



Study of Aquatic Fungi and Their Role in Putrefaction of Allochthonous Leaves at Hanna Lake (Balochistan)

Saadullah Khan Leghari¹, Mudassir Asrar^{1*}, Sana-ur-Rehman Sheikh²,
Tariq Ismail¹ and Anwar Khan¹

¹Department of Botany, University of Balochistan Quetta, Pakistan.

²Institute of Management Sciences, University of Balochistan, Quetta, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SKL and MA designed the study, developed protocol and wrote the manuscript. Authors SRS, TI and AK managed literature survey and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The micro fungal flora of the mud and water of Hanna Lake in Quetta district was investigated during 2015, using Baiting techniques. Apart from *Mycotypha* sp, the flora of Hanna Lake was essentially same and similar to those recorded by other authors for temperate lakes and river. *Fusarium solani* was the only species isolated consistently from mud and water through out the year and it is a true aquatic fungus. Other species showed a distinct seasonal pattern. Many of these included common phylloplane fungi such as *Alternaria* sp and *Botrytis cinerea*. The changes occurred in the fungal flora on leaves of *Eucalyptus* sp after immersion in water was studied and it was shown that phylloplane population declined quite rapidly. There was evidence that the decaying leaves were colonized by *Fusarium solani* and *Pythium proliferum*.

Keywords: Water; mud; temperature; pH; electrical conductivity; *Eucalyptus* sp.

*Corresponding author: E-mail: mudassirisrar@hotmail.com;

1. INTRODUCTION

Fungi presented in soil and have been recovered from a wide range of aquatic habitats including lakes, streams, distribution systems, drinking water and also on the surface of drinking water reservoirs [1]. Water fungus is habitually minute living organisms, which have do not have fruiting bodies however they reproduce asexually. The fungi in aquatic habitat grow very abundantly and require specialized technique for their culture and identification. The fungal diversity plays important role in determining environmental pollution. Water bound fungus are usually recognized like "Phycomycetes", which are a specific group including "True fungi (Eumycota)" and fungi resembling living things that is correlated with Chromista. Previously the kingdom of fungi comprises of; Bacteria, Amoeba and Mysomycetozoea, these are now placed/ documented as separate Taxa. At the same time the "Fungi perfect" are the allied group of animals such as "Oomycetes" these are chemically and biologically dissimilar fungus groups, however having similar shape size and habitat and ecology [2]. Two other workers [3,4] reported that there are about 200 species that grows on fresh water, mud and soil habitats and many of these are parasitic in nature. About 1.5 million species of fungus flora including species from soil and out of these approximately 3000 fungal species were recognized as a aquatic fungi and only 465 species are reported from sea waters [5,6]. Fungi associated with leaves decomposing in streams have received considerably more attention than any other fungal decomposers in aquatic ecosystems [7,8]. Dick, 2001 has suggested a role in leaf decomposition for the Oomycetes, but to date significant involvement in the process has not been convincingly demonstrated [9]. The water fungi which characteristically decompose the remains of leaves and timber by their network of hyphal structure are identified as "Aquatic Hyphomycetes". The hyphomycetes of water are grow in fresh, healthy, oxygen containing and moving waters as prescribed by other researchers [10,11]. Water habitat are diverse from place to place, time to time and much fluctuate in their physic-chemical and facial appearance. As a result, symphony and quality and quantity of water fungi is different considerably between these habitats as also described by other authors [12]. Fungi are ubiquitous and can easily grow in all kinds of environment and habitats found in nature, occasionally as symbiotic in nature and usually as molds. The occurrence of fungi growing

epiphytically on plants in ponds and lakes has nuisance value and their identification and ecology requires additional research [13].

Hanna Lake is one of the foremost attractions near Quetta, capital city of Balochistan, the largest province of Pakistan as shown in Fig. 1. The lake is situated 14 km from Quetta in the Urak valley. Hanna Lake is a natural lake a dam was constricted on it in 1894 at the time of British Empire. On the lakesides there are beautiful picnic attractions for children, eating places with decorated swings under the shadow of Pine and Eucalyptus trees. The water from Urak valley is diverted and stored in this little dam, this water is coming from Zargoan mountainous range through streams to the Hanna Lake.



