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Isolation and Identification of Mycoflora in Mangroves Ecosystem in Mumbai Region

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Abstract: *Covering a quarter of the world's tropical coastlines and being one of the most threatened ecosystems, mangroves are among the major sources of terrestrial organic matter to oceans and harbor a wide microbial diversity. In order to protect, restore, and better understand these ecosystems, researchers have extensively studied their microbiology, yet few surveys havefocused on their fungal communities. Our study indicates the presence of several species on this mycobiome that were not previously reported as mangrove-associated. In particular, we detected representatives of several commercially-used fungi, producers of secreted cellulases and anaerobic producers of cellulosomes. These results represent additional insights into the fungal community of the gray mangroves of the, and show that they are significantly richer than previously reported.*

Keywords: Aeromycoflora, Aeromicroflora, Bio allergens, Microbial study, etc

I. INTRODUCTION

The Mangrove area account for 0.7% of total tropical forest of the world. The largest extent of the mangroves is found in Asia (42%) followed by Africa (20%), North and central America (15%), Oceania (12%) and South America (11%), mangrove forests originated in southeast Asia. But they are constantly under threat and destroyed to make way for road and buildings, commercial aquaculture and by marine pollution.

Garbage has been dumped into these intertidal areas, upsetting the salinity of the seawater and choking off Mangrove tree roots knowledge about the benefits of mangroves increased significantly in Mumbai (as well as the rest of India) After the tsunami of 2004. The villages of pichavaram and muthupet in the southern state of Tamilnadu were protected by mangroves and suffered less damage than villages without this natural barrier. In 2005, the Bombay high court ruled to prevent any further destruction of the city's mangroves.It cited India's forest conservation act of 1980as well as the coastal regulation zone notification of 1991.

In 2005 data of survey of India shows an extent of 4,445 SQ. KM. Mangrove areas in India. Out of the total mangrove's acreage, 57% are found on the east coast, 23% on the west coast and the remaining 20% on Andaman and Nicobar Islands. Mangroves lies in India's eastern and western coasts. The Sundarbans, located on the delta of the river Ganges, is the world's largest mangrove forest, covering parts of west Bengal and Bangladesh. Mumbai, in the west coast of India, has between 35 and 45 SQ.KM. of mangrove forest. This is all that remains after almost 70% was destroyed in land reclamation project according to the survey conducted by a Mumbai-Based environmentalist.

The Mangrove flora of the world is resented by about 65 species. If the vivipary and breathing roots were taken into consideration, there would be 55 species in the world. The floral diversity of mangroves in India is great. In India mangroves are represented by approximately 59 species (inclusive of some mangrove associates) from 29 families, of the 59 species, 34 species belonging to 21 families are present along the west coast and 48 species belonging to 32 families are present along east coast.Having specialized root systems and other morphological adaptations, mangroves from dense forest on the shore lines, creating a secured habitat for a variety of fauna. Since mangroves are transition ecosystems, they give refuge to terrestrial marine/brackish water as well as purely intertidal organisms, making itself richly diverse ecosystem. The muddy or Sandy sediments of the mangrove forest are home to a variety of epibenthic, in faunal, and meiofaunal invertebrates.

Fungi area unit typically plant morbific. There area unit relatively few species that area unit morbific to animals, particularly mammals. in keeping with Hawksworth (1992), there area unit or so one. 5 million represented species of

