

The Occurrence of Keratiophilic Fungi and Related Dermatophytes from sewage water discharge points

Y. Avasn Maruthi¹*, D. Apta Chaitanya², Kaizar Hossain³, A Sravani⁴ and S Jagadish⁵

Dept. of Environmental Studies, Institute of Science, GITAM University, Visakhapatnam, A.P., INDIA

ABSTRACT

*Keratiophilic fungi display potentially pathogenic properties to animals, including human beings. Studies of these fungi in the environment are therefore of hygienic and epidemiological importance. The sewage water and sewage sludge, which are rich in organic matter, are habitat for many groups of microorganisms, such as viruses, bacteria, fungi, algae, protozoa and worms. Out of them some are frequently distributed in waste contaminated soils. Total 24 species of 9 genera were identified in total of 50 soil samples of 5 different Polluted Beach Sands. 14 dermatophytic species of 6 genera and 10 non-dermatophytic species of 3 genera were among them. Sampling site wise study of all the 5 sites reveal the order of dominance of each site as follows, based on their RIVs. Polluted Beach Sands 1 (Near Fishing Harbour): *T. rubrum* (61.64) > *Aphanoascus fulvescens* and *Aspergillus nidulans* (56.16) > *M.gypseum* (54.10) > *C.tropicum* (53.01) > *M. canis* (45.57); Polluted Beach Sands 2: *Myceliophthora vellera* (65.98) > *Aspergillus flavus* (57.69) > *Fusarium oxysporium* (56.83) > *F. solani* (55.98) > *Aphanoascus keratinophilus* and *A.versicolor* (54.27); Polluted Beach Sands 3: *C.tropicum* (71.25) > *C. pannicola* (68.60) > *A.nidulans* and *F.oxysporium* (63.97) > *Aphanoascus fulvescens* (55.29) > *Arthroderma quadrifidum* (53.97); Polluted Beach Sands 4: *C.keratinophilum* (78.09) > *C.pannicola* (70.9) > *A.flavus* (57.51) > *T.mentagrophytes* (5.78) > *C.indicum*, *M. canis*, *A.fumigatus* and *A.versicolor* (54.62); Polluted Beach Sands 5: *Aphanoascus keratinophilus* (68.51) > *C.indicum* (67.09) > *M.gypseum*, *A.niger* and *Fusarium oxysporium* (64.25) > *Arthroderma quadrifidum* (57.09) > *Aphanoascus fulvescens* (55.67). It can be expected, therefore, that the sludge on a wastewater treatment plant area or applied to land poses an elevated health risk to immunocompromised individuals*

Key words: Keratinophilic Fungi, Dermatophytes, Polluted Sand Soils and waste water.

INTRODUCTION

Keratinophilic Fungi are the finest Keratin degraders, prevalent in Keratin rich environments. The waste water and sewage are rich in organic mater, are habitat for many [1] & [2] and domestic sewage is a rich source of Keratin, Cellulose and Lignin etc. where the occurrence of Keratinophilic fungi can be easily expected.

Surveys of Keratinophilic fungi from different habitats have indicated that several species of dermatophytes and non-dermatophytic fungi inhabiting soil [3], air [4] and sewage sludge [5] & [6].

In general, the qualitative and quantitative composition of Keratinophilic fungi can be multifunctional bioindicator of environmental pollution with waste. It means that the composition indicates not only the presence of keratin remnants and faecal contaminants in the environment but also respond to the changes in environmental conditions. Additionally, the fungal growth indices inform us about the infection risk associated with the contamination of the environment with potential fungal pathogens [7] & [8]

By keeping in view all the above, this work was carried out to screen the waste water contaminated sand soils for Keratinophilic Fungi.

MATERIALS AND METHODS

To carryout this study total of 11 sampling points were selected out of a coastal stretch of 16km. Sampling points were chosen based on sewage and waste water discharge points. Simultaneously the control area (which was free from waste water discharge) was also identified and soil samples were collected from this area also.

Soil from each sampling site was collected at three levels (Surface soil, 5cm depth and 10-15cm depth and 250gm of soil from each) with sterile spatulae into sterile screw cap tubes. Then they were carefully brought back to the laboratory. All the samples were set for fungal identification in two ways, viz. Surface Soil Dilution Plating method (SSDP) [9] and Hair Baiting Technique [10].

The fungal species from the resulted growth were observed by their colonial morphology, stained with Lacto phenol Cotton blue dye and photographed by using Binocular Digital Research Microscope (from Labomed, USA) and analyzed by using a software *Progres Capture Pro*. The microphotographs were identified by comparing with available literature. Fungal indices used in this study were FI, NS-Number of isolated sps., NA-Number of fungal strains, FIPS-Frequency of Isolation of Predominating Fungal sps., and LI-L index [11].

Physical factors like soil water content, pH, Organic matter percentage of all the soil samples were estimated [12].