Fig. 1. A view of Hanna Lake
(Internet source)

The core aims and objects of this study were to isolate and identify the micro fungi of Hanna Lake and to find out the chemical properties of water that effect micro fungal flora. This study was also undertaken to determine the possible role of fungi in the decomposition of allochthonous leaves in lake water.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 180 water, submerged leaves and mud samples collected in the sterile bottles with screw-capped from under water surface of Hanna Lake on monthly basis from June to December 2015. Three different parts of Hanna Lake were selected for the water and mud samples. Bottles with their caps were dipped about 30 cm in side the water surface and then capes were removed inside for the water to collect water. Fully filled bottles with water were recapped, while still in water. The water samples were stored in the refrigerator until analysis was done. The temperature and the pH level were recorded at the spot.

2.2 Fungi Isolation by Baiting Technique from Water, Leaves and Mud Samples

For the identification of aquatic fungi from Hanna Lake water, submerged leaves and mud samples were collected. From each samples 50 ml of water, a leaf and 5gm of mud was taken and were introduced into a sterile, clean, 12 cm Petri-dish (3 replicates). To the samples in each Petri-dish 20 ml of sterile distilled water was added to soak the content. Few sterilized hemp-seeds and *Zea mays* grains were introduced into each as baiting substances dish [14]. The dishes were incubated at room temperature for 24-48 hours than the colonized seeds and grain were transferred to sterile distilled water in Petri-dishes which contained crystalline Penicillin (2000 units/L-water) to depress the bacterial growth only [15]. The Petri dishes were then incubated at room temperature for about 4-6 weeks to get maximum fungal growth colonizing the seeds and grains these were examined on weekly basis. After each examination, the colonized seeds and grains were again transferred to clean sterile Petri-dishes containing sterile distilled water.

2.3 Identification of Fungi

The aquatic fungi recovered from mud and water samples were purified on glucose-peptone (GP) agar medium [16]. For identifications of fungal flora subsequent references were used for the present inquiry [17-28].

2.4 Decomposition of Leaf Material in the Lake Water

The leaves of *Eucalyptus tereticornis* L. were often blown by wind into the lake during the autumn, which could be seen floating on surface and on the bottom in large number, leaves from trees surrounding the lake together with the fallen parts from other sources e.g. dead organic plants (Macrophytes and Algae), dead animals and animals feces appeared to form the bulk of the organic input to the lake. Leaves falling in to the water already possess a mycoflora and the main objective of this part of the study was to determine whether or not the existing leaf mycoflora population played any role in decomposition of leaf materials in water. Therefore, it was suggested to check the changes that occurred in the existing leaf fungal population and subsequent colonization of the leaves by aquatic fungi when leaves from tree were submerged in lake water, for this study

Eucalyptus leaves was collected in a sterilized jar from the lake by a standardized technique. These leaves and water were taken to the laboratory. About 100 randomly leaves were put in to the jar containing lake water. The jar was left on a laboratory at room temperature for 30 weeks. Five leaves were taken from the jar at the interval of 2, 6, 14, 30 weeks, leaves were washed with sterilized distilled water. 5 discs were punched from each leaf and were placed on nutrient agar plates. The fungal populations on these leaves were determined.

2.5 Correlation Determination

Statistical Correlation with pH, Temperature, Electrical Conductivity and total number of Fungi isolated from water and mud sample was calculated by axel program in computer.