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fungi. over four hundred species area unit glorious to cause illness in animals, and much fewer of those species can specifically cause illness in kinsmen. Several of them can cause solely superficial varieties of diseases that area unit a lot of a cosmetic than a pathological state. Thus, there don't seem to be several species of fungi that area unit morbific to human that may be fatal. The study of Fungi as animal and human pathogens is understood as medical phytology. There's conjointly a branch referred to as veterinary phytology however the kinds of diseases that area unit found within the pets area unit typically a similar as area unit found in kinsmen. attributable to the rarity of human diseases caused by Fungi, there's less information of such diseases. In 1674, AntonVan Leuwenhoek became the primary person to examine and describe numerous microorganisms. He continuing to watch the microorganisms till his death in 1723. In 1841, David Grubby incontestable for the primary time that a flora infection of the scalp, referred to as fungal infection, was caused by a flora (in Rippon, 1988). In 1890 Sabouraud began business enterprise giant numbers of articles on flora disorders of the skin and eventuallygave monumental contributionto the sphere ofmedical phytology. It'd not be till 1934 that species ideas of dermatophytes would be redefined by Chester Emmons, in keeping with the foundations of botanic terminology& current mycological standards of reproductive structure morphology and therefore the structures on/in that they were borne.The first case of desert rheumatism was represented in Argentina shortly before 1890; the patient suffered for seven years before finally dying and by 1915, there have been forty glorious cases of this illness, that was thought to be a rare and universally fatal. However, by now it had been already glorious that there was an illness referred to as mycosis, that wasn't associated, at that point, with C. immitis. It'd not be till Dickson (1937) that it had been accomplished that mycosis was simply a milder style of desert rheumatism, that was conjointly represented by Fiese, M. J. 1958. Dickson & Gifford (1938) closing coccidioidin diagnostic test of oldresidents of Jerome Kern County incontestable that 50-70% have, at it slow been infected by this flora.Aspergillus fumigates may be a species advanced instead of one species, its truly composed of 10 species. These species area unit unremarkably found in decaying vegetation, particularly once the latter is undergoing microbiological heating, as a result of this advanced isthermophilic, tailored to growing at high temperatures fifty - 55ºC (120 -130ºF).

II. MATERIALS AND METHODS

Isolation of mycoflora was done by using soil sample from mangrove areas. For this Nutrient Agar plates and potato dextrose plate was used.

For preparation of nutrient agar, 14gms of nutrient agar was added to 1l distilled water and the medium was sterilized at 120°*c* and 15 lbs pressure. 20 ml of sterilized NA was poured into sterile petri plates and medium was allowed to cool till solidified.

For preparation of potato dextrose agar,15gms of nutrient agar was added to 1l distilled water and the medium was sterilized at 120°*c* and 15 lbs pressure. 20 ml of sterilized NA was poured into sterile petri plates and medium was allowed to cool till solidified.

2.1 Methods

Soil samples were collected from different locations and bought to the laboratory for further detection. The suspension of soil sample was made and hence streaked using the nichrome loop on the nutrient agar plates and potato dextrose agar plates under aseptic conditions. The plates were kept for incubation at 37□C for 24 hours and results were observed the next day. The isolated colonies were observed and hence suspension was made for gram staining.The gram stained slides were observed under microscope and the fungal species was detected

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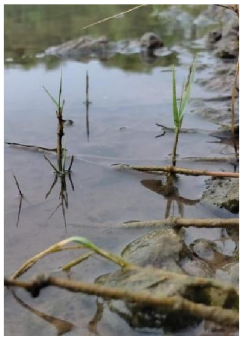
Sample Collection from Gorai beach Mangrove Zone, Mumbai


Location: 1


Location: 2


Location: 3

Photoplate.1: Collection of samples at different Zones of Mangrove

III. RESULT AND DISCUSSION

Table 1: Characters and Identification of Fungal Organisms

Sr. No.	Characters		Name of the Organism
	Mycelium	**Spores / Conidia**	
1	Colonies olive black, velvety.	Conidiophores short, simple, unbranched; conidia forming branched chain of 2 to 10 with 3 to 8 transverse septa in each; conidia golden brown, pale, 20-63 X 9-13µm.	*Alternaria alternata*(Fr.) Keissl., *Beihefte Bot. Centralbl.,* Abt. 129(2): 434(1912). Ellis, p. 466 (1971).
2	Colonies brown at first but turning into black.	Conidiophores course, Head varying in size, biseriate but some having phialides borne directly on the vesicle, Phialides 7 – 10 X 2 – 2.5 µm, Conidia globose or sub globose, sometimes elliptic, 3 – 6 µm in diameter formed in chains giving rise to ornamented conidia.	*Aspergillus carbonicus*Gallo, A. et. al. *Int. J. Food Microbiol.* 179, 10-17 (2014).
3	Colonies yellow at first but turning into bright to dark yellow green.	Conidiophores course with length of 1mm & diameter of 19 – 20 µm, Head varying in size, loosely radiate / splitting / columnar, biseriate but some having phialides borne directly on the vesicle, Phialides 7 – 10 X 2 – 2.5 µm, Conidia globose or sub globose, sometimes elliptic, 3 – 6 µm in diameter	*Aspergillus flavus* (Raper and Fennell, 1965; N. K. Udaya Prakash, 2004)
4	Growth spreading, dark smoky green, more or less velvety, Young heads bluish green, Conidial heads columnar with varying length,	Conidiophores smooth, short, often greenish, 2–8 µm diameter, Vesicles flask shaped, fertile on upper half of / 3 quarters, often greenish, phialides borne directly on vesicles, closely packed, lower ones deflected upwards, 6 – 8 X 2 – 3 µm. Conidia small, globose, smooth, mostly 2.5 – 3 µm in diameter	*Aspergillus fumigatus* (Raper and Fennell, 1965; N. K. Udaya Prakash,2004)
5	Colonies light green, smooth, velvety; developing dirty white patch from the center outwards; reverse deep red to purple;	Conidial heads columnar, short, brown, with distinct foot cells, usually short, phialides biseriate, conidia globose, rough about 2.5 – 4 µm in diameter	*Aspergillus nidulans* (Raper and Fennell, 1965; Raper, 1966b; Clutterbuck, 1974)