RESULTS AND DISCUSSION

Table – 1 represents the physico-chemical parameters of the test soil samples. Nine species of Fungi, belonging to five genera were isolated from soil samples by using both hair baiting and surface dilution plating techniques (Table – 2). Three of these genera were dermatophytes (*Tricophyton mentegrophytes*, *T.rubrum*, *Microsporum gypseum*, *M.nannum*, *Chrysosporium pannicola*) and other species were related Keratinophilic Fungi (*Aspergillus flavus*, *A.candidus*, *A.fumigatus*, *Fusarium oxysporium*).

Using Hair Baiting Technique, nine species of Keratinophilic fungi were recorded from sandy soils of HF (Heavy Flow of waster water) and MF (Medium Flow of waster water). Dermatophytes and other related Keratinophilic fungi were represented by five out of nine species. These comprised of 55.55% of the soil Keratinophilic mycobiota (Table – 2).

Table – 1: Physico-Chemical parameters of test soils

S.No.	Site	Parameter			
		Soil Temperature (°C)	% Water Content	% Organic Matter Content	pH
1	DA1	16.5±0.2	20.7	0.47	7.4-7.6
2	DA2	16.1±0.1	24.6	0.49	7.5-7.6
3	HF1	15.2±0.3	85.6	0.72	8.1-8.2
4	HF2	15.8±0.2	88.1	0.81	7.9-8.1
5	HF3	16.0±0.3	82.7	0.74	8.2-8.3
6	HF4	15.5±0.3	84.5	0.72	8.7-8.9
7	HF5	15.7±0.1	83.0	0.91	7.9-8.3
8	HF6	15.1±0.1	87.2	0.95	7.4-7.5
9	HF7	15.4±0.2	89.5	0.89	8.6-8.9
1	MF1	16.0±0.3	42.3	0.54	7.5-7.6
11	MF2	15.9±0.2	54.5	0.62	7.8-7.9
12	CS	17.0±0.5	63.8	1.53	6.9-7.1

*DA=Dried and Abandoned Drainage

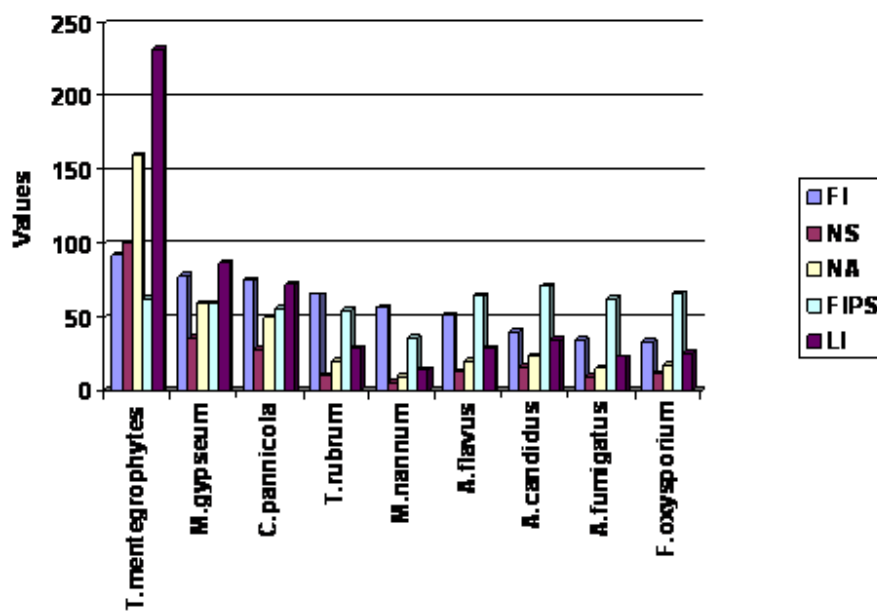
HF=Heavy Flow Drainage

MF=Medium Flow Drainage

CS=Control Soil

Table – 2: Keratinophilic Fungi isolated from soils of sampling sites by Hair Baiting Technique (Number of isolated colonies, their total number, and % in different soils, number of positive soils and their percentage of all soils, Relative Importance Value of species – RIV.

Isolated Species	Sample Sites												Tot. Colony	%	+ ve Occurrence No.	%	RIV
	DA1	DA2	HF1	HF2	HF3	HF4	HF5	HF6	HF7	MF1	MF2	CS					
I. Keratinophilic Fungi related Dermatophytes																	
<i>Trycophyton mentegrophytes</i>	-	1	3	3	2	1	1	2	3	1	1	-	18	26.86	10	83.33	110.19
<i>Microsporium gypseum</i>	1	1	2	3	3	2	1	1	-	-	1	-	15	22.38	9	75.0	97.38
<i>Chrysosporium pannicola</i>	1	-	-	-	2	2	2	1	-	1	1	-	10	14.92	7	58.33	73.25
<i>T.rubrum</i>	2	1	-	-	-	3	4	2	1	-	2	-	15	22.38	7	58.33	80.71
<i>Microsprium nanum</i>	-	-	1	1	1	-	2	2	1	1	-	-	9	13.43	7	58.33	71.76
Total Species per site	3	3	3	3	4	4	5	5	3	3	3	-	-				
Total Isolates	4	3	6	7	8	8	10	8	5	3	5	-	67				
II. Other Keratinophilic Fungi																	
<i>Aspergillus flavus</i>	3	2	2	2	1	1	2	3	1	1	2	3	23	37.70	12	100.0	137.70
<i>Aspergillus candidus</i>	1	2	-	1	2	2	1	1	2	-	-	2	14	22.95	9	75.0	97.95
<i>Aspergillus fumigatus</i>	1	1	1	1	-	-	3	-	2	1	1	4	15	24.59	9	75.0	99.59
<i>Fusarium oxysporum</i>	1	-	-	-	1	2	3	-	1	-	-	1	9	14.75	6	50.0	64.75
Total Species per site	4	3	2	3	3	3	4	2	4	2	2	4	-				
Total Isolates	6	5	3	4	4	5	9	4	6	2	3	10	61				