3. RESULTS AND DISCUSSION

The results obtained in this study showed that there were variety of fungi present in water and mud from Hanna Lake. It was reported that the range of species obtained was much more limited. A total of 14 and 11 species of fungal flora were recorded from water and mud, respectively (Tables 1 and 2). Among more frequently isolated species in the lake were *Achyra* sp, *Aspergillus niger*, *Aspergillus flavipes*, *Botrytis cinerea*, *Cladosporium* sp, *Fusarium solani*, *Fusarium culmorum*, *Mucor himalis*, *Penicillin expansum*, *Penicillium chrysogenum*, *Pythium proliferum*, *Phoma* sp, *Saprolegnia ferax*, *Rhizopus nigricans* and *Alternaria* sp. These species however represented only a relatively small portion of the total mycoflora. Similar observations were reported by others [29-32] they found only 31 fungal species belongs to 20 fungal genera out of total 389 fungal colonies. They also reported that *Aspergillus niger* was most frequent species with (83.33%), *Rhizopus* sp. (75%) followed by *A. flavus* and *A. fumigatus* with (58.33)% frequency. In most respect the types of fungi isolated from mud and water samples of Hanna Lake were essentially similar. The observation of other researcher [33] supported these results. They exhibited that the water fungi healthier from the mud samples of river Nile were almost similar to those reported in the water sample of river Nile ((Egypt) collected from same locations. A part from, *Fusarium solani*, *Fusarium culmorum*, found in Hanna Lake, all the fungi recorded from water sample were envoy of lower fungi. Most of those

presented were *Saprolegnia* and *Achya* sp. El-Hissy 1982 [33] reported a total of 7 species of *Saprolegnia* when they were investigating the Nile water. Laila and Nasser [34] also found that the genera *Aspergillus* (9 species out of 45 species, 166 colonies out of 400 fungal total counts) and *Penicillium* (7 species, 68 colonies) had the greatest diversity of the isolated species as well as the highest fungal total count. As per as mud samples were concerned, a different range of species apart from *Fusarium* spp were found when results were compared with water samples. The records of fungi from mud and water showed some evidence of a seasonal pattern in occurrence. Some species occurred constantly throughout the sampling period, for example *Fusarium solani*, *Saprolegnia ferax* and *Pythium proliferum* as shown in Tables 1 and 2.

The total numbers of species were larger during August and September, and the micro-fungal population was declined during late autumn. In contrast to this observation, EL-Shaeouny in 1980 [35] reported that in the soils of Egypt *Pythium* was comparatively common in the mounths of cold seasons and approximately they were missing during months of summer.

The data illustrated in Table 3 indicated that the majority of fungi isolated from decomposing leaves of *Eucalyptus* sp were found to be more or less the same as found in water and mud, e.g. *Aspergillus* spp, *Penicilium* spp, *Pythium*, *Fusarium* spp, *Alternaria* sp. These results were more or less the same as recorded by other researchers [36]. This shows that there is no

Table 1. Monthly recorded fungi isolated from water samples of Hanna Lake (Percentage frequency)

	Name of species	June	July	Aug	Sept	Oct	Nov	Dec
1	<i>Achya</i> sp	1	4	6	10	-	-	3
2	<i>Aspergillus flavipes</i>	2	-	8	-	10	-	-
3	<i>Aspergillus niger</i>	-	7	9	3	8	2	3
4	<i>Cladosporium</i> sp	9	9	1	10	1	1	-
5	<i>Fusarium solani</i>	6	14	11	1	5	3	-
6	<i>Mucor himalis</i>	-	-	-	-	-	14	-
7	<i>Penicilium chrysogenum</i>	-	3	8	1	4	2	-
8	<i>Pythium proliferum</i>	12	13	16	5	22	-	-
9	<i>Saprolegnia ferax</i>	2	3	2	1	1	16	4
10	<i>Alternaria</i> sp	-	10	4	14	3	17	1
11	<i>Botrytis cinerea</i>	-	3	2	-	-	-	4
12	<i>Penicilium expansum</i>	-	3	2	4	-	-	-
13	<i>Penicilium chrysogenum</i>	6	5	9	-	2	-	-
14	<i>Rhizopus nigricans</i>	-	-	1	3	-	3	2
	Total No. of species isolated	7	11	13	10	9	8	6

Table 2. Monthly records of fungi isolated from mud samples of Hanna Lake (Percentage frequency)