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6	Colonies spreading rapidly, with mycelium white to dark brown, black to purple heads, Conidial heads globose, radiate	Conidiophores arise from substratum varying from 200 µm to several mm long, 10 – 20 µm in diameter, smooth, vesicles globose, phialides borne directly on the vesicle or metulae present, metulae vary in length 10 – 15 µm; Conidia small, globose, rough, 4 - µm in diameter	*Aspergillus niger* (Raper and Fennell, 1965)
7	Colonies yellowish to orange brown, reverse orange red to maroon.	Conidial heads few, scattered; pale, grey, green in colour; conidiophores hyaline, thin walled; vesicles about 21-27 X 15-18 µm; phialidsuniseriate; conidia elliptical, spinulose, orange brown in colour.	*Aspergillus ruber* Thom & Church, *The Genus Aspergillus:* 112 (1926).
8	Colonies grayish black; diffused.	Conidiophores solitary, straight; pale, brown, large, dark; conidia typically curved, navicular; olive brown with pale ends; mostly 35-45 µm long and 20-24 µm wide.	*Bipolarispapendorfii* (Aa) Alcon, *Mycotaxon*17: 68 (1983); Ellis, p.413 (1971).
9	Colonies white to cream coloured, slow growing, smooth; mycelium hyaline, submerged; pseudohyphae and true hyphae also seen.	Budding cells (blastoconidia) of varying shapes; usually round or short oval, 2.8-10.5 µm in diam; chlamydospores round, large, thick-walled and usually terminal.	*Candida albicans* (C.P. Robin) Berkhout, *De Schimmelgesl. Monilia, Oidium, OosporaenTorula, Disset. Ultrecht: 44 (1923);* Watanabe, p. 212 (2002).
10	Colonies golden yellow with production of ample conidial masses; ascomata globous, dark brown without peridial hairs.	Ascospores brown, smooth walled, ellipsoidal, formed singly, spherical, nearly hyaline, thin-walled.	*Corynacussepedonium*(C.W.E mmons) Arx, *Proc. K. Ned. Akad. Wet., Ser. C, Biol, Med. Sci.* 76(3):292(1973); Domsch et.al., Vol.1.p.232(1980).
11	Colonies dark grey, velvety with branched, septate mycelium.	Conidiophores long; conidia elliptic curved; septa usually 3 with 3rd cell broader and darker than the others.	*Curvularialunata*M. B. Ellis, *Mycol. Pap.* 106:34(1966).
12	Colonies yellowish brown with similar colour on reverse.	Sporodochia pulvinate, visible as black dots; conidia globose usually 15 to 25 µm in diam.	*Epicocum nigrum* Link., *MagazinGes. naturf. Freunde, Berlin* 7:32(1815); Ellis p. 72(1971).
13	Colony peach colored, conidiogenous cells hyaline, enteroblastic, mono or polyphialidic	Macroconodia abundant, typically falcate with foot cell, tapering at both the ends, 4 septate	*Fusarium equisetii* (Booth, 1971; John Webster 1980; Barnett and Hunter, 1987)
14	Colony salmon pink colored, conidiogenous cells hyaline, enteroblastic, mono or polyphialidic.	Straight, tapering, fusiform, 5 septate macroconidia	*Fusarium moniliforme* (Booth, 1971; John Webster, 1980; Barnett and Hunter, 1987)
15	Colonies white with purple violet tinge; reverse dark purple.	Conidiophores unbranched; microconidia abundant, ellipsoidal; macroconidia 2 to 5 septate, fusiform curved.	*Fusarium oxysporum* Schlecht, *Flora Beroliensis*2:139(1824).
16	Colonies thick olive green colour with reddish brown reverse.	Conidiophores formed on surface usually terminal; conidia ellipsoidal and smooth.	*Penicillium chrysogenum* Thom, *Bull. Bur. Anim. Ind. US Dep. Agric.*

