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STUDY OF FUNGAL DIVERSITY OF SOME SELECTED NATURAL SPOT OF EAST KOLKATA WETLAND

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Abstract: *Biological diversity - or biodiversity - is a term we use to describe the variety of life on Earth. Microbes are one of the dominant life forms in the earth. Their contribution to the earth and human being is beyond the imagination. Their science is concerned with their form, structure, reproduction, physiology, metabolism and classification. It also includes their distribution in nature, their relationship with each other and other living organisms, their effects on human beings, other animals and plants. Biological diversity (biodiversity) encompasses the variety of life forms occurring in nature, from the ecosystem to the genetic level, as a result of evolutionary history (Wilson 1992). Microbial Diversity is an integral part of biodiversity which includes bacteria, archaea, fungi, algae, protozoa and protests. Fungi constitute a major portion of natural resources likely to provide innovative applications useful to man. Fungi are one of the major sources of antimicrobial agents and produce a wide range of other important medicinal compounds, industrially important biomolecules, novel enzymes, insecticides the microbial level is beginning to be recognized, but this richness of diversity amongst bacterial, fungal and virus species has yet to be catalogued particularly in West Bengal. The East Kolkata Wetland (EKW) is situated at 880 20' E - 880 35' E and200 25' N -200 35' N. Climate here is more or less sub-tropical with the annual mean rainfall around 200 cm. The maximum temperature during summer rises around 39^0 C. while minimum temperature during winter is around 10^0 C. The average temperature during most part of the year is around 30^0 C during day time with a fall in temperature of 5^0-6^{0}C at night. East Kolkata Wetland shows an immense diversity of flora and fauna both at the macro and micro level. Microbial richness of a region is its unseen asset that needs to be explored and conserved. Soil samples collected from East Calcutta Wetland shows the presence of various new strains of microbes which are not only ecologically important but also have commercial value. Isolation, characterization, documentation and conservation of these resources are important considering their strategic importance for future generation as well as complimentary economic growth and prosperity. In this present work several fungi were isolated and purified from diverse area of East Calcutta Wetland out of which about 10 organisms was identified by microscopic studies. Among the isolates it is expected that one or two new genus may obtained.*

Key Words: *East Kolkata Wetland, Biodiversity, Fungal Biodiversity, Isolation*

Introduction

Microbial Diversity is an integral part of biodiversity which includes bacteria, archaea, fungi, algae, protozoa and protists. East Kolkata Wetland shows an immense diversity of flora and fauna both at the macro and micro level. Microbial richness of a region is its unseen asset that needs to be explored and conserved. Soil samples collected from ECW shows the presence of various new strains of microbes which are not only ecologically important but also have commercial value. They are capable of degradation of toxic chemicals like nitrophenol, nitroaromatic compounds,

pesticides etc. , bioremediation of heavy metals, oil contaminated soil and toxic compounds , degradation and recycling of woody tissues of plants, and nitrogen fixation along with the cyanobacters; other bacteria playing important roles in metal accumulation, oil degradation, antimicrobial compound production, enzyme production etc.

Fungi constitute a major portion of natural resources likely to provide innovative applications useful to man. Fungi are one of the major sources of antimicrobial agents and produce a wide range of other important medicinal compounds, industrially important biomolecules, novel enzymes, insecticides the microbial level is beginning to be recognized, but this richness of diversity amongst bacterial, fungal and virus species has yet to be catalogued particularly in West Bengal. Isolating, culturing and cataloguing of fungi are a daunting task and started recently with the development of new technology. But microbial diversity including fungi is one of the difficult areas of biodiversity research. Extensive exploration is required for understanding the biogeography, community assembly and ecological processes which will be for isolating and identifying the fungi, vitamins immunosuppressant and immune modulators. The enormous diversity available at.

The biological diversity of the Indian subcontinent is one of the richest in the world. India is recognized as one of the 12 mega diversity regions of the world. Nearly 72% of India's bio-wealth is constituted by fungi (~18%), insects (~40%) and angiosperms (~13%). Thus, India's contribution to the global diversity is around 8%. The most important mega-diversity centers are Western Ghats, Northeastern hill regions, Andaman Nicobar islands, mangrove forests of Sunderban area, silent valley of Kerala, Chilka lake of Orissa, Sonar Lake of Maharashtra , the Himalayan region, East Kolkata Wetland etc. India's rich microbial diversity (14,500 species of fungi, 2000 lichens, 17,000 flowing plants are currently known), has not been adequately enumerated and catalogued. Apart from this there is no such recognized data bank of microbial resources specially on bacteria and fungi. Very few works has been done in the field microbial biodiversity in West Bengal. So it might be an important work for future to work in this field.