	Name of Species	June	July	Aug	Sept	Oct	Nov	Dec
1	<i>Alternaria</i> sp	2	-	3	14	9	5	6
2	<i>Aspergillus flavipes</i>	1	-	4	3	7	-	8
3	<i>Aspergillus niger</i>	-	1	1	10	5	6	-
4	<i>Botrytis cinerea</i>	-	1	2	5	-	-	-
5	<i>Cladosporium hrbarium</i>	-	2	4	-	10	-	-
6	<i>Fusarium solani</i>	1	8	3	4	5	3	10
7	<i>Fusarium culmorum</i>	-	2	1	2	-	3	6
8	<i>Phoma</i> sp	-	2	10	4	10	-	10
9	<i>Pythium proliferum</i>	3	6	5	15	6	4	-
10	<i>Penicilium chrysogenum</i>	1	2	-	3	-	10	-
11	<i>Sterile hypha</i>	-	-	10	4	10	-	-
	Total no. of species isolated	5	8	10	10	8	6	5

clear cut distinction between truly aquatic, semi aquatic and terrestrial fungi. The maximum number (6-7) of decomposer species on were noted during 6, 14 and 30th weeks and most frequently distributed species were *Fusarium solani*, *Mucor hiemalis*, *Penicilium* sp (Table 3). Many zoo-sporic fungi commonly associated with aquatic habitats and considered for a long time, as being confined to water, have been shown by a number of authors [16]. These species were regarded as aquatic and soil saprobes or plant parasites [37].

The parameters such as: pH, Temperature and Electrical Conductivity thought to be important factors that are threatening the biodiversity, ecosystem functioning and the distribution of micro-flora in water [38-40]. An increase in temperature generally increases metabolic rates [41] probably explaining the accelerated leaf decomposition driven by aquatic fungi at higher temperatures [42-44]. In this study, the pH, Temperature, Electrical Conductivity were recorded and found to be uniform through out the study period in mud and water samples (Tables 4 & 5). This was not surprising since the lake was characterized by widespread currents, wave action and lake was thermo-cline throughout the year. Even though variation in flora and Physico-chemical values on any given date were slight, seasonal variations were great. Values of pH were higher in summer (8.0-8.2) as presented in Tables 4 & 5 and early fall, which was possibly a response to the unusually high number of Algae

present during these months. Sparrow (1960) [20] reported that depletion of dissolved CO₂ by Algae (Predominantly HCO₃ at the prevailing pH of this Lake), can raise the pH considerably during the summer months. Anthropogenic acidification has deleterious effects on both structure and functioning of surface water ecosystems [45]. In the present study the greater number of species isolated during the spring and fall (10-13) from water samples and (8-10) from mud samples. This might be accounted for, in part, by fungal propagates being transported into the Lake by run off water carrying nutrients and decaying plant debris. The correlation between pH and Fungal population in water and mud sample was 0.59 and 0.55% (Table 6). These results express the highest micro-fungal population during august (13 and 10) in water and mud sample, respectively and the number of species become declined towards the more acidified (December, pH 6.3) and alkalinity (June, 8.2) condition as illustrated in Table 4 and Tolkkinen in 2015 [46] stated that the naturally acidic streams supported distinct fungal assemblages with many OTUs (operational taxonomic unit) unique to thee streams. In this study the species which were isolated from mud and water were broadly similar with little variation as the statistical Correlation value (0.89%) indicated in Table 6. In Egyptian soils is in the support of these results [35]. On the other hand some species were only appeared at certain times of the periods. Among these were *Botrytis cinerea*, *Cladosporium* sp, which were

Table 3. Fungi isolated from the leaf discs (Percentage frequency)

Name of species	Weeks				
	0	2	6	14	30
1 <i>Alternaria</i> sp	7	6	-	-	-
2 <i>Aspergillus niger</i>	3	-	5	4	-
3 <i>Botrytis cinerea</i>	-	2	8	3	-
4 <i>Fusarium solani</i>	5	6	11	5	4
5 <i>Mucor flayus</i>	-	-	4	-	5
6 <i>Mucor hiemalis</i>	-	-	-	11	3
7 <i>Penicilium</i> sp	2	-	3	3	11
8 <i>Pythium proliferum</i>	-	-	-	4	5
9 <i>Rhizopus nigricans</i>	-	5	6	5	3
Total no. of species	4	4	6	7	6

Table 4. Monthly, temperature, pH, electrical conductivity (variation) and total number of fungi species obtained from water samples

Parameters	June	July	Aug	Sept	Oct	Nov	Dec
pH	8.2	8.1	8.0	8.1	8.0	7.4	6.3
Temperature °C	28	33	25	23	20	15	12
Electrical conductivity	0.25	0.55	0.75	0.45	0.95	0.28	0.48
Total no. of species	7	11	13	10	9	8	6

Table 5. Monthly, temperature, ph, electrical conductivity (variation) and total number of fungal species obtained from mud sample

Parameters	June	July	Aug	Sept	Oct	Nov	Dec
pH	8.2	8.1	8.0	8.1	8.0	7.4	6.3
Temperature °C	28	33	25	23	20	15	12
Electrical conductivity	0.25	0.55	0.75	0.45	.95	0.28	0.48
Total no. of species	5	8	10	10	8	6	5

Table 6. Correlation with pH, temperature, electrical conductivity and total number of fungi isolated from water and mud sample (%)

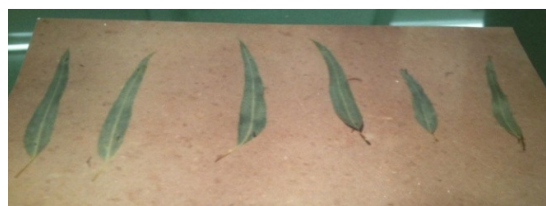
Samples	Correlation between pH and no. Sp	Correlation between temperature and no. Sp	Correlation between electrical conductivity and no. Sp	No. of Spp correlation between water and mud samples
Water sample	0.59	0.57	0.50	0.89
Mud sample	0.55	0.37	0.55	

particularly evident during summer and early autumn. Further the occurrence of species with the highest frequency in the summer and autumn. Their absence from samples collected during late winter and spring showed great seasonal variation. The pH during summer and autumn ranged from 8.0-8.2. The temperature was also ranged from (20-24°C). During late autumn the pH was (6.3). The temperature was recorded as 12°C (Tables 4 & 5).

Perhaps these were the major agents which checked the micro-fungal distribution. Many other researchers [47,42,43] reported that the Temperature affects the distribution, growth and reproduction of aquatic hyphomycetes. In common it is able to be realistic that water loving fungi depicted from the mud samples of Hanna Lake was almost similar to those detected in the water and mud of river Nile [33]. In this study *Botrytis cinerea*, *Cladosporium herbarium*, *Fusarium* spp, normally regarded as terrestrial fungi, was frequently isolated from the water of lake. It has been observed [20] that fungi such as *Phytophthora* and *Pythium* survive well in aquatic as well as terrestrial habitats and these may be regarded as semi-aquatic or amphibious fungi, having the characteristics of aquatic as well as terrestrial fungi. In this study a number of fungi from water and mud samples were recorded. Some were found relatively in frequently and others with great regularity, e.g. *Alternaria* sp, *Fusarium*, *Saprolegnia* and *Pythium* etc.

Although no cultural studies were undertaken to confirm the idea, it is tempting to suggest according to Park's since, that those fungi which were found constantly may represent in dwellings

or true aquatic fungi. *Fusarium solani* generally recorded as typical terrestrial fungus was also isolated throughout sampling period from water and mud. The rest of fungi which were isolated in frequently did not apparently fulfill park's criteria for truly aquatic fungus. These may perhaps be regarded as immigrant forms with only transient existence in water. Supporting evidence for this view comes from the observation that in this study the occurrence of some spp with high frequency is apparently correlated with autumn leaf fall, including among those spp, as *B. cinerea*, *Cladosporium* sp. Further investigation in and on substrates in water would seem to be a field in which further useful study should be made.

**Fig. 2. Eucalyptus tereticornis L. leaves collected from Hunna Lake****Fig. 3. Decomposing leaves**

4. CONCLUSION

In conclusion, our results indicated that the flora of Hanna Lake was essentially same and similar to those recorded by other authors for temperate lakes and river. Data suggest that pH, temperature and substratum (leave) are important drivers of fungal mediated leaf decomposition and fungal distribution, with leaf quality being more important than temperature. Fungal frequency tended to be higher in the alkalinity as compared to acidity. Best Temperature and pH level for fungal richness in mud and water body is 25-33°C and 8.0-8.1, respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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