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			118:58(1910); Pitt, p. 328 – 331 (1979).
17	Mycelium with no obvious pattern, whitish in colour.	Chlamydospores abundant, delimited from mycelium with septum, usually yellowish brown in colour; sporangia abundant, ellipsoidal with a prominent papilla.	*Phytophthora palmivora*(E. J. Butler) E. J. Butler, *Science Rep. agric. Res. Inst. Pusa:*82 (1919)
18	Mycelium with abundant branching, thread like curve.	Sporangia spherical terminal; conidia colourless terminal with thick wall.	*Pythium debaryanum* R. Hesse, *Inaug. Diss., Halle:*14-34(1874); Gilman, p.158(1957); Waterhouse, p.19-20(1968).
19	Presence of the rhizoids at the base of sporangiophores, Stoloniferous habit, an aerial hypha grows out and where it touches the substratum it bears rhizoids and sporangiophores. The growth is repeated.	Sporangiophores in groups from stolon, opposite to rhizoids; sporangium spherical, brown with well-developed Columella	*Rhizopus stolonifer* (N. K. Udaya Prakash, 2004)
20	Colonies loose, white turning greenish on maturation.	Conidiophores branched; conidia conspicuously rough, globose; bluish green in colour.	*Trichoderma viride*Pers., *Syst. Mycol.* (Lundae) 3:215 (1794); *Mycol. Pap.* 116: 1-56(1969); Subramanian, p. 653-655 (1971).