The East Kolkata Wetlands (EKW), located on the eastern fringes of Kolkata city is one of the largest assemblages of sewage fed fish ponds spread over an area of 12,500 ha. These wetlands form a part of the extensive inter-distributory wetland regimes formed by the Gangetic Delta. EKW sustains the world's largest and perhaps oldest integrated resource recovery practice based on a combination of agriculture and aquaculture, and provides livelihood support to a large, economically underprivileged population of around 20,000 families which depend upon the various wetland products, primarily fish and vegetables for sustenance. Based on its immense ecological and socio cultural importance, the Government of India declared EKW as a Wetland of International Importance under Ramsar Convention in 2003. The wetland system currently produces over 15,000 MT per annum from its 264 functioning aquaculture ponds, locally called bheries. Additionally, nearly 150 MT of vegetables are produced daily by subsistence farmers. Needless to say, EKW serves as the backbone of food security of Kolkata City. EKW is a classical example of harnessing natural resources of the wetland system for fisheries and agriculture through ingenuity of local communities with their traditional knowledge.

There are some reports on microbial biodiversity at national and international level. The biota of marine microorganisms has developed unique metabolic and physiological functions that not only ensure survival in extreme habitats, but also offer a potential for the production of novel enzymes for potential exploitation. Mangrove ecosystem is nutritionally very rich and widely diverse group of organisms can survive in this extreme habitat. Out of the large number of species examined, only a fraction of marine bacteria have been isolated and cultured. Among them, alkaliphilic *Bacillus* strains are of considerable importance in biotechnological applications (Fritze et al., 1990, Kumar. and Takagi, 1999, Kumar et al. 2004). A novel *B. lehensis* (MLB2 (T)) was reported recently

from Leh region, Jammu and Kashmir and *B. licheniformis* SPT27, a producer of extracellular alpha amylase was isolated from the alkaline soil of the eastern coastal region of Bombay, Gujarat. However, in an extensive survey of microbial diversity at marine salterns near Bhavnagar, Gujarat, no *Bacillus* sp. was documented (Ghosh, et al. 2007, Aiyer, 2004, Deve, and Desai, 2006).

Pushpangadan and Narayanan (2001) made attempt to organize systematics and biodiversity research in India. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates was studied by Parthasarathi et al. (2007). They isolated and identified the strains able to fix nitrogen, produce extracellular enzymes like protease, cellulase, xylanase, and amylase, and solubilize inorganic phosphates. Molecular analysis of microbial diversity of Lonar soda lake (Indian Soda Lake) was analysed by Wani et.al. (2006). Bacterial diversity of East Calcutta wetland and their possible identification was done by Ghosh et.al. (2007).

Surajit Das et al. (2006) reported about the marine microbial diversity & its importance. Halobacteria (Halococcus) isolated from mangrove sediments produce L-asparaginase etc. Joshi et al. (2008) reported the cultivable bacterial diversity alkaline Lonar Lake, India. Most of their isolates produced biotechnologically important enzymes at alkaline pH, while only two isolates (ARI 351 and ARI 341) showed the presence of polyhydroxyalkcanoate (PHA) and exopolysaccharide (EPS), respectively.

East Calcutta Wetland is low lying area of about 12500 hectares on the eastern region of Calcutta. It is acting as a natural sewage treatment plant to the city and side by side generates product like paddy, vegetables and fish utilizing the sewage. It receives effluents from domestic activities, industries, tanneries, battery manufacturing units as well as health sectors. The purification of the waste products is mainly based on microbial activity. The hot and humid climate all throughout the year favors this site to act as an incubator for diverse group of microbes. Thus the site was selected to explore wide variety of microbes which can be applicable in biotechnology and bioremediation. Bacterial diversity of East Calcutta wetland and their possible identification was done by Ghosh et.al. (2007).

Main objective is to isolate the biologically diverse group of fungi from locality of selected natural spot of East Kolkata wetland of South 24-parganas. The purification and indexing of the isolates will be done thereafter. The microbes will be preserved for future use. The identification and cataloguing of the isolated microbes will be done. As there is no such report on the micro fungi of above said area, this study will help to prepare a catalogue/data bank of total fungi including micro fungi of the selected area.

