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Fungal Analysis of Water from Yusmarg Health Resort of Kashmir Valley

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Abstract—To assess the density and diversity of fungal flora, a study of aquatic fungi along with some physical parameters like temperature and pH was carried out during the month of November and December 2010, in Yusmarg area of Kashmir valley at four sites differing from each other markedly in terms of biotic and abiotic factors. During the study the fungal flora showed variation in relation to the physical parameters. The colony count was maximum at Site IV Reservoir outlet (197) followed by Site III Reservoir Inlet (75), Site I Dudhganga (67) and site II Tank Area (52). The total fungal population was maximum at Site IV Reservoir outlet (1.0×10^4 in Nov and 0.9×10^4 in Dec.) and minimum at Site II Tank Area during both the months (0.3×10^4 in Nov and 0.2×10^4 in Dec.). Among the different colonies identified it was found that about 53% of the isolated colonies belong to *Pencillium s*pp. 31% belongs to *Aspergillus* spp. and 16% were identified as *Candida* spp.

Index Terms—Fungi, Water, Yusmarg, *Pencillium, Aspergillus, Candida*, CFU/ml, Colony count, Pollution.

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1 INTRODUCTION

The importance of water can simply be understood by the fact that life originated in water. Water is and has always been mankind's precious resource. It is recognized as the key environmental issue of the 21st century and a key to poverty alleviation. Water is vital to the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more water of high quality for domestic purposes and economic activities. Water abstraction for domestic use, agricultural production, mining, industrial production, power generation, and forestry practices can lead to deterioration in water quality and quantity that impact not only the aquatic ecosystem (i.e., the assemblage of organisms living and interacting together within an aquatic environment), but also the availability of safe water for human consumption. It is now generally accepted that aquatic environments cannot be perceived simply as holding tanks that supply water for human activities. Rather, these environments are complex matrices that require careful use to ensure sustainable ecosystem functioning well into the future. In fact, without water life could not exist. Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication (Hughes and Koplan, 2005).

Water serves as the second best natural medium for the growth of micro organisms. The word microbes or microorganisms refer to a group of extremely tiny living organisms, which can only be seen with the aid of a microscope (Agbonlahor, 1998). Microbes consist of bacteria, fungi, viruses and protozoa. They are adapted to the various environments on earth: some living in water, some in soil, others in air, plants and animals including man. According to Rosebury (1961), microbes are not only ubiquitous in human, but they also abound in numerous numbers on and in his body, while he is in the best of health. Those that cause diseases are called Pathogens. Microbial communities of aquatic environments include viruses, bacteria, fungi, algae and other microbes. Bacteria and fungi mostly dominate the aquatic systems. Water fungi and fungus-like organisms as a biological factor of ecological water systems have significant influence on the environment and its modification. They decompose necrosis substrates found in water bodies. Fungi also can act as facultative parasites and then frequently occur on their hosts. Looking into the adverse impacts of fungi, it is important to study the occurrence of fungi in drinking water because they are capable of producing mycotoxins in water which are secondary metabolites of these filamentous fungi (Hamid et al. 2013). The intrusion of biological agents into water systems can pose serious public health risks because these agents cannot be easily detected and can remain hidden until a widespread contamination exists.

2. MATERIAL AND METHODS:

LOCATION AND SITE DESCRIPTION

Yusmarg is a set of meadows surrounded by Pine trees and mountains. It is about 47Km away from Srinagar and lies in the district Budgam of Jammu and Kashmir. There were three main water sources: Dudhganga river (Site I), Tank Area (Site II), Reservoir, which was divided into two sites: Reservoir Inlet (Site III) and Reservoir Outlet (Site IV). Dudhganga lies between the geographic coordinates of 33°50' 34.30" N and 74°39' 46.63" E at an elevation of about 2275m a.s.l. This is a mighty river which makes a little white foam so it is called Dudhganga. The geographic coordinates of Tank Area are 33°49' 53.26"N and 74°40' 25.46" E with an altitude of 2407m a.s.l. The tank receives water from Kalnag and Fransnag (two tributaries of Dudhganga) through pipes. The tank remains twelve months operative and is a gravity based system. The Reservoir is fenced. The geographic coordinates of Reservoir Inlet are 33°49'30.56"N and 74°40'11.01"E with an elevation of 2364m a. s. l. and that of Reservoir Outlet are 33°49' 25.72" N and 74°40' 25.46" E with an elevation of about 2362 m a. s. l. The bottom of reservoir was lined by silt.