Isolated Keratinophilic Fungi

Fig.1: Indices of Fungal Growth

*FI = Fungal Index, NS = No. of Isolated species, NA = No. of Fungal species, FIPS = Frequency of Isolation of Predominating Fungal species, LI = L-Index.

The most prevalent species found were in the order *T.mentegrophytes* (RIV=110.19; FIPS=62.5), *M.gypseum* (RIV=97.38; FIPS=60.0), *T.rubrum* (RIV=80.71; FIPS=56.0), *C.pannicola* (RIV=73.25; 55.0), *M.nannum* (RIV=71.76; FIPS=36.0) among the dermatophytes and *A.flavus* (RIV=137.70; FIPS=65.0), *A.fumigatus* (RIV=99.59; FIPS=70.8), *A.candidus* (RIV=97.95; FIPS=62.5) and *Fusarium oxysporium* (RIV=64.75; FIPS=66.66) among the other Keratinophilic related fungi (Table-2, Fig-1).

[13] was carried out a study on ecology of cycloheximide – resistant fungi in field soils receiving raw city wastewater normal irrigation water and reported that *Alternaria alterna*, *Aspergillus candidus*, *Geotrichum candidum*, and *Paecilomyces lilacinus* are the species most commonly found in those habitats included.

Microsporium gypseum is a geophilic dermatophyte relatively frequently isolated from skin lesions. Therefore, this species is of special epidemiological importance. The waste water favored the growth of *Microsporium gypseum* on Keratinous substrata in a wide temperature range. It can be expected, therefore, that the waste water on waste water treatment plant area or applied to land poses an elevated health risk to immune compromised individuals [7].

The comparative qualitative analysis of microscopic fungi showed that quantity of fungi was very different and oscillated in untreated waste water from $31 \times 10^3 / \text{cm}^3$ to $167 \times 10^3 / \text{cm}^3$, in treated waste water – from $200 / \text{cm}^3$ to $750 / \text{cm}^3$, and finally in sewage sludge – from $43 \times 10^3 / \text{g}$ of dry solids to $182 \times 10^3 / \text{g}$ of dry solids and *Penicillium* was dominant [1].

CONCLUSION

Keratiophilic fungi display potentially pathogenic properties to animals, including human beings. Studies of these fungi in the environment are therefore of hygienic and epidemiological importance. The importance increases in highly populated and industrialized areas, because of their high organic and inorganic contamination considerably impacting microbial communities, including those of Keratinophilic fungi. An essential element of these studies is evaluation of these effects of waste management and industrial contaminants on the distribution of Keratinophilic fungi on the areas.

Acknowledgments

We are thankful to All GITAM University Management for providing necessary facilities to carryout this work.

REFERENCES

- [1] Kacprzak M, Neczaj E, Okoniewska E. *Desalination*, **2005**, 185(1-3), 363-370
- [2] Adewoye S O and Adegunlola G A. *Advances in Applied Science Research*, **2010**, 1 (1), 9-13
- [3] Kushawaha RKS. *Mycosen*, **1981**, 26, 324-326.
- [4] Marchisio VF, Fuscoui A, Rigo S. *Mycopathologia*, **1994**, 127, 103-115.
- [5] Muhsin T M and Hadi R B. Dermatophytes and other keratinolytic fungi from sewage sludge at Basrah, Iraq. *Mycoses.*, **2000**, (in press).
- [6] Tawfik Muhsin M and Rawa Hadi B. *Maycopathologia.*, **2002**, 154, 185-189.
- [7] Ulfing K, *Polish journal of Env. Studies*, **2003**, 12(4), 461-466.
- [8] Samuel P, Prince L and Prabakaran P. *European Journal of Experimental Biology*, **2011**, 1 (3):130-138
- [9] Ali-Shtayeh M S, Chee Len Lm-Ho and Dick M W. *Transactions of the British Mycological Society*, **1986**, 86, 39-47.
- [10] Rahul Sharma and Rajak R C. *Resonance*. **2003**, 28-40.
- [11] Ulfing K. *Revista Iberoamericana de Micologia*, **2000**, 44-50.
- [12] Webber J R. Soil properties, herbicide sorption and soil system. In:Southern, T. Research methods in weed science., **1977**, second editions. USA: Science Society.
- [13] Ali-Shtayeh M S, Rana Jamous M F and Abu-Ghdeib S I. *Mycopathologia.*, **1999**, 144, 39-54