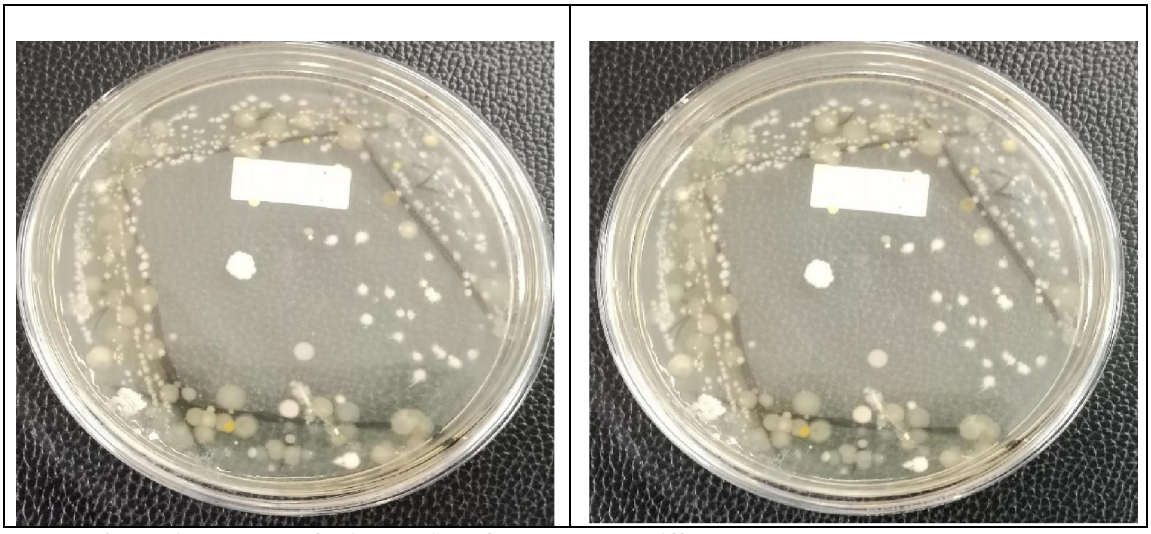

Photo Plate 2: Petri plates kept for incubation after exposed at different beach

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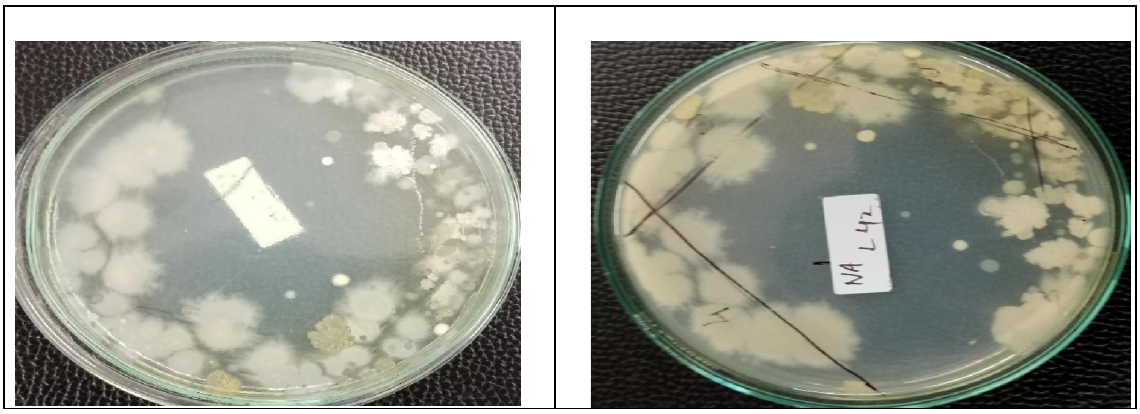
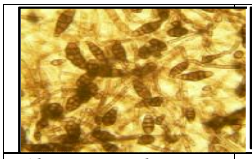
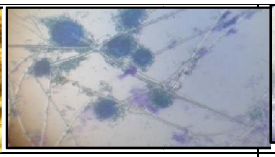
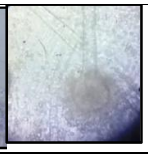
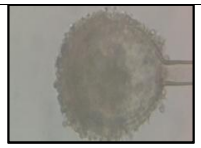
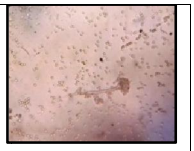
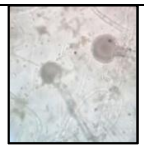
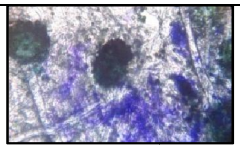
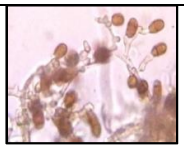
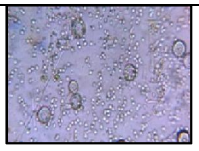
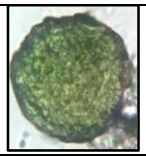
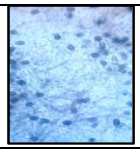
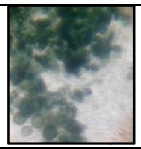
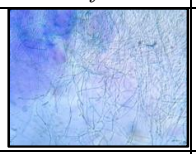
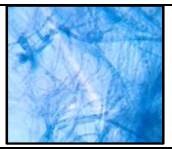
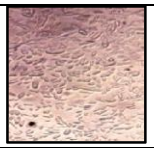
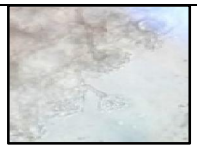
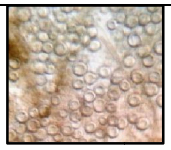
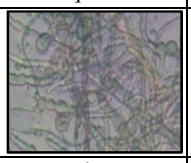


Photo Plate 3: Colony Cultures of the Fungi

				
Alternaria alternata	*Aspergilluscarbonicus*	*A. flavus*	*A. fumigatus*	*A. nidulans*
				
A. niger	*A. ruber*	*Bipolarispaperd orfii*	*Candida albicans*	*Corynacusseped onium*
				
Curvularialunata	*Epicoccum nigrum*	*F. equisetii*	*F. moniliforme*	*F. oxysporum*
				
P. chrysogenum	*Phytophthora palmivora*	*Pythium debaryanum*	*R. stolonifer*	*Trichoderma*

Photoplate 4: Microscopic Characters of the Fungi

Table 2: Characters and Identification of Bacteria

Sr. No	Colony Characteristic	Family	Organism
1)	Round, smooth, convex, glistening with entire edge. *S. aureus* from cattle, human and other domestic animals produces golden yellow coloured colonies in nutrient agar.	*Micrococcaceae*	*Staphylococcus aureus*