COLLECTION OF SAMPLES (SAMPLING)

Samples of water from all the sites under consideration were collected in 1 litre plastic bottles, which were previously cleaned and rinsed with ethanol and then three to four times with distilled water and then three to four times with the site water (A.P.H.A 1998). The samples of water were collected at the depth of 1-10 cm from the water surface. During collection of samples, extreme care was exercised to avoid contamination of the parts of bottle. The collected samples were then processed for microbial analysis.

LABORATORY ANALYSIS

Microbiological analysis of water samples was done by plate count test using the methodology of APHA (1998). The most important method used for the measurement of microbial community is plate count technique which measures the number of viable cells. In this technique it is important that only a limited number of colonies, 10-100 (Below 10 TFTC: Too few to be counted; Above 100 TNTC: Too numerous to be counted) should develop on a plate (APHA, 1998). Otherwise it may lead to in accuracies in the count. Rose Bengal Agar was used for enumeration and cultivation of fungi. About 15 to 20 ml of medium was poured in each Petri plate on a laminar flow cabinet. The medium after cooling got solidified. The Petri plates were incubated over night to check the contamination if any, inside the media. Before inoculation, the samples were diluted to different levels, in order to get the approximate number and density of the bacteria easily. Spread plate technique was followed for inoculation of water samples. This technique involves distribution of water samples (0.1 ml) over surface of prepared agar plate. This technique allows the microbial colonies to grow over the surface of the medium and eventually counting becomes easier. After inoculation the culture plates were incubated in an incubator in inverted position at a temperature of 28ºC for 48 hours to assess the growth of colonies. Pure cultures were obtained by platinum loop through streaking technique (APHA, 1998). Micro-organisms were transferred from one medium to another for the preparation of pure cultures. Colonies that developed on agar plates were counted with unaided eyes as per key given by Johnson

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and Case, 1995. The counts were expressed as CFU/ml (Colony Forming Unit) of water sample. The number of colonies counted were expressed as CFU/ml and were calculated by using the following formula.

$$CFU/ml = n \times d$$

Where, n = number of colonies
d = dilution factor = 1/dilution (10^{-1}, 10^{-2}, 10^{-3}….).

3. RESULTS AND DISCUSSION

Different types of colonies at different sites were obtained during the study period. A total of 19 types of fungal colonies were obtained during the study period which were assigned the names from F1 to F19 as shown in Table 1. Among the different colonies identified it was found that about 53% of the isolated colonies belong to Pencillium spp. 31% belongs to Aspergillus spp. and 16% were identified as Candida spp. (Table 4). The data recorded on Fungi reveals that maximum number of fungal population was recorded during November at Site IV (1.0×10^4) and minimum at Site II (0.3×10^4). Similar trend was seen during December with maximum equal to 0.9×10^4 CFU/ml and minimum equal to 0.2×10^4 CFU/ml. This can be attributed to temperature and organic matter content. Temperature is a key factor determining the activity of organisms in ecosystems (Friberg et al. 2009), with higher temperatures stimulating biological activities at least within physiological limits (Bergfur & Friberg 2012). Temperature affects the distribution (Suberkropp 1984), growth and reproduction (Rajashekhar & Kaveriappa 2000; Fernandes et al. 2009) of aquatic hyphomycetes. An increase in temperature generally increases metabolic rates (Sokolova & Lannig 2008). Among the colonies identified, all have been found to grow well at the pH obtained at various Sites (6.83-7.14). Sharma et al (2011) while studying the effect of temperature and pH Combinations on Growth Pattern of Dermatophytes have found that majority of fungi grow well at a pH range from 4.2-9.3. Usually too alkaline and too acidic solutions are not favourable for the growth of fungi. Most of the fungi, however, tend to grow better on the acidic side. Cochrane (1958) states that many fungi, with few exceptions, grow best on media with an initial pH of 5.0 to 6.5. From the table 2 it is also clear that during both the months maximum types of colonies were present at Reservoir outlet (Site IV) and minimum in Tap water. Candida spp. was absent at Site II during both the months and thus the drinking water supplied from the tank was free from it. At Site IV there was comparatively a little decrease in the colony count during December. This may be attributed to the factor that the reservoir outlet was undergoing construction and had huge anthropogenic pressure and cattle disturbance. Furthermore the water was muddy; there may be the chances of high organic carbon where the fungi act as decomposers. According to APHA (1998), increasing numbers of Fungi usually indicate increasing organic loading in water. The occurrence of fungi and fungus-like organisms in water reservoirs is of great importance for sanitary and epidemiological reasons, as some of the fungi are pathogenic to humans. Man lives in a close contact with fungi throughout life. Fungi and fungus-like organisms regarded as important etiological factors of mycotic infections are identified in fresh and salt waters. The most commonly encountered fungi in various ecosystems include such pathogenic species as Aspergillus candidus, Candida albicans, Penicillium mycetomagenum, and Trichosporon cutaneum. Candida albicans and Trichosporon cutaneum induce mycotic infections of skin, circulation systems, and organs. (Rozga et al. 1999; Kiziewicz & Czeczuga 2001). The species of Pencillium and Aspergillus were isolated which are usually found in polluted lake waters (as opined by Kellermann and McBeth, 1912), and these genera also have been reported frequently from the drain waters with maximum densities during higher pollution (Khulbe and Durgapal, 1994).These species can thus act as a good indicators of water pollution (Cook, 1954; Harbola and Khulbe, 1989). Heavily polluted water bodies have large number of soil fungi (APHA, 1998). Thus the reservoir outlet was most polluted.