Materials and methods

Slide were prepared by staining the mycelium by cotton blue dye and observed under oil immersion microscope. Identification of the microbes have done morphologically by light microscopy, Phase contrast Microscopy and 3-D Transmission electronic microscope (model no-TM1500) and picture available in **Pictorial Atlas Of Soil and Seed Fungi (**Author- Tsugeo watanabe)

Isolation of soil sample:

Soil samples were collected from different corners of at least one meter distance from 6 inches depth and mixed well and put in sterile plastic containers. These were then shifted to the laboratory for further analysis.

Soil sample were collected from the localities like Goltala fishery , Natar very , Bantala tannery , Bamanghata , Natar vari ala , Khasmahal , Kumarpukuria , Thardha(hargar) , Dhapa , Gadakhali , Matla bridge (canning) , Sonakali bridge , Motghara , Purbaballatta etc. from EKW area.

Isolation of fungi:

Fungus were isolated by 10 gms dry soil in 90 ml sterile distilled water, shake well for 15 minutes and kept for one hour for settling. After that serial dilution were made

in 9 ml sterile distilled water. 0.1 ml sample were poured in agar plates and spread well by glass spreader and kept in incubator at 32^0 C.

Optimization of the growth media were done by different media like Potato Dextrose Media, Molt Agar Media and Czapek-Dox agar media and Czapek-Dox agar media was found to be most suitable. Cultivations were made on both still culture or shaking culture methods. Purifications were done by tube dilution methods.

Pure culture preparation of isolated fungi:

Organisms were Purified by serial dilution method and inoculated in a slant (Fig-1).

