical South America. Robertson and Blaber (1992) suggested that the contribution of plankton to total net production in mangrove habitats ranges from 20 to 50%. Careful measurements are verifying that predication for large systems. Phytoplanktons are responsible for 20% of the total production in mangrove estuaries in the Fly River Delta in Papua New Guinea (Robertson *et al* 1991) and 20-22% of the total production in the Pichavaram mangroves of south India (Kawabata *et al* 1993). In the present studies the 30 species of cyanobacteria were isolated from Manakudy mangrove.

Of the total estimated area of 150 million sq km of the earth, about 70% is occupied by oceans. However, of all the total photosynthetic productivity of 555.2 billion tons of dry weight/year on earth, only 34.4% is contributed by the oceans (Bassham 1975). India has a vast coastline of over 7500 km; in addition it has many lakes, ponds, puddles, backwater areas and a tropical climate that results in abundance of natural populations of varied organisms. Cyanobacteria are widespread and abundant in most marine habitats. Their ability to grow in seawater is presumably related to a preference for alkaline conditions and an ability to tolerate high salt concentrations. The resistance, which many species show towards osmotic shock, extremes of temperature and reducing conditions, suits their existence in a variety of intertidal habitats. Desikachary (1959) suggested that probably 20% of all known cyanobacteria occur in saline conditions and a majority of them are truly marine area. However, little work has been done to understand the cyanobacterial biodiversity of marine environments of India (Thajuddin *et al* 2000 & 2002).

Table 1. Physico-chemical analysis of Manakudy mangrove water (2014 - 2015)

S. No	Parameters	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	pH	7.6 ± 1.26	8.2 ± 1.84	8.7 ± 2.16	7.9 ± 1.54
2	Atmospheric Temp. (^{0}C)	28.5 ± 2.57	30.9 ± 2.18	34.2 ± 2.97	32.1 ± 2.14
3	Surface water Temp. (^{0}C)	27.4 ± 2.06	28.3 ± 2.11	33.4 ± 2.56	31.1 ± 2.17
4	EC (mho/cm)	288 ± 5.14	313 ± 5.06	392 ± 6.17	306 ± 5.84
5	Turbidity (NTU)	5.25 ± 3.08	6.12 ± 3.17	7.87 ± 2.87	6.51 ± 3.31
6	Total dissolved solids	854 ± 1.21	883 ± 1.81	910 ± 1.12	892 ± 1.65
7	Alkalinity	15.9 ± 1.28	20.8 ± 1.33	26.1 ± 1.18	21.7 ± 1.87
8	Free carbon dioxide	1.45 ± 2.14	1.69 ± 2.18	1.91 ± 2.45	1.58 ± 2.22
9	Dissolved Oxygen	6.5 ± 0.12	6.1 ± 0.65	5.1 ± 0.98	5.9 ± 0.87
10	Ammonia	7.3 ± 6.71	6.5 ± 6.12	5.8 ± 6.17	6.2 ± 6.42
11	Nitrate	17 ± 4.19	12 ± 4.67	9.1 ± 4.81	10 ± 4.27
12	Nitrite	5.7 ± 5.17	4.9 ± 5.26	3.4 ± 5.81	4.7 ± 5.28
13	Total hardness	1050 ±0.11	1125 ± 0.34	1210 ± 0.81	1150 ±0.22
14	Calcium	675 ± 0.47	697 ± 0.22	794 ± 0.84	710 ± 0.35
15	Magnesium	324 ± 0.18	345 ± 0.14	372 ± 0.31	354 ± 0.15
16	Chloride	124 ± 1.51	151 ± 1.87	195 ± 1.34	167 ± 1.28
17	Sulphate	15.7 ± 1.22	17.2 ± 1.42	18.7 ± 1.46	17.9 ± 1.85
18	Salinity (ppt)	23.8 ± 1.44	25.9 ± 1.31	29.1 ± 1.25	26.7 ± 1.13
19	Silicate	3.87 ± 1.17	4.13 ± 1.28	5.18 ± 1.54	4.54 ± 1.11
20	Total Phosphorus	1.655±0.15	1.340±0.28	0.901±0.84	1.420±0.24
21	Inorganic phosphorus	1.091±0.24	0.926±0.86	0.605±0.22	0.978±0.29
22	Organic phosphorus	0.620±0.33	0.480±0.14	0.301±0.56	0.495±0.41
23	BOD	8.8 ± 1.11	11.8 ± 1.42	14.2 ± 1.27	12.3 ± 1.38
24	COD	59.7 ± 1.63	66.8 ± 1.44	73.2 ± 1.64	67.4 ± 1.85

Monsoon (Oct-Dec); Post-Monsoon (Jan-Mar); Summer (Apr-June); Pre-Monsoon (July-Sep)

* Except pH and temperature, all values expressed in mg^{-1}

Table 2. Bacterial flora in the Manakudy mangrove

S. No	Name of bacteria	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	*Streptococcus mutans*	+	+	-	+
2	*Escherichia coli*	+	+	+	+
3	*Vibrio anguillarum*	+	+	+	+
4	*V.alginolyticus*	+	+	-	+
5	*Pseudomonas aeruginosa*	+	+	-	+
6	*P. fluorescens*	+	+	+	+

7	*Aeromonas salmonicida*	-	+	+	+
8	*A. hydrophilia*	+	+	+	+
9	*Edwardsiella tarda*	+	+	-	+
10	*Enterobacter aerogenes*	+	-	+	+
11	*Lactobacillus plantarum*	-	-	+	-
12	*Bacillus pumilus*	-	+	+	+

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

Table 3. Fungal Flora in the Manakudy Mangrove

S. No	Name of Fungi	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	*Aspergillus niger*	+	+	+	+
2	*A. flavus*	+	+	+	+
3	*A. fumigatus*	-	-	+	-
4	*A. luchensis*	+	+	+	+
5	*A. nidulans*	-	-	+	-
6	*A. terreus*	+	+	+	+
7	*Trichoderma viride*	+	+	+	+
8	*Fusarium sp*	+	-	-	-
9	*Fusarium oxysporum*	+	-	-	-
10	*Curvularia sp*	-	-	+	-
11	*Candida albicans*	+	+	+	+
12	*Xylaria sp.*	+	-	-	-
13	*Verticillium sp*	-	-	+	-
14	*Geotrichum candidum*	-	-	+	-
15	*Penicillium citrinum*	+	+	+	+
16	*P. javanicum*	+	+	+	+
17	*P.javanicum*				
18	*Helminthosporium oryzae*	-	+	+	-
19	*Rhizobus stolonifer*	-	-	+	-
20	*R. oryzae*	-	-	+	-

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

Table 4. Cyanobacterial Flora present in the Manakudy mangroves

S. No	Name of cyanobacteria	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	*Oscillatoria amoena*	+	+	+	+
2	*Oscillatoria tenuis*	+	+	+	+
3	*Oscillatoria formosa*	+	+	+	+
4	*Anabaena sphaerica*	-	+	+	+
5	*Nostoc muscorum*	+	+	+	+
6	*Chamaesiphon sideriphilus*	+	+	+	+
7	*Xenococcus acervatus*	+	+	+	+
8	*Phormidium valderianum*	+	+	+	+
9	*Phormidium fragile*	+	+	+	+
10	*Trichodesmium erythraeum*	+	+	+	+
11	*Richelia intracellularis*	+	+	+	+
12	*Hapalosiphon welwitchii*	+	+	+	+

13	*Dichothrix bauriana*	-	+	+	+
14	*Spirulina subsalsa*	+	+	+	+
15	*Spirulina platensis*	+	+	-	+
16	*Spirulina subtilissima*	+	+	+	+
17	*Lyngbya majuscula*	+	+	-	+
18	*Lyngbya hieronymusii*	+	+	+	+
19	*Stichosiphon sansibaricus*	+	+	+	+
20	*Nodularia spumigena*	+	+	+	+
21	*Microcoleus chthonoplasts*	+	+	+	+
22	*Myxosarcina concinna*	+	+	+	+
23	*Merismopedia glauca*	+	+	+	+
24	*Chroococcus turgidus*	+	+	+	+
25	*Gomphospaeria aponina*	+	+	+	+
26	*Microcystis pulverea*	-	+	+	+
27	*Microcystis lamelliformis*	-	+	+	+
28	*Microcystis flos-aquae*	-	+	+	+
29	*Synechocystis pevalekii*	+	+	+	+
30	*Gloeothece linearis*	+	+	+	+

+, Present; -, Absent

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

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