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Table 1: Colony morphology and microscopic examination of isolates from four sites.

S.No.	Appearance	Margin	Elevation	Colour	Species Identified	As-signed Name
1	Circular	Filamentous	Convex	Dark Green	*Pencillium* sp.	F_1
2	Circular	Filamentous	Convex	Green	*Pencillium* sp.	F_2
3	Circular	Filamentous	Convex	White	*Aspergillus* sp.	F_3
4	Circular	Filamentous	Convex	L. Green	*Aspergillus sp.*	F_4
5	Circular	Filamentous	Convex	Yellow	*Pencillium* sp.	F_5
6	Circular	Filamentous	Convex	Yellowgreen	*Pencillium* sp.	F_6
7	Circular	Filamentous	Convex	Black	*Pencillium* sp.	F_7
8	Filamentous	Filamentous	Convex	White	*Pencillium* sp.	F_8
9	Circular	Entire	Convex	Orange	*Aspergillus* sp.	F_9
10	Circular	Entire	Convex	Yellow	*Pencillium* sp.	F_{10}
11	Circular	Entire	Convex	Red	*Aspergillus* sp.	F_{11}
12	Filamentous	Filamentous	Umbonate	Green	*Pencillium* sp.	F_{12}
13	Filamentous	Filamentous	Raised	White	*Aspergillus* sp.	F_{13}
14	Circular	Entire	Flat	Creamish	*Candida* sp.	F_{14}
15	Circular	Entire	Flat	Pink	*Candida* sp.	F_{15}
16	Circular	Curled	Flat	Red	*Pencillium* sp.	F_{16}
17	Circular	Raised	Curled	Pink	*Aspergillus* sp.	F_{17}
18	Irregular	Filamentous	Convex	Green	*Pencillium* sp.	F_{18}
19	Rhizoid	Filamentous	Flat	Pink	*Candida* sp.	F_{19}

Table 2: Colony Count, number of isolates and CFU/ml at all the sites during both the months.

Site	November			December			Grand Total
	Number of isolates	Colony count	CFU/ml	Number of isolates	Colony count	CFU/ml	
Site I	5	38	0.4×10^4	3	29	0.3×10^4	67
Site II	4	32	0.3×10^4	3	20	0.2×10^4	52
Site III	5	40	0.4×10^4	4	35	0.3×10^4	75
Site IV	8	100	1.0×10^4	7	97	0.9×10^4	197

Table 3: Temperature and *p*H at all sites during the two months.

	Temperature (ºC)		pH	
	November	December	November	December
Site I	8.5	1.5	7.14	6.86
Site II	7.3	1.0	7.12	6.80
Site III	7.9	1.1	7.0	7.10
Site IV	9	2.5	7.1	6.83
Average	8.15	1.52	7.09	6.90

Table 4: Percentage of different fungal species during two months.