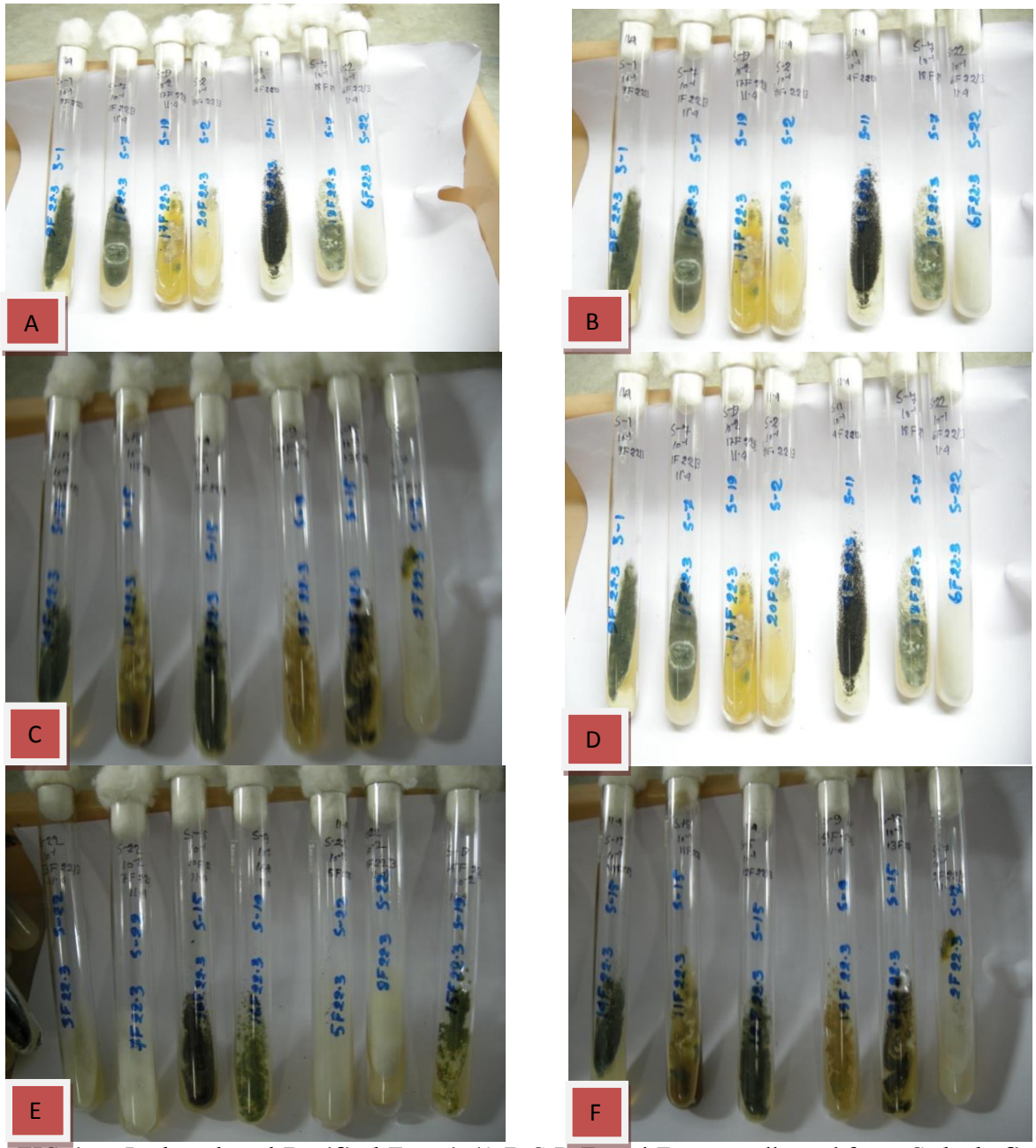

FIG-1 : Isolated and Purified Fungi (A,B,C,D,E and F were collected from **Goltala fishery, Natar very , Bantala tannery, Bamanghata , Khamahal, Natar vari ala,) ..**

Identification of some isolated fungi:

Slides were prepared from petridishes and Pictures were taken in 3D Transmission Electronic Microscope (Model No.-TM 1500,).

Based on microscopically examination (3-D Transmission Electronic Microscope Model No - TM1500) and picture available in **Pictorial Atlas of Soil and Seed Fungi (**Author- Tsugeo watanabe) some fungi are identified (Table-3, Fig-2 and Fig-3).