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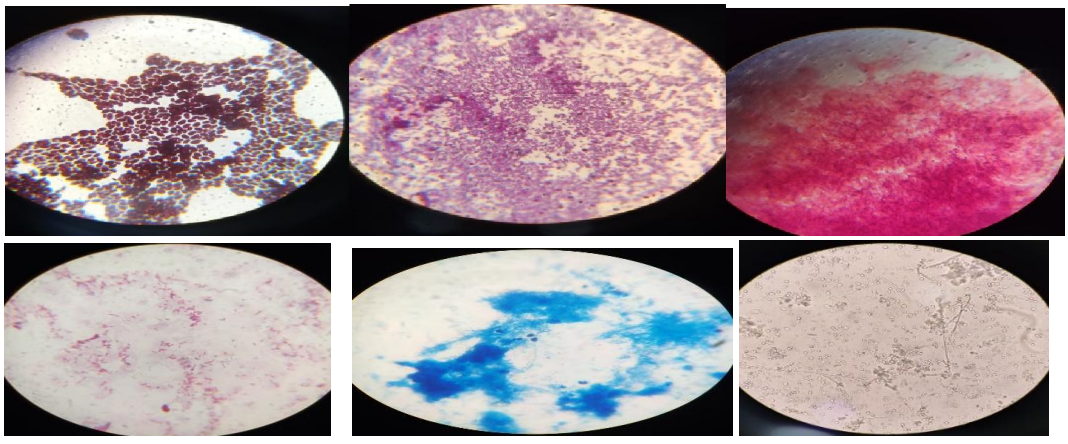
2)	Dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish color and have a diameter of ≥ 0.5 mm, and are surrounded by a zone of β-hemolysis that is often two to four times as large as the colony diameter.	*Enterococci*	*Streptococcus pyogenes*
3)	Off-white or beige in color with a shiny texture. It often looks like mucus or a cloudy film over the whole surface of the plate. An E. coli colony is slightly raised and has an entire, fixed margin and a steady growth pattern, creating concentric growth rings in the colony.	*Enterobacteriaceae*	*Escherichia coli*
4)	Rod-shaped and Gram-positive, when cultured on ordinary nutrient agar, the morphology circular colony of this bacteria is rough, opaque, fuzzy white or slightly yellow with jagged edges.	*Bacillaceae*	*Bacillus sp.*
5)	Gram-negative, oxidase negative, catalase positive, citrate positive, indole negative, rod-shaped bacterium. The bacterium is approximately 1-3 microns in length, and is capable of motility via peritrichous flagella.	*Enterobacteriaceae*	*Enterobacter aerogenes*
6)	Small Gram-negative rods, 0.3 - 1µm in diameter and 1 - 6µm in length, appearing singly, in pairs and in chains. Shigella species are facultative anaerobes and are non-spore formers. Shigella species do not possess flagella and hence are non-motile.	*Enterobacteriaceae*	*Shigella sp.*



Photoplate 4: Microscopic Characters of the Bacteria

IV. DISCUSSION

This was in accordance with the research of Yulma et al., that there Werebacteria's genus found in sediments in the Mangrove, namely Bacillus, Enterobacteria, Listeria, Micrococcus. Bisset et al. stated that the diversity of sedimentary microorganism communities was very high. This is due to the content of sediments Supporting and assisting in the formation of aerobic and anaerobic Microenvironment cooperation. For example, a decrease in oxygen levels due to

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microorganism activity in a room rich in organic matter will form an anaerobic microenvironment that supports facultative and obligate anaerobic microorganism activities. This causes the emergence of groups of microorganisms with certain specific Physiological properties that are in accordance with the conditions of the microenvironment.

V. CONCLUSION

Mangrove regions are unique swampy regions with water region being alkaline in nature and sediment or soil region having a neutral to slightly acidic pH. Since mangrove environment is prevalent to stress conditions such as salt stress, microorganisms growing under such stress conditions could have a potential for bioremediations programmes. The soil isolates were halo-tolerant and could tolerate relatively high concentrations of heavy metals. Mangroves are saline coastal ecosystem rich in Carbon and other nutrients. They harbor large numbers of population of unique bacteria. The present study reveals the mixed population of bacteria of Gorai mangroves area. Further studies on these organisms and more evaluation of their stress tolerance could make them applicable for various industrial applications.

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