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Aquatic fungi diversity of upper lake and Hathaikheda reservoir of Bhopal Madhya Pradesh

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Abstract
The fungal flora of two water bodies Upper Lake and Hathaikheda Reservoir of Bhopal Madhya Pradesh were isolated during the course of study from January 2017 to December 2018. During the present study, total 600 isolates, comprising of 17 genera and 24 species of aquatic and terrestrial fungi were recorded from two water bodies. These fungi belonged to various orders of the group Oomycota, *Ascomycota* and Zygomycota. Members of Saprolegniales were dominant over the rest of the orders of fungi at both the water bodies. Upper Lake showed a great fungal diversity compared to that of Hataikheda Reservoir as total 332 isolated of fungi were isolated from Upper Lake, while as only 268 isolated of fungi were isolated from Hathaikheda Reservoir. Majority of the fungal species were common at both the water bodies, but *Aphanomyces laevis, Aphanomyces invades, Saprolegnia hypogyanna, Pythium undulatum and Aspergillus terrus* were only specific to Upper Lake.

Keywords: Aquatic fungi, diversity, upper lake and Hathaikheda reservoir

Introduction
Aquatic fungi, as the word suggests require aquatic environment for the growth and development of their various life processes, but it is difficult to give a précis and universal definition of aquatic fungi because they show great diversity. These include Yeasts, some *Zygomycotina*, *Deuteromycotina*, *Mastigomycotina* (Zoosporic fungi), *Ascomycotina*, and few *Basidiomycotina*. Some of them may occupy water for the whole of their lives, others may be amphibious and some may have temporary aquatic existence, probably brought on a substratum by wind or swept by floods into water. Aquatic funguses are habitually microscopic organisms, which do not produce visible fruiting body but grow asexually (anamorphic fungi). Their occurrence in water is rather faint and specialized methods are required to observe their diversity, population structure and ecological utility. In the aquatic system, they greatly differ between substrates (Mille-Lindlom *et al.,* 2006) [16] and vary with the physic-chemical characteristics of the individual habitats, such as water flow (Baldy *et al.,* 2002) [3], dissolved oxygen level (Medeiros *et al.,* 2009) [15], nutrient concentration (Rankovic, 2005) [18], Salinity (Roache *et al.,* 2006) [20], depth of water body (Wurzbacher *et al.,* 2010) and temperature (Barlocher *et al.,* 2008) [4]. Therefore, fungal communities potentially differ between streams, shallow water lakes and wetlands, deep lakes, and other habitats such as salt lakes and estuaries. Water associated fungi are historically known as "phycomycetes", a functionally defined group consisting of "true fungi" (Eumycota) and "analogously evolved fungus-like organisms" belonging to Chromista (Oomycetes, Thraustochytridiomycetes). Oomycetes or water molds, are now known to show great similarity with diatoms and brown algae, so phylogenetically grouped with them (Beakes *et al.,* 2012; van West 2006) [5, 25]. They represent a continuing threat for global food protection, as being most challenging group of disease causing organisms in both agriculture and aquaculture (Derevnina *et al.,* 2016) [10]. (Shearere *et al.,* 2007) have reported occurrence of approximately 3000 species of fungi in all the three combined biodiversity i.e. marine habitats, freshwater and brackish; however (Jones *et al.,* 2014b) [13] puts it between 3069 and 4145. Biodiversity of aquatic fungi in aquatic habitat is low compared to terrestrial habitats, which is in contract with other organisms e.g., insects where freshwater are habitat to 6% of all insect species even though covering merely one percent of earth's surface (Dijkstra *et al.,* 2014) [11]. Processes concerning aquatic fungi are significant to the implementation of aquatic ecosystems but have received modest concentration in the literature.

Along with bacteria and protozoa, aquatic fungi are known to play great roles in the functioning of reservoirs; they fulfill important functions as associations in the food chain and in the flow of matter in the biosphere (Buessing and Gessner, 2006) [6]. Aquatic fungi are also known to decompose cellulose, hemicelluloses and many xenobiotics (Augustin *et al.*, 2006, Baldrian, 2006; Steinberg, 2007) [1, 2, 24]. However, existing knowledge on aquatic fungi is scrappy and it is anticipated that only about seven percent of all the species of aquatic fungi have been recognized and described to date (Mueller and Schmit, 2007) [17]. The present Piece of work was designed to study the distribution and occurrence of aquatic fungi of two water bodies Upper Lake and Hataikheda Reservoir of Bhopal Madhya Pradesh.