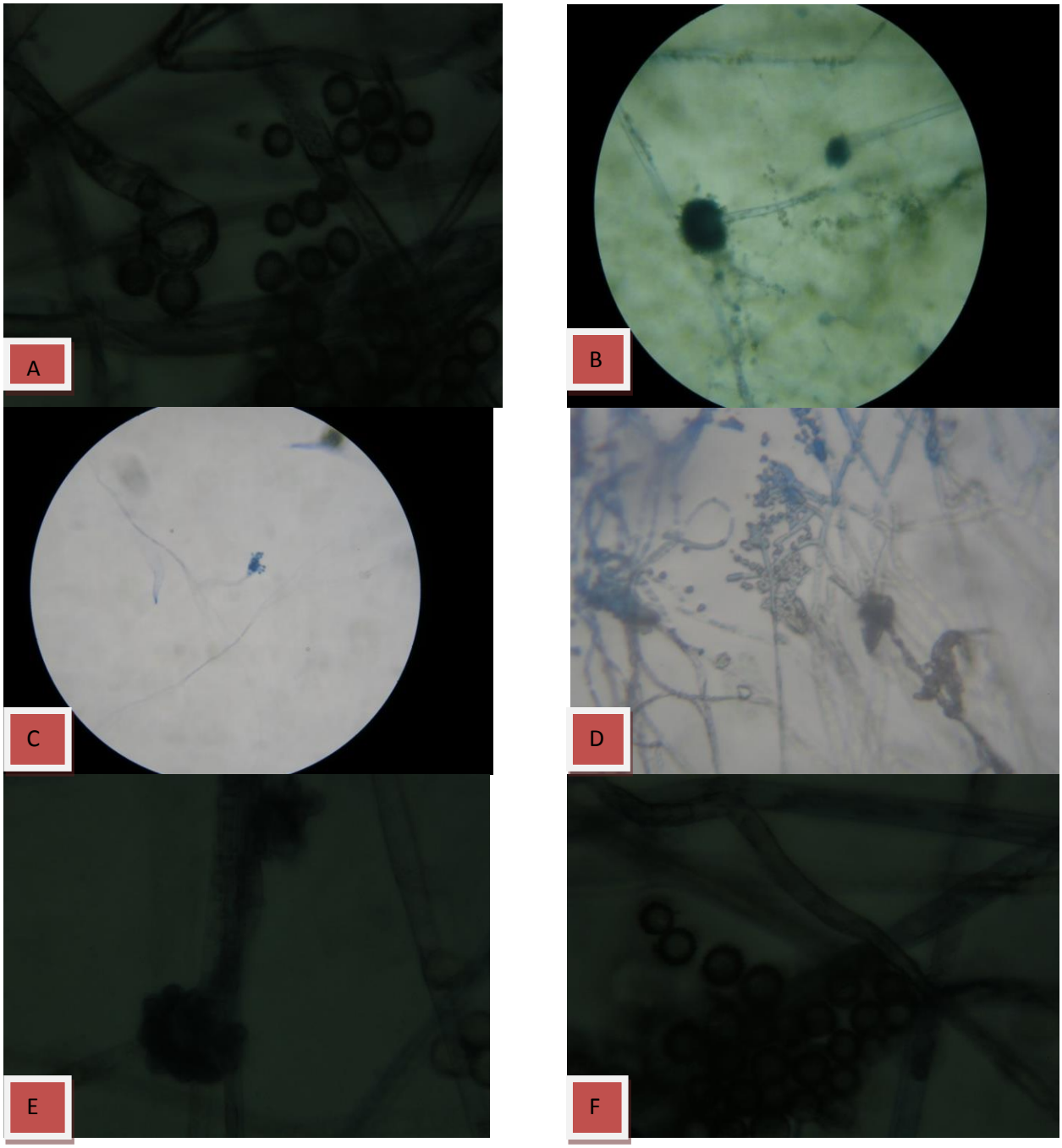

FIG-2: [A- 3F4.4 (adakhali fari ghat); B- 16F4.4 {Malta bridge (canning)}; C 5F4.4 (Gadakhali fari ghat); D- 7F4.4 (Gadakhali fari ghat); E- 17F4.4 (Malta bridge (canning); F- 14F4.4 (Gadakhali near sundori tree).]