S. No.	Assigned name	Species identified	Percentage
1	F_1	*Pencillium* sp.	53 %
2	F_2	*Pencillium* sp.	
3	F_5	*Pencillium* sp.	
4	F_6	*Pencillium* sp.	
5	F_7	*Pencillium* sp.	
6	F_8	*Pencillium* sp.	
7	F_{10}	*Pencillium* sp.	
8	F_{12}	*Pencillium* sp.	
9	F_{16}	*Pencillium* sp.	
10	F_{18}	*Pencillium* sp.	
11	F_3	*Aspergillus* sp.	31 %
12	F_4	*Aspergillus* sp.	
13	F_9	*Aspergillus* sp.	
14	F_{11}	*Aspergillus* sp.	
15	F_{13}	*Aspergillus* sp.	
16	F17	*Aspergillus* sp.	
17	F_{14}	*Candida* sp.	16 %
18	F_{15}	*Candida* sp.	
19	F_{19}	*Candida* sp.	

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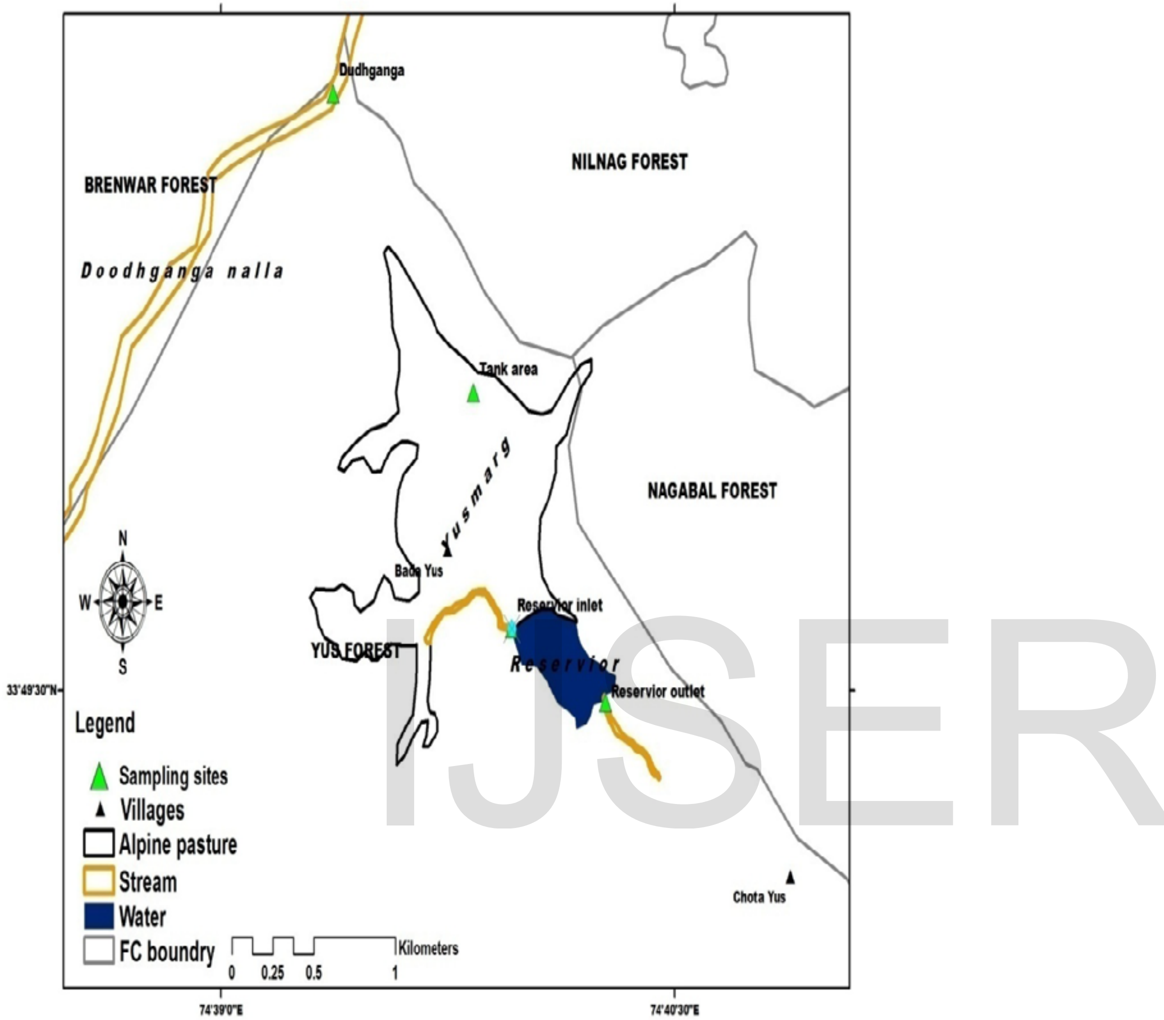
Fig. 1: Map of Yusmarg showing study area and sampling sites.



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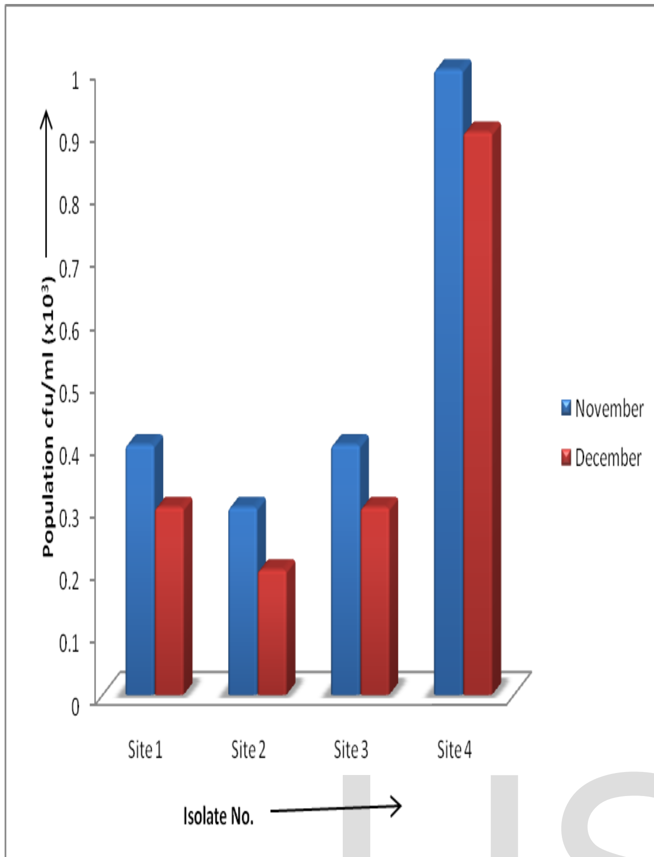

Fig.2: Graphical representation of CFU/ml at all sites.

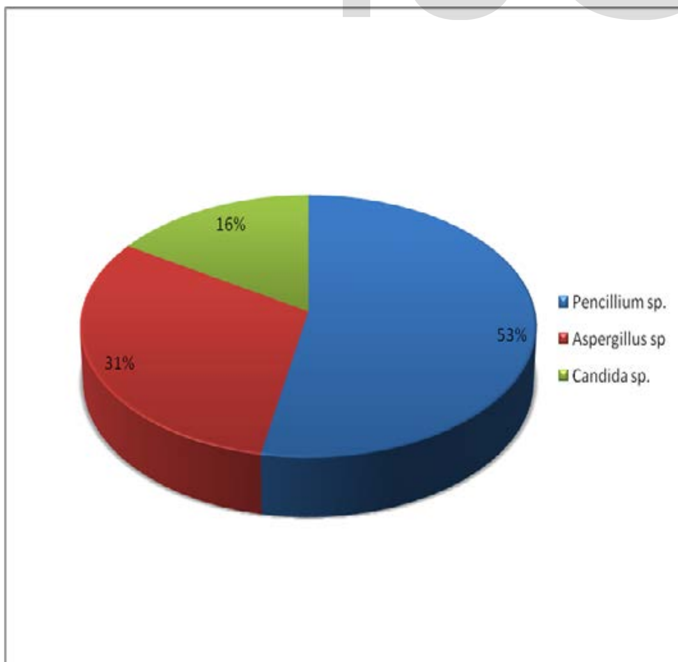

Fig. 3: Percentage contribution of identified species of fungi.

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