Material and Methods

Isolation of fungi from water samples using baiting technique

Collected water samples were poured in separate Petri dishes of the size 9 cm x 2 cm and baited with soyabean, mustard and jawar seeds. After 24 hours baits were removed and washed by using sterilized distilled water and after that placed separately in small Petri dishes containing 20-30 ml sterilized distilled water. After this they were incubated at 15 to 22 °C temperature in the BOD incubator. The growth of colony was observed after 48 hours.

Purification and Identification

Pure and bacteria free cultures were prepared by following the methods of Coker (1923) [8], Raper (1937) [19], Johnson (1956) [12], Scott (1961) [21].

Antibiotics: Tetracycline 100 mg/l and Streptomycin sulphate 100 mg/l were used to avoid bacterial contamination.

Baits used: Soyabean seeds, jowar seeds and cotton seeds.

Staining of Cultures: Mycelium can be stained with the aid of cotton blue for permanent or temporary mount. Neutral red is useful to stain living cultures for study and photography. Following methods were used for staining of cultures:

Preservation: The dead specimen of living cultures on which the taxonomic description is based (the type) is deposited at least in one of the major herbaria. The cultures are preserved in formalin (10%) in ordinary or specimen tube, or in alcohol 70-90%.

Slide Preparation: Small baits of sterilized soya bean/jawar seeds are added in the water culture, and allowed to develop sex organs, when mature sex organs are formed, the entire colony is immersed for 2 hours in a dish containing 70% F.A.A. (90 ml of 70% ethyl alcohol, 5 ml of glacial acetic acid, & 5 ml of formalin). Then, a slide is put below the colony and picked up in such a way so as to have the entire colony spread out on the slide in the desired position. A mounting medium of eosin-Y or basic fuchsine in 5% glycerin or cotton blue in lacto phenol is added to the slide, and the culture is enclosed with a cover slip. After 5-8days, when water is evaporated from the colony and mounting medium, the cover slip is sealed with balsam or nail polish or DPX mountant.

Results

The fungal flora of two water bodies Upper Lake and Hataikehda Reservoir of Bhopal Madhya Pradesh were isolated during the course of study from January 2017 to December 2018. In the present study, total 600 isolates, comprising of 17 genera and 24 species of aquatic and terrestrial fungi were recorded from two water bodies. At Upper Lake a total number of 332 isolates were isolated which belonged to 17 genera and 24 species. Among 24 species isolated 13 species belonged to Oomycota, 9 *Ascomycota* and 2 species to Zygomycota (Table-1). At Hataikehda Reservoir a total number of 268 isolates were isolated which belonged to 10 genera and 19 species. Among 19 species isolated 09 species belonged to Oomycota, 8 *Ascomycota* and 2 species to Zygomycota (Table-2).

Table 1: Showing occurrence and distribution of aquatic fungi at Upper Lake

Name of fungi	2017												2018												Total no of occurrence	Frequency %
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D		
Heterokontophyta																										
Saprolegniales *(Saprolegneaceae)*																										
Achlya Americana	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	13	54%
Achlya apiculata	+	+	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	11	46%
A. flagellate	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	12	50%
A. prolifera	+	+	+	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+	-	+	+	14	58%
A.klebsiana	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	+	-	-	+	+	10	42%
Aphanomyces laevis	+	+	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	12	50%
A. invades	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	11	46%
Saprolegnia diclina	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	15	62%
S. ferax	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	+	+	10	42%
S. parasitica	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	16	67%
S. hypogyana	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	+	+	13	54%
Peronosporales (Pythiaceae)																										
Pythium afertile	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	7	29%
P. undalatum	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	6	25%
Ascomycota																										
Eurotiales (Trichocomaceae)																										
Aspergillus fumigatus	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	18	75%
A. flavus	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	16	67%

Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	24	100%
A. sydowii	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	15	62%
A. terreus	+	+	+	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	13	54%
Penicillium sp	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	9	37%
Pleosporales (Pleosporaceae)																										
Alternaria sp.,	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	20	83%
Curvularia sp.	-	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	-	14	58%
Hypocereales (Nectriaceae)																										
Fusarium sp	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	22	92%
Zygomycotina (Mucrales)																										
Rhizopus	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	16	67%
Mucor sp	+	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	+	15	62
Total no. of occurrence of fungi during the months	23	22	17	12	5	3	2	13	19	6	21	22	21	19	15	11	5	6	2	15	19	7	24	23	332	

Table 2: Showing occurrence and distribution of aquatic fungi at Hataikehda Reservoir.

Name of fungi	**2017**												**2018**												**Total no of occurrence**	**Frequency %**
	J	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**		
Heterokontophyta																										
Saprolegniales (Saprolegneaceae)																										
Achlya americana	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	10	42%
Achlya apiculata	+	+	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	+	+	10	42%
A. flagellate	+	+	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	+	+	9	37%
A. prolifera	+	+	+	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	13	54%
A.klebsiana	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	9	37%
Saprolegnia diclina	+	+	+	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	15	62%
S. ferax	+	+	+	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	+	12	50%
S. parasitica	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	17	71%
Peronosporales (Pythiaceae)																										
Pythium afertile	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	8	33%
Ascomycota																										
Eurotiales (Trichocomaceae)																										
Aspergillus fumigatus	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	17	71%
A. flavus	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	16	67%
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	24	100%
A. sydowii	+	+	+	-	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	14	58%
Penicillium sp	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	9	37%
Pleosporales (Pleosporaceae)																										
Alternaria sp.,	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	20	83%
Curvularia sp.	+	+	+	+	+	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	14	58%
Hypocereales (Nectriaceae)																										
Fusarium sp	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	22	92%
Zygomycotina (Mucrales)																										
Rhizopus sp	+	+	+	+	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	16	67%
Mucor sp	+	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	+	-	+	+	13	54%
Total no. of occurrence of fungi during the months	19	18	14	9	5	1	2	10	15	6	18	17	16	14	11	10	5	6	2	9	16	8	19	18	268	

It was found that Upper Lake had richer fungal flora as compared Hataikehda Reservoir. Majority of the fungal species were common at both the water bodies, but *Aphanomyces laevis, Aphanomyces invades, Saprolegnia hypogyanna, Pythium undulatum and Aspergillus terrus* were only specific to Upper Lake.

At Upper Lake maximum number of fungi 23, were recorded in the year 2017 in the month of January, while the minimum number was 2 recorded in the month of July 2017. Similar trend was found in the year 2018, maximum 24 species were found in November 2017 and only 2 species were recorded in the month of July 2018 (Table-1).

At Hataikehda Reservoir maximum number of fungi 19, were recorded in the year 2017 in the month of January, while the minimum number was 1 recorded in the month of June 2017. Similar trend was found in the year 2018, maximum 19 species were found in November 2018 and only 2 species were recorded in the month of July 2018 (Table-2).

At Upper Lake *Aspergillus niger* was found to be the most dominant species with a 100% frequency, apart from this species *Fusarium sp 93%, Alterneria sp* 83% and *Aspergillus fumigatus* 75% were the co-dominant species; among the conidial fungi. Among the Saprolegniales; *Saprolegnia parasitica* was dominant with frequency of 67%, followed by *Saprolegnia diclina* 62% *Achlya prolifera* with 58% frequency each (Table -1)

At Hataikehda Reservoir *Aspergillus niger* was found to be the most dominant species with a 100% frequency, apart from this species *Fusarium sp 92%, Alterneria sp* 83% and *Aspergillus fumigatus* 71% were the co-dominant species; among the conidial fungi. Among the Saprolegniales; *Saprolegnia parasitica* was dominant with frequency of 71%, followed by *Saprolegnia diclina* 62% *Achlya prolifera* with 54% frequency each (Table -2)

At Upper Lake the aquatic fungi isolated during the study period by order wise was that 137 isolates were of Saprolegniales, 13 Peronosporales, 95 Eurotiales, 34 Pleosporales, 22 Hypocreales and 31 Mucorales (Table -1)
At Hataikehda Reservoir the aquatic fungi isolated during the study period by order wise was that 95 isolates were of Saprolegniales, 8 Peronosporales, 80 *Eurotiales*, 34 *Pleosporales*, 22 *Hypocreales* and 29 *Mucrales* (Table -2).

Discussion

The data obtained from analysis of two water bodies regarding occurrence and distribution of aquatic fungi reveals that from the total fungi isolated common genera of fungi recorded were, *Achlya, Alternaria, Pythium, Saprolegnia, Aspergillus, Pencillium, Mucor and Fusarium*. These genera were found frequently at both the water bodies and exhibited wide range of tolerance to temperature, pH and other parameters. Hataikheda Reservoir showed less fungal diversity compared to that of Upper Lake which was less polluted water body where all the water parameters were in tolerance limit for growth of aquatic fungi. These observations are in the agreement with the findings of Harvey,1952 and Khan,1981 who reported the occurrence of large number of water molds from non-polluted and clear water and very few from heavily polluted water. In polluted waters bacteria may inhibit the growth of fungi.
From both the water bodies' genus *Achlya* and *Saprolegnia* contributed maximum isolates. These observations are in agreement with the reports of (Chauhan and Qureshi, 2012). Maximum fungal species were recorded during winter months when temperature was low and minimum during rainy season followed by summer season. Higher temperatures in summer and rainy season have also been reported unfavorable for most of aquatic fungi by (Dayal and Tandon, 196; Shrivastava, 1967 and Manoharachary, 1981).

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