

Diversity of zoosporic and terrestrial fungi in As-Sant Canal at Assiut, Egypt

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Abstract: Monthly samples were collected from water and submerged mud of As-Sant Canal 5 km North East of Assiut City, Egypt during the period from November 2012 to October 2013. Using sesame seeds as baits, 18 species belonging to 7 genera of zoosporic fungi were isolated from water. The broadest spectrum of these fungi (7 species) was observed in January whereas the narrowest (3 species) was in May. *Achlya* followed by *Saprolegnia* and *Dictyuchus* were the most common genera being recovered through 11, 9, and 7 months respectively. The genus *Achlya* prevailed during August to October 2013 due to the high counts of *A. klebsiana*. *Saprolegnia* was dominant during November 2012 to May 2013 being affected by the counts of *S. ferax* and *S. furcata*. *Dictyuchus monosporus* was appeared in relatively high counts during June. Other fungi including *Aphanomyces*, *Pythium*, *Phytophthora* and *Thraustotheca* were less frequently observed. Terrestrial mycobiotawere represented by 112 species and 49 genera in case of water samples and 99 species belonging to 45 genera in case of submerged mud. *Acremonium*, *Aspergillus*, *Fusarium*, *Galactomyces*, *Mucor*, *Penicillium*, *Talaromyces* and *Trichoderma* were the most common genera in water and mud samples.

Keywords: Zoosporic and terrestrial fungi, fresh water and submerged mud.

Introduction

Research regarding the abundance and taxonomic identification of aquatic fungi in various types of waters and particularly in rivers and lakes with varied trophic status (Pietryczuk *et al.*, 2013, 2014) has been increasingly frequent in the recent years. Fungi can grow on dead plant remains and decompose cellulosic materials of seeds, fruits, petals, leaves, twigs and other elements of plants fallen into water (Wurzbacher *et al.*, 2010). Many aquatic fungal species have been reported as potential fish pathogens (Fadaeifard *et al.*, 2011) and are involved in superficial and systemic diseases to animals and humans (Hageskal *et al.*, 2009). Fresh water fungi comprise hundreds of species that are fully adapted to the aquatic environment as well as many terrestrial fungal species that are translocated from the soil into water and end up growing in the water just like other aquatic fungal species. Sources of terrestrial fungi into fresh water habitats are animals, plants and soil. The occurrence, distribution, and seasonal variations of aquatic fungi in relation to water and soil characteristics, as well as to the various geographical regions all over the world have been carried out (Rattan *et al.*, 1980; El-Hissy and Khallil, 1989 and Czeżuga, 1996). The fresh water fungi including those of strictly aquatic habitats and those of terrestrial habitats are commonly found in pools, ponds, lakes, rivers and streams. Many researchers noted the dependence of the growth and development of zoosporic fungi on the season. Some species develop predominantly during warm seasons, others during cold seasons (Dasgupta and Rachel, 1990; Pystina, 1998). Several zoosporic and

terrestrial fungi were recently recovered from some rivers of central Europe by Pietryczuk *et al.* (2018). Representative of *Achlya*, *Pythium* and *Saprolegnia* (zoosporic) as well as *Acremonium*, *Alternaria*, *Aspergillus*, *Exophiala*, *Microsporum* and *Penicillium* were found in these rivers. The prevalence of these genera in water samples from Iraq (Shaker and Sharif, 2012), India (Smily *et al.*, 2014; Thomas and Sangauel, 2017) and Brazil (Gomes *et al.*, 2008; Silveira *et al.*, 2013; Doi *et al.*, 2018) has been confirmed.

In Egypt, zoosporic fungi have been isolated and studied from waters of the River Nile, Ibrahimia canal, in some ponds and irrigation canals (El-Hissy, 1974; El-Hissy *et al.*, 1982; El-Nagdy and Abdel-Hafez, 1990; El-Nagdy and Khallil, 1991; Khallil *et al.*, 1991). The occurrence and distribution of aquatic fungi were investigated by El-Hissy and Abd-Elaah (1989) in 315 soil samples collected from all over Upper Egypt from Aswan to El-Giza including El-Fayoum Governorate, some aquatic fungi (*Achlya americana*, *Dictyuchus sterile*, *Saprolegnia parasitica*, *Saprolegnia ferax*, *Pythium* sp. and *Allomyces arbuscula*) were isolated from water, mud and soil samples. Nine years later, Abd-Elaah (1998) identified 19 species recovered from 14 soil samples collected from different sites of the Red Sea Governorate, the aquatic fungal genera were *Allomyces*, *Dictyuchus*, *Saprolegnia* and *Pythium*, while the terrestrial fungal genera were *Aspergillus*, *Penicillium*, *Fusarium*, *Neurospora* and *Rhizopus*. Furthermore, Abd-Elaah (1999) isolated 36 species, which belong to 18 fungal genera from cultivated clay soil at Sohag. The isolated fungi included *Allomyces*, *Saprolegnia*, *Achlya*, *Pythium*,