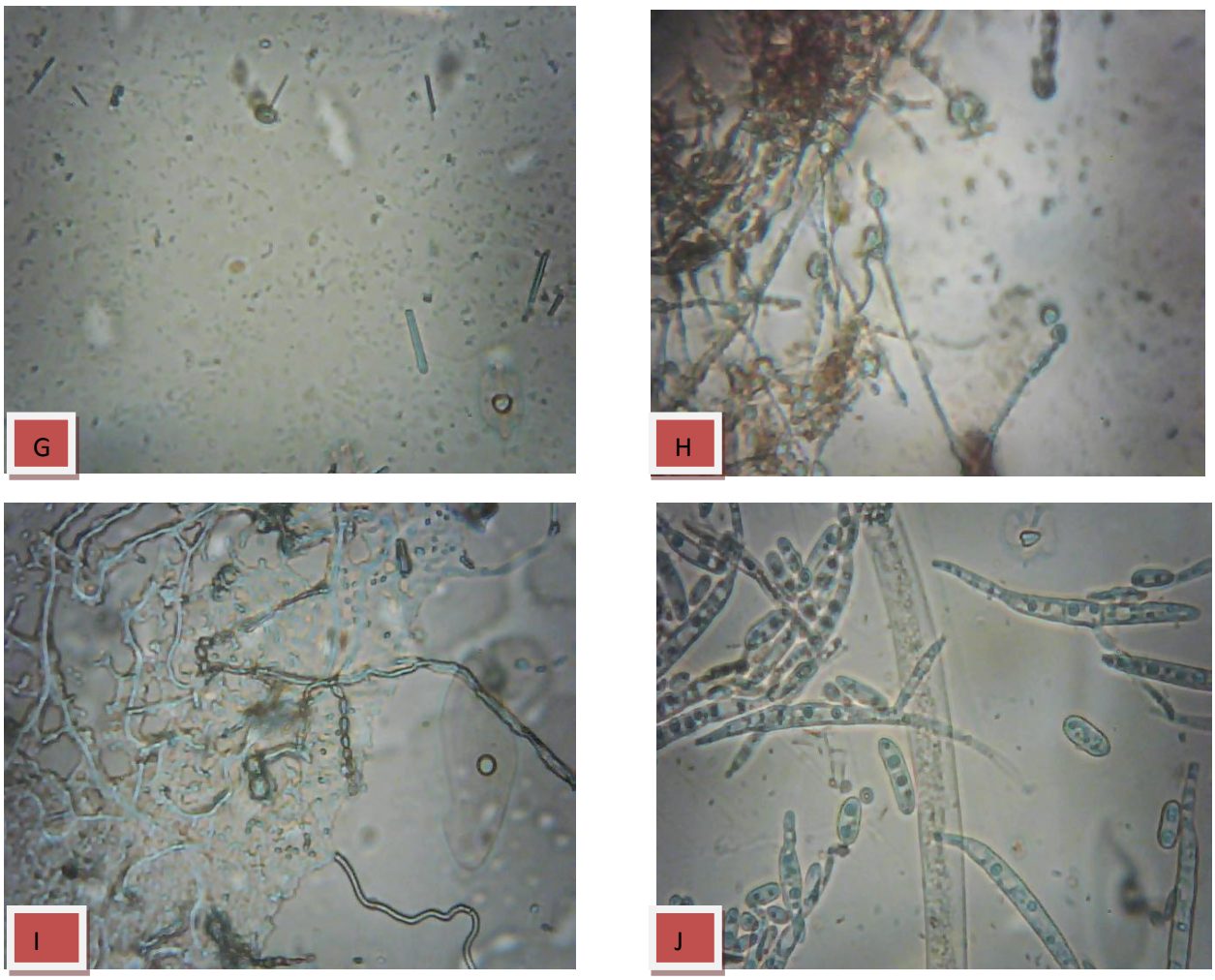

FIG-3: [G- 26F4.4 (Motghora); H- 30F4.4 (Gadakhali); I- 23F4.Motghora); J- 20F4.4 {Motghora (pond side)}].

Result and Discussion

More than hundreds of soil samples were collected from different locality of EKW. About three hundreds of visually different fungi were isolated from the agar plates and were purified by dilution method. They were then studied under microscope after preparation of the slides.

The pH was measured by dissolving 10 gms dry soil in 90 ml sterile distil water, shake well for 15 minutes and kept for one hour for settling. pH was measured by pH-meter. Visually different fungi were isolated and catalogued (Table-1). Colony characters were recorded (Table -2) Microscopic examinatios were done after slide preparation and probable identification were recorded in Table -3.

Table – 1
Plate count and pH record of the isolates

Soil Sample No	Locality	Soil Type	pH	Number of fungi at					
				10^2 Dilution			10^3		
				72h	96h	120h	72h	96h	120h
S-7	Bantala (fishery fedding cannel)	Wet	5.2	4	7	12	2	4	6
				6	10	15	4	5	7
S-9	Kumarpukuria (rice cropland)	Dry	6.0	2	4	5	1	2	3
				1	3	4	0	1	3
S-11	Kumarpukuria (swage cannel)	Wet	5.8	1	3	5	1	2	3
				2	3	6	0	1	3
S-13	Thardha PS-Bhargar	Wet	6.9	1	2	4	0	1	2
				0	1	3	0	0	1
S-10	Kumarpukuria (Besides fiishary)	Wet	6.2	2	4	5	1	2	4
				0	2	3	0	1	2
S-1	Dhapa (decomposed garbage)	Wet	6.3	4	6	10	2	4	7
				5	6	11	1	4	6
S-5	Bantala (Besides sewage desposal cannel)	Wet	6.5	1	5	7	1	3	5
				2	6	8	1	2	4
S-3	Goltala fishery	Wet	6.5	1	3	5	0	1	3
				0	1	3	0	0	1
S-2	Natar veri	Wet	6.8	0	3	5	0	1	2
				1	3	7	0	2	4
S-20	Bantala tannery chemical contaminated field	Dry	7.0	2	5	9	1	3	5
				3	7	11	1	4	6
S-19	Bantala tannery field soil	Dry	6.5	3	4	7	1	2	4
				2	3	4	1	1	2
S-15	Bamanghata waste water cannel side	Wet	6.5	4	7	14	2	5	9
				3	6	11	1	4	7
S-1	Natar variala (fishery ponds side)	Wet	6.2	0	1	3	0	1	2
				0	1	2	0	1	1
S-22	Bantala tannery chemical waste cannel side	Wet	6.5	4	7	13	2	5	9
				6	9	15	3	7	11
S-11	Khamahal	Dry	6.0	1	4	5	0	3	5
				2	5	7	1	4	4
S-21	Bantala tannery	Wet	8.3	0	1	2	0	1	1
				0	1	3	0	0	1
S-31	Gadakhali near fari ghat	Dry	6.5	2	6	9	4	6	7
				1	4	7	1	4	6
S-25	Gadakhali fari ghat	Dry	6.3	2	10	12	2	8	11
				1	5	8	1	5	7
S-33		Wet	6.0	2	4	6	0	2	4

	Gadakhali, near sundari tree			1	4	6	1	3	5
S-42	Matla bridge (canning)	Dry	6.7	2	2	3	0	1	3
				0	1	2	0	1	2
S-38	Sonaakhali Bridge	Dry	6.5	0	1	2	0	1	2
				0	3	5	0	1	1
S-34	Motghara pond side	Wet	6.0	13	20	26	0	2	3
				8	15	21	0	1	3
S-41	Malta Bridge canning	Wet	6.5	2	5	8	0	0	1
				0	1	2	0	0	0
S-36	Motghara	Dry	5.5	8	20	32	4	6	10
				20	27	35	11	17	26
S-39	Purbaballatta pond side	Wet	5.5	9	16	21	0	1	3
				7	15	18	0	2	4
S-24	Gadakhali near fari ghat	Wet	6.5	10	22	28	2	5	6
				15	17	20	0	1	3

Table - 2
Colony Morphology

Sample no	**Isolate no.**	**Appearaence in slant**	**Colour**		**Colour change**	**Morphology**	**Growth Rate**
			Colony colour	**Slant back side colour**			
S-7	2F3.11	Velvet like	Deep green	Yellow	1st light green then deep green	Mycelium growth	High
S-9	8F3.11	Powder like	Brown	Black	_	Mycelium growth	Slow
S-7	3F3.11	Powder like	Black	White	_	Mycelium growth	High
S-11	10F3.11	Powder like appearance	Green	Brown	_	Mycelium growth	Slow
S-9	6F3.11	Velvet like	Blackish green	Black	1st green then black green	Mycelium growth	Slow
S-7	5F3.11	Velvet like	Light brown	Deep brown	_	Mycelium growth	Slow
S-13	12F3.11	Powder like	Brown	White	_	Mycelium growth	High
S-9	7F3.11	Powder like	Deep brown	Light brown	_	Mycelium growth	Slow
S-11	11F3.11	Powder like	Black	Brown	_	Mycelium growth.	High
S-11	9F3.11	Cotton like	White	Yellow	_	Mycelium growth	Profuse

S-7	1F3.11	Cotton like	Green	Yellow	-	Mycelium growth	High
S-7	4F3.11	Powder like	Green	Brown	_	Mycelium growth	High
S-22	8F22.3	cotton like appearance	White	Off white	1st milky white after that off white	Mycelium growth	Profuse
S-15	10F22.3	Velvet like appearance	Brown	White	_	Mycelium growth	Slow
S-7	2F22.3	Cotton like appearance	Off white	Brown	_	Mycelium growth	Slow
S-7	1F22.3	Powder like appearance	Green	Black spot	1st yellow then green	Mycelium growth	High
S-22	6F22.3	Cotton like	White	White	_	Mycelium growth	High
S-22	3F22.3	Cotton like	Off white	Brown	_	Mycelium growth	High
S-22	7F22.3	Cotton like	Light green	Deep brown	_	Mycelium growth	Slow
S-9	15F22.3	Velvet like	Green	White	_	Mycelium growth	Slow
S-19	17F22.3	Velvet like	Brown	Deep brown	_	Mycelium growth	Slow
S-1	9F22.3	Powder like	Blackish dgreen	Brown	_	Mycelium growth	Slow
S-15	11F22.3	Velvet like	Brown	Brown	-	Mycelium growth	Slow
S-15	13F22.3	Velvet like	Chacolet	White	_	Mycelium growth	Slow
S-7	18F22.3	Velvet like	Green	Brown	_	Mycelium growth	Slow
S-2	20F22.3	Cotton like	White	Brown	_	Mycelium growth	Profuse
S-15	14F22.3	Cotton like	Green	White	_	Mycelium growth	Slow
S-11	4F22.3	Powder like	Black	White	_	Mycelium growth	Slow
S-22	5F22.3	Cotton like	White	White	_	Mycelium growth	High
S-15	12F22.3	Velvet like	Green	Off white	_	Mycelium growth	Slow
S-25	4F4.4	Powder like	Yellowish green	White	1st yellow then green	Mycelium growth	Slow
S-25	`3F4.4	Cotton like	Off white	Light pink	-	Mycelium growth	High
S-25	3F4.4	Cotton like	Yellowish white	Light yellow	_	Mycelium growth	High
S-25	1F4.4	Powder like	Brown	White	_	Mycelium growth	High
S-31	10F4.4	Velvet like	Green	White	_	Mycelium growth	Slow
S-42	17F4.4	Cotton like	Yellow	Yellow	_	Mycelium growth	Slow
S-31	6F4.4	Cotton like	Black	Black	_	Mycelium growth	High

S-38	15F4.4	Cotton like	Gray	Black	1st white then gray	Mycelium growth	Slow
S-33	13F4.4	Velvet like	Green	Yellow	_	Mycelium growth	High
S-42	16F4.4	Cotton like	Milky white	Brown	_	Mycelium growth	High
S-31	7F4.4	Powder like	Deep brown	White	_	Mycelium growth	High
s-31	8F4.4	Velvet like	Grey	green	-	Mycelium growth	Slow
s-33	12F4.4	Cotton like	Milky white	black	-	Mycelium growth	High
s-25	5F4.4	Powder like	Green	white	-	Mycelium growth	Slow
s-33	14F4.4	Powder like	Black	black	-	Mycelium growth	High
s-31	9F4.4	Powder like	Grey	white	-	Mycelium growth	High
s-33	11F4.4	Velvet like	Milky white	Light	-	Mycelium growth	Slow
s-24	18F4.4	Powder like	Green colour with white margin	white	First white then green	Mycelium growth	Slow
s-34	19F4.4	Velvet like	Green	white	-	Mycelium growth	High
s-34	21F4.4	Powder like	White	white	-	Mycelium growth	Slow
s-34	22F4.4	Powder like	Light green	Light yellow	-	Mycelium growth	Slow
s-34	20F4.4	Cotton like	White	Deep brown	-	Mycelium growth	Slow
s-41	27F4.4	Powder like	brown	Deep brown	First yellow then brown	Mycelium growth	Slow
s-36	23F4.4	Cotton like	Yellowish white	Yellowish white	-	Mycelium growth	Slow
s-36	24F4.4	Powder like	Grey	white	-	Mycelium growth	Slow
s-36	26F4.4	cotton like	Milky white	white	-	Mycelium growth	High
s-36	25-F4.4	Velvet like	Green with yellow margin	Light brown	First yellow then green	Mycelium growth	High
s-33	31F4.4	Cotton like	White	Light brown	-	Mycelium growth	High
s-33	30F4.4	Velvet like	Red	red	-	Mycelium growth	Slow
s-31	28F4.4	Velvet like	Yellowish green	brown	First yellow then green	Mycelium growth	High
s-31	29F4.4	Velvet like	Black	black	-	Mycelium growth	Slow

Table – 3
Probable Identification

Soil sample no.	Collection spot	Isolate no.	Morphological character	Possible identified genus
S-25	Gadakhali fari ghat	3F4.4	Mycelium attached with conidiophores	*Aspergillus sp.*
S-25	Gadakhali fari ghat	5F4.4	Mycelium attached with conidiophores	*Penicillium sp.*
S-31	Gadakhali	7F4.4	Mycelium attached with conidiophores	*Penicillium sp.*
S-33	Gadakhali near sundori tree	14F4.4	Mycelium and round Shape echinulate spore	*Sphaeropsis sp.*
S-42	Malta bridge (canning)	16F4.4	Conidiophores bearing conidia	*Pericouic sp.*
S-42	Malta bridge (canning)	17F4.4	Conidiophores bearing conidia	*Llelicocephalum sp.*
S-34	Motghora(pond side)	20F4.4	Ascus with ascospores	*Fusarium sp.*
S-36	Motghora	23F4.4	Mycelium with branched conidia	*Xylohypha sp.*
S-36	Motghora	26F4.4	Cylindrical spore	*Cylindrocladium sp.*
S-33	Gadakhali	30F4.4	Mycelium with chlamydospore	*Umbelopsis sp.*

Conclusion

Study of microbial biodiversity is of vital importance to the understanding of the different processes of the earth and which may present potent novel microorganisms for screening of bioactive compounds. The diverse area of locality of selected natural spot of 24-parganas including East Kolkata wetland of West Bengal region is almost unexplored. There are no such records of available fungi of such area. Isolation, documentation and conservation of these resources are important. The study from these diverse conditions might be an important work for future in this field.

In this present work more than hundreds of soil samples were collected from different locality of EKW. About three hundreds of visually different fungi were isolated from the agar plates and were purified by dilution method in Czapex Doc slant. They were then studied under microscope after preparation of the slides. Out of which probable identification were made by microscopic studies and with the help of picture available in **Pictorial Atlas of Soil and Seed Fungi (**Author- Tsugeo watanabe). Among the isolates it is expected that one or two new genus may obtained. Few characteristic features like cellulose degradation, pesticide degradation capabilities PHB degrading abilities is under progress.

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