Dictyuchus and *Isoachlya*. In a subsequent investigation thirteen species belong to six genera of zoosporic fungi were isolated from 30 soil samples collected from Sohag city. The dominant species were *Allomyces*, *Saprolegnia*, *Achlya* and *Pythium* (Abd-Elaah and Galal, 2006).

The aim of the present work is to study the biodiversity of zoosporic and terrestrial fungi in the fresh water and submerged mud of As-Sant irrigation Canal near Assiut City. Prevalence and monthly variations of the counts of these fungi were also investigated. Contamination of water with certain fungal species involved in human, animal or fish diseases was also evaluated.

Materials and methods

Collection of water and submerged mud samples

Monthly samples were collected from As-Sant irrigation Canal approximately 5 km North East of Assuit city, Egypt during the period from November 2012 to October 2013. A total of 24 samples were taken from water and submerged mud (12 samples for each). Water and submerged mud samples were kept in sterile one litre capacity glass bottles. All samples were transferred to the laboratory and preserved in a fridge till mycological analysis.

Physicochemical characteristics of water and submerged mud

Monthly measurements of temperature and pH of water, moisture content and pH of submerged mud were recorded. Water temperature was measured in situ using a thermometer. A pH meter (Jenway 3510, pH/mV/Temperature bench meter, Essex, CM6 3LB, England) was used to measure the pH of water and diluted (1:5) mud samples. Moisture content of mud was calculated by subtracting the dry weight of mud (24 hrs at 105°C) from the fresh weight (50 g) and the data were represented as percentages.

Isolation of Zoosporic fungi from water samples:

Aliquots of 30 ml of water samples were poured into sterilized 10 cm diameter Petri-dishes in which 10 seeds of sesame were placed as baits (El-Hissy and Khallil, 1989). Five replicates were prepared for each sample and the seeded plates were incubated at 20°C. After 24 and 48 hours baits colonized with fungal hyphae were washed with sterilized distilled water and transferred to Petri-dishes containing sterile water to which chloramphenicol (250 mg antibiotic/l) was added to suppress bacterial growth. The incubation period extended to 4 weeks to allow good growth and sporulation of zoosporic fungi. Colonized sesame seeds were counted and the data were expressed as the number of colonies per 25 seeds.

Isolation of terrestrial fungi from water samples:

For the recovery of terrestrial fungi, 1 ml of each water sample was transferred to 10 cm sterile Petri dish followed by addition of 20 ml of an appropriate agar medium just above solidifying temperature. Five replicates were prepared and incubated at 28°C for 1-2 weeks during which the developing fungi were counted and isolated.

Isolation of terrestrial fungi from submerged mud samples

The dilution-plate method was used for enumeration of different fungal species as described by Johnson and Curl (1972). After culturing and incubation at 28°C for 1-2 weeks the growing fungi were counted as CFU/gram of fresh mud.

Media used for isolation of fungi

a- Dichloran Rose Bengal Chloramphenicol Agar (DRBC)

This medium was prepared as described by King *et al.* (1979) with the following composition: (g/l) peptone, 5.0; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.5; glucose, 10.0; agar, 15.0, to which rose bengal (25 µg/ml) and chloramphenicol (100 µg/ml) as bacteriostatic agents and dichloran (20 µg/ml) were used which restricts the growth of mucoraceous species without affecting the other species.

b- Dichloran Chloramphenicol Peptone Agar (DCPA)

This medium contained (g/l) peptone, 15; KH₂PO₄, 1; MgSO₄.7H₂O, 0.5; dichloran (20 µg/ml), 1 ml; chloramphenicol, 0.2 mg; agar, 20. The medium was sterilized at 121°C for 15 min; the final pH was 6.2 (Andrews & Pitt, 1986).

c- Cellulose-Czapek's Agar

To allow the growth of cellulose decomposing fungi a medium described by Eggins & Pugh (1962) was prepared. The following ingredients were used (g/l): cellulose powder, 10; NaNO₃, 3.0; K₂HPO₄, 1.0; MgSO₄.7H₂O, 0.5; KCl, 0.5; FeSO₄.7H₂O, 0.01; agar, 20; chloramphenicol, 0.25 (as a bacteriostatic agent).

Morphological identification of fungal genera and species

Identification of fungal genera and species was done according to their macro- and microscopical characteristics as described by Coker (1923), Johnson (1956), Seymour (1970), Waterhouse (1956), Scott (1961), Morton and Smith (1963), Raper and Fennell (1965), Ellis (1971, 1976), Pitt (1979), Plaats-Niterink (1981), Moubasher (1993), Gams and Bissett (1998), de Hoog *et al.* (2000), Zare and Gams (2004), Leslie and Summerell (2006) and Domsch *et al.* (2007).

Molecular identification of fungal isolates

Fungi were individually grown in plates containing Czapek's yeast agar (CYA) incubated at 28°C for 7 days. A small amount of fungal growth was scraped and suspended in 100 µl of distilled

water and boiled at 100°C for 15 minutes and stored at -70°C. Dead fungal cultures were then sent to SolGent Company, South Korea for DNA extraction as well as amplification and sequencing of rDNA. Two universal primers ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5' - TCC TCC GCT TAT TGA TAT GC - 3') were used (White *et al.*, 1990). Gene amplification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTP (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25 µl, template 1.0 µl, DW to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20 sec, annealing at 50 °C for 40 sec and extension at 72°C for 1 min, with a final extension step of 72°C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. The purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1 % agarose gel. Then these bands were eluted and sequenced in the sense and antisense directions.

Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained were subjected to Clustal W

analysis using MegAlign (DNASStar) software version 5.05 for the phylogenetic analysis (Thompson *et al.*, 1994).

Results and discussion

Physicochemical characteristics of water and submerged mud

Regular and marked variations were observed in the water temperature with the water temperature with the highest (33°C) being recorded in July 2013 (one of the summer months) and the lowest during December 2012 and January 2013 (winter months) as shown in table (1). The water pH was generally in the alkaline side with the highest value (8.79) being recorded in November 2012 and the lowest (6.92) in May 2013.

The pH values of submerged mud were almost similar to those of water ranging between 6.55 in May 2013 and 8.43 in July 2013. The Moisture Content of submerged mud ranged from 20.7 % in July 2013 and 49.3 % in March 2013. During 8 out of 12 months experiment, the moisture content was over 30 %. As mentioned by Voronin (2008) the maximal diversity of saprolegnial fungi (except for the species of the genus *Achlya*) was observed at a temperature range of 12.2 to 18.0 °C. At low temperatures, 0.1°C to 0.9°C, species of *Achlya*, *Aphanomyces* and *Dictyuchus* were significantly more frequent. The species *Aphanomyces laevis*, *Dictyuchus monosporus* and *Saprolegnia parasitica* are listed as eurythermic and are often found at water temperatures ranging from 0.1°C to 23°C.

Table 1: Physicochemical characteristics of water and mud samples during the period From November 2012-October 2013

Samples characteristics	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Water Temperature	19	18	18	18.5	19	24	25	25.5	33	29	25.5	25
Water pH	8.79	7.97	7.08	7.15	8.11	8.48	6.92	7.18	8.21	7.33	7.18	7.75
Mud pH	7.97	8.08	6.99	6.58	7.60	8.45	6.55	7.34	8.43	7.71	7.69	7.95
Mud Moisture Content	36.6	30.9	31.9	44.9	49.3	36.6	47.2	23.0	20.7	24.1	40.96	26.86

Diversity of zoosporic fungi in water samples

Using sesame seeds, it was possible to catch 18 species belonging to 7 genera of zoosporic fungi. The broadest spectrum of these fungi (7 species) was found in January whereas the narrowest (3 species) was in May (Table 2). *Achlya* followed by *Saprolegnia* and *Dictyuchus* were the most common genera being recovered during 11, 9, and 7 months respectively. *Achlya* species attained their highest counts during August to October 2013 when the water temperature ranged from 25°C to 29°C. The counts of *Achlya* were relatively high

during April to July 2013 when the temperature fluctuated between 24°C to 33°C. Five species of *Achlya* were isolated of which *A. klebsiana* occurred in relatively high counts during August and September 2013. *A. debaryana* appeared in high counts during April, May and July 2013. *A. diffusa*, dominated in September 2013. The genus *Saprolegnia* prevailed during the period from November 2012 to May 2013 when the water temperature ranged from 18°C to 25°C. It was represented by five species of which *S. ferax* and *S. furcata* were more frequent especially during the

period from November 2012 to April 2013. *Dictyuchus* was encountered in relatively high counts during June, July and September 2013 recording its peak in June due to the high counts of *D. monospora* and *Aphanomyces* sp. was moderately recovered from 6 water samples with relatively high counts in December 2012 and July 2013. *Pythium* was intermittently encountered during the period of study registering its peak in June 2013. Two species of *Pythium* were identified as *P. middleton* and *P. vexans*. Species of *Phytophthora* and *Thraustotheca* were of low incidence in the tested water samples. Indian researchers Gupta and Mehrotra (1989) distinguished four groups of zoosporic fungal species in terms of their dependence on temperature: fungi that prefer low temperatures of 14.2°C to 18.0°C; low to moderate temperatures of 14.2°C to 30.0°C; moderate to high temperatures of 21.4°C to 34.8°C; and eurythermic species (14.2°C to 34.8°C).

Zoosporic fungi are found in all kinds of aquatic habitats. They are saprobes or parasites on animals or plants and can be isolated from its environments by baiting technique, where baits like pollen grains, insect larvae and cellulose are placed in streams and lakes. Seasonal variations and occurrence of zoosporic fungi in various water areas were studied by several investigators. Reports from Poland (Kiziewicz, 2006) revealed the isolation of various zoosporic fungal species belonging to *Achlya*, *Aphanomyces*, *Dictyuchus*, *Pythium* and *Saprolegnia*. *D. monosporus* and *S. ferax* were among the common fungi in Polish water. Godlewska *et al.* (2009) performed a quantitative analysis of species composition of fungi and straminipilous organisms in four ponds situated in Białystok, Poland. They obtained several fungal species belonging to the genera *Achlya*, *Aphanomyces*, *Pythium*, *Saprolegnia* and *Thraustotheca*. Some fungi such as *Achlya klebsiana*, *Dictyuchus monospora* and *Saprolegnia ferax* which were identified from Polish Ponds occurred also in As-Sant canal near Assiut. Recently Pietryczuk *et al.* (2018) isolated several zoosporic from some rivers of central Europe (Augustów Lakeland, NE Poland) which included *Achlya americana*, *A. flagellata*, *A. oligacantha*, *Pythium debaryanum* and *Saprolegnia parasitica*. In Iraq Farkha and Ahmadi (2011) isolated many species of aquatic fungi from surface water of streams and springs. They noticed that the maximum number of fungal species was recorded at autumn and winter months. The most dominant species were belonging to *Saprolegnia*, *Pythium*, *Achlya*, *Allomyces* and *Aphanomyces*. The interaction of physicochemical factors greatly influences the occurrence of various species of aquatic fungi.

In Egypt, El-Hissy *et al.* (2000) isolated seventeen aquatic fungi from fresh water samples collected from Aswan High Dam Lake (AHDL) during the period from November 1992 to October 1993. Monthly and vertical (from surface to 20 m depth) samples were taken to determine fluctuations of these fungi in the lake (10 km south of Aswan High Dam). The physicochemical characteristics of the collected watersamples were also taken. The fungal population showed marked vertical variations during the period of study. Surface water samples yielded the highest number of genera and species (8 genera, 13 species), while water samples collected from near the bottom (16-20 m deep) were poor (3-4 genera or species). This reduction in fungal taxa was correlated markedly with the reduction in the amount of dissolved oxygen and organic matter. The most common genera were *Achlya*, *Aqualinderella*, *Pythium* and *Saprolegnia* (moderate occurrences). *Allomyces*, *Aphanomyces*, *Dictyuchus* and *Pythiopsis* were of rare occurrence and irregularly distributed in vertical strata. During the previous study water and submerged mud was receiving industrial effluents of Kima Factory for fertilizers near Aswan City, Egypt, El-Hissy *et al.* (2001) found that *Pythium* and *Saprolegnia* occurred in high incidence whereas *Aphanomyces* and *Dictyuchus* were less frequent. Ali and Abdel-Raheem (2003) identified 26 species belonging to 8 genera of zoosporic fungi from mud samples of Egyptian lakes of which *Aphanomyces*, *Dictyuchus*, *Pythium* and *Saprolegnia* were among the isolated fungi.

Diversity of terrestrial fungi in water samples

A total of 112 species belonging to 49 genera were recovered on the three medium types. The highest gross total count (459.6 CFU/ml in all samples) was obtained on DRBC medium whereas the lowest (373.8 CFU/ml) occurred on Cellulose-Czapek's agar as shown in (Table 3 and Figs 1 & 2).

a- On DRBC agar

It was possible to collect 81 fungal species belonging to 35 genera representing different fungal taxa. *Aspergillus* was the leading genus being found in all water samples accounting for 26.15 % of total fungal population. It was represented by 14 species of which *Aspergillus niger*, *A. brasiliensis*, *A. flavus* and *A. sydowii* were the most common (66.67 % - 91.67 % of samples). Each of *Mucor* and *Penicillium* appeared in 91.67 % of water samples matching respectively 3.87 % and 10.88 % of the total count of fungi.

Among the 3 species of *Mucor*, *M. circinelloides* and *M. racemosus* occurred in high incidence (91.67 % and 58.33 % of samples respectively).

Table 2: Counts (CFU/25 seeds) and Incidence (I out of 12 months) of zoosporic fungi recovered from fresh water of As-Sant Canal

Taxa	Month												Total		Incidence		OR
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	CFU	%CFU	I	I %	
<i>Achlya</i>	1	0.0	2	1	1	11	11	6	8	22	19	15	97	33.68	11	91.67	H
<i>A. Americana</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6	0.0	0.0	0.0	0.0	6	2.08	1	8.33	L
<i>A. debaryana</i>	0.0	0.0	0.0	0.0	0.0	11	11	0.0	8	0.0	0.0	0.0	30	10.42	3	25.00	L
<i>A. diffusa</i>	0.0	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	0.0	11	3.82	2	16.67	L
<i>A. klebsiana</i>	0.0	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0	17	9	3	30	10.42	4	33.33	M
<i>Achlya</i> sp.	1	0.0	1	1	0.0	0.0	0.0	0.0	0.0	5	0.0	12	20	6.94	5	41.67	M
<i>Aphanomyces</i> sp.	1	3	1	0.0	0.0	0.0	0.0	1	5	2	0.0	0.0	13	4.51	6	50.00	M
<i>Dictyuchus</i>	0.0	1	0.0	1	0.0	0.0	0.0	8	5	1	6	1	23	7.99	7	58.33	H
<i>D. monospora</i>	0.0	0.0	0.0	1	0.0	0.0	0.0	8	1	0.0	1	0.0	11	3.82	4	33.33	M
<i>Dictyuchus</i> sp.	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	4	1	5	1	12	4.17	5	41.67	M
<i>Phytophthora</i> sp.	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0	2	0.0	0.0	0.0	3	1.04	2	16.67	L
<i>Pythium</i>	0.0	0.0	2	0.0	0.0	2	1	6	2	0.0	0.0	4	17	5.90	6	50.00	M
<i>P. middleton</i>	0.0	0.0	0.0	0.0	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	2	0.69	1	8.33	L
<i>P. vexans</i>	0.0	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	0.69	1	8.33	L
<i>Pythium</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	1	6	2	0.0	0.0	4	13	4.51	4	33.33	M
<i>Saprolegnia</i>	20	19	19	22	24	12	12	4	0.0	0.0	0.0	5	137	47.58	9	75.00	H
<i>S. anisopora</i>	0.0	0.0	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	1	0.35	1	8.33	L
<i>S. ferax</i>	0.0	0.0	3	0.0	9	11	12	4	0.0	0.0	0.0	5	44	15.29	5	41.67	M
<i>S. furcata</i>	19	17	13	0.0	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	58	20.14	4	33.33	M
<i>S. uliginosa</i>	0.0	0.0	2	22	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25	8.68	3	25.00	L
<i>Saprolegnia</i> sp.	1	2	1	0.0	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9	3.13	4	33.33	M
<i>Thraustotheca</i> sp.	0.0	0.0	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	0.35	1	8.33	L
Total CFUs/ml	22	23	24	26	25	25	24	25	22	25	25	25	291	100.00	---	---	---
No. of genera (7)	3	3	4	5	2	3	3	5	5	3	2	4	---	---	---	---	---
No. of species (18)	4	4	8	5	5	4	3	5	6	4	4	5	---	---	---	---	---

CFU, Colony Forming Units; % CFU, their percentages; I, Incidence; OR, Occurrence Remark; H, High; M= Medium; L= Low.

The genus *Penicillium* was represented by 11 species of which *Penicillium aurantiogriseum* and *P. solitum* were the most common (83.33 % and 66.67 % of samples matching 8.09 % and 1.17 % of total fungal population respectively). The genera *Acremonium*, *Cladosporium*, *Fusarium*, *Galactomyces*, *Talaromyces* and *Trichoderma* were also encountered in high incidence (58.3 % - 83.3 % of samples). Two identified species namely *G. candidus* and *T. harzianum* in addition to white yeasts and sterile mycelia were recovered from 66.7 % - 83.3 % of samples. The following fungal taxa were moderately isolated during this part of study. These were *Aspergillus versicolor*, *Fusarium proliferatum*, *Mucor hiemalis* and *Talaromyces duclauxii* (50 % of samples for each). Other species as *Aspergillus terreus*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Penicillium chrysogenum* and *Talaromyces purpurogenus* appeared each in 41.67 % of samples. Also, *Alternaria alternata*, *Aspergillus nidulans*, *A. ochraceus*, *Fusarium solani*, *Penicillium oxalicum* and *Phoma* species were recovered from 33.3 % of the surface water samples of As-Sant Canal (Table 3).

b- On DCPA agar

As shown in table (3), the mycological analysis of fresh water samples revealed the isolation of 88 fungal species attributed to 43 genera.

Eight fungal genera occurred in high incidence namely, *Aspergillus* (91.67 % of samples), *Penicillium* (83.3 %), *Chaetomium*, *Galactomyces* and *Scopulariopsis* (75 % for each), *Talaromyces* (66.67 %) and *Sarocladium* (58.3 %).

The genus *Aspergillus* was represented by 15 species of which *Aspergillus flavus*, *A. niger* and *A. sydowii* were the most common (83.33 %, 75.0 % and 66.67 of samples respectively). The moderately occurring species were *A. nidulans* (50 %), *A. brasiliensis* and *A. versicolor* (41.67 % each). The remaining species of *Aspergillus* appeared in low incidence (8.3 % - 25.0 %).

Out of the 9 *Penicillium* species identified, only *Penicillium aurantiogriseum* and *P. solitum* were of high incidence being recovered from 83.3 % and 66.6 % of water samples respectively. The rest of *Penicillium* species appeared in low frequencies ranging from 8.33 % to 25.0 % of samples. *Chaetomium piluliferum*, *Galactomyces candidus* and *Scopulariopsis brevicaulis* were isolated from 75.0 %, 75.0 % and 58.3 % of samples respectively.

The following genera and species were moderately encountered from the tested water

samples: *Fusarium*, *Trichoderma*, *T. harzianum* (50.0 % of samples), *Acremonium*, *Humicola*, *Mucor*, *M. circinelloides*, *Sarocladium strictum* and *Talaromyces stipitatus* (41.6 % each), *Alternaria alternata*, *Cladosporium*, *C. cladosporioides*, *C. herbarum*, *M. hiemalis*, *M. racemosus*, *Phoma*, *Scopulariopsis candida*, *S. koningii*, *Talaromyces dauclauxii* and *Verticillium* sp. (33.3 % each).

Unidentified fungal species belonging to white yeasts (66.67 %) and sterile mycelia (41.67 %) were also isolated from fresh water samples. The remaining genera and species shown in table (3) were of low incidence.

c- On Cellulose-Czapek's agar

Over a period of 12 months, it was possible to isolate 63 fungal species belonging to 28 genera from fresh water samples cultured on Cellulose-Czapek's agar (Table 3). *Aspergillus* was the most prevalent genus being recovered from 100 % of water samples. It was represented by 13 species among which *Aspergillus flavus* and *A. niger* were the commonest (75 % of samples for each) and *A. nidulans* (58.33 %). *A. brasiliensis* and *A. sydowii* appeared in moderate incidence (50 % for each). The remaining species of *Aspergillus* occurred in low frequency.

Each of *Mucor* and *Trichoderma* appeared in 83.3 % of samples. The three representative species of *Mucor* were of high incidence (58.3 % - 75.0 % of samples). On the other hand, *Trichoderma harzianum* was the most common 75.0 % among the four species of the genus *Trichoderma*. *Fusarium*, *Penicillium* and *Scopulariopsis* occurred also in high incidence (75.0 %, 75.0 % and 58.3 % of samples respectively). Of these genera *F. proliferatum*, *P. aurantiogriseum* and *S. brevicaulis* appeared in 66.67 %, 75.0 % and 41.67 % of water samples respectively.

The following fungal genera and species were moderately recovered during this part of study; *Acremonium* sp., *Chaetomium piluliferum*, *Cladosporium*, *C. cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Emericellopsis minima*, *Sarocladium*, *S. strictum* and *Stachybotrys* in addition to unidentified yeasts and sterile mycelia. The remaining genera and species listed in table (3) were of low incidence.

Twelve fungal species appeared only on DRBC but were missed on DCPA and Cellulose agar: *Absidia cylindrospora*, *Acremonium potronii*, *Acrostalagmus luteoalbus*, *Aspergillus unguis*, *Exserohilum rostrata*, *Fusarium circinatum*, *Mycogone rosea*, *Paraconiothyrium estuarinum*, *Penicillium italicum*, *P. lividum*, *P. simplicissimum* and *Phoma herbarum*.

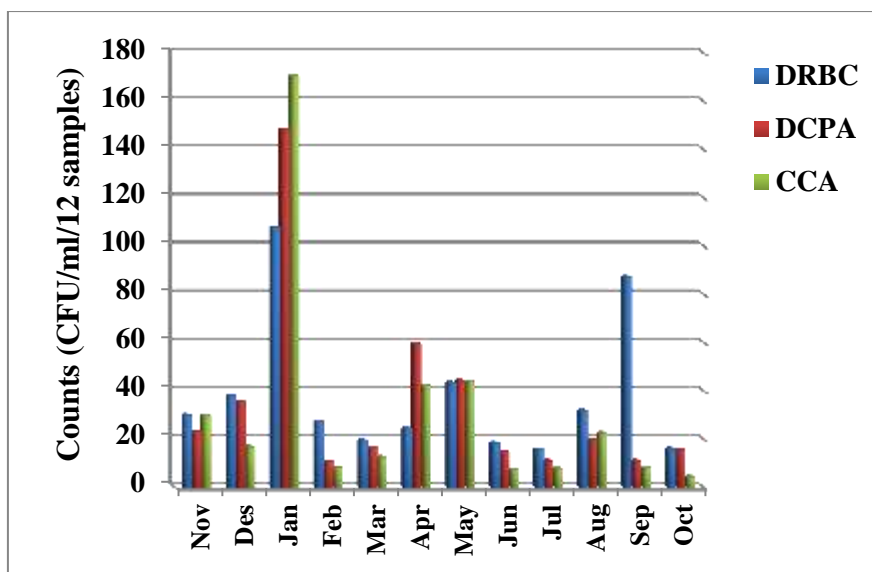


Figure 1: Monthly variations in the counts of terrestrial fungi in water samples.

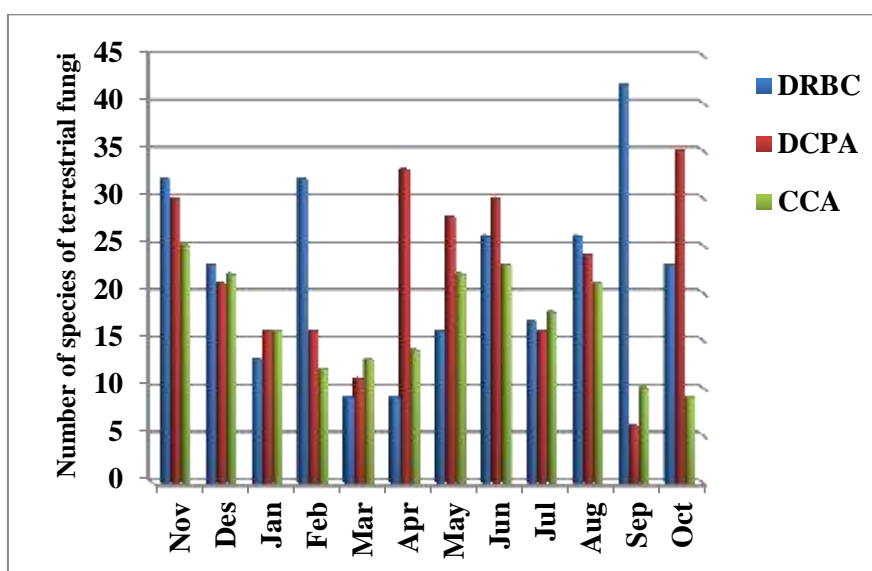


Figure 2: Monthly variations in the no. of species of terrestrial fungi in water samples.

On the other hand 5 fungal species occurred on DCPA and Cellulose but not on DRBC. These are *Aspergillus parasiticus*, *Emericellopsis minima*, *Purpureocillium lilacinum*, *Scopulariopsis brumptii* and *Verticillium* sp.

It appears that the medium composition affects to a great extent the prevalence of fungi where some ingredients enhance or reduce the vegetative growth of certain fungi. For example the genera *Galactomyces* and *Talaromyces* which prevailed on both DRBC and DCPA (8-9 months) appeared only in one month in case of Cellulose-Czapek's agar which did not contain peptone among its ingredients.

Similar results were reported from Brazil by Gomes *et al.* (2008) who analyzed water with high salinity and alkaline pH from Casa Caiada

Beach in Orlinda, Pernambuco State. They isolated 50 species of fungi; the most frequently isolated were *Aspergillus*, *Penicillium* followed by *Fusarium*, *Trichoderma*, *Cladosporium*, *Curvularia* and *Paecilomyces*. Other reports from Brazil (Silveira *et al.*, 2013) showed the isolation of *Paecilomyces* from water from an estuary of the Patos Lagoon in the state of Rio Grande do Sul. More recently, Doi *et al.* (2018) studied the density and diversity of filamentous fungi in the water of Araca Bay in Sao Paulo, Brazil. They reported that *Aspergillus* was represented in 54.5 % of the water samples, followed by *Penicillium* at 25.0 %. In the summer, 20.8 % of the fungal species found in the water were determined to be from the genus *Cladosporium* and 12.5 % were found to be from the genus *Aspergillus*. In Iraq, Shaker and

Sharif (2012) isolated both filamentous and yeast fungi from Al-Sader water treatment plant. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Cladosporium* spp., *Penicillium* spp., *Candida* spp. and *Rodotorula mucilaginosa* were listed among the common fungi. In Pakistan, Leghari *et al.* (2016) studied the mycobiota of water of Hanna Lake and reported that *Aspergillus niger*, *A. flavipes*, *Cladosporium* sp., *Fusarium solani*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Phoma* sp., *Rhizopus nigricans* and *Alternaria* sp. were more frequently isolated. In India, Singh *et al.* (2014) isolated 23 fungal species from Ganga River. Their list included *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *A. fumigatus*, *A. terreus*, *Alternaria alternata*, *Curvularia lunata*, *Chaetomium globosum*, *Fusarium oxysporum*, *Penicillium citrinum* and *Trichoderma koningii*. Also, Smily *et al.* (2014) reported that the fungi of the river system appeared to prefer the period from August to November within which the most preferred period was October. The common fungi comprised *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Curvularia lunata*, *Fusarium oxysporum*, *Penicillium citrinum*, *Rhizopus arrhizus*, *Trichoderma viride* and *Ulocladium chartarum*. Other reports from India given by Thomas and Sangavel (2017) revealed the prevalence of *Aspergillus niger* (50 %) succeeded by *Penicillium* sp. (40 %), *Paecilomyces* sp. and *Aspergillus flavus* (22.5 % each). They also mentioned that the period from April-June was the richest in fungal species (13 species) especially those belonging to *Alternaria*, *Aspergillus*, *Chrysosporium*, *Curvularia*, *Fusarium*, *Paecilomyces*, *Penicillium* and *Scopulariopsis*. Water samples taken during July-September yielded more colonies (139 CFU) followed by those collected during April-June (89 CFU), October-December (82 CFU) and January-March (56 CFU).

It is worth mentioning that some fungal species which were rarely isolated in the present study have been reported as potentially pathogenic to humans and animals. Among these fungi are *Exophiala spinifera* and *Rhinocladiella aquaspersa*. *E. spinifera* is cosmopolitan in soil, plants, water and decaying wood materials and is involved in cutaneous infections as well as deep subcutaneous abscesses (Lee *et al.*, 2018). *Rhinocladiella aquaspersa* was frequently reported from cases of chromoblastomycosis (Gonzalez *et al.*, 2013).

Diversity of terrestrial fungi in submerged mud samples

A total of 45 genera represented by 99 species were isolated from the monthly samples of submerged mud. The highest total number of fungal propagules was recovered on DRBC

(1091200 CFUs) while the lowest count was recorded on Cellulose Czapek's agar (772400 CFUs). The broadest spectrum of genera and species (35 genera and 76 species) was also collected on DRBC and the narrowest (22 genera and 48 species) occurred on Cellulose Czapek's agar (Table 4 and Figs 3&4).

Aspergillus, *Mucor* and *Penicillium* were the most prevalent being recovered during 10-12 months on the three types of media. The genera *Acremonium*, *Cylindrocarpon*, *Fusarium* and *Humicola* appeared in moderate to high incidence depending on the isolation medium. Each of *Galactomyces* and *Talaromyces* was completely absent on Cellulose Czapek's agar but were commonly isolated from 5 to 8 mud samples on DRBC or DCPA.

a- On DRBC agar

Aspergillus (represented by 18 species) was the most frequent genus. It was encountered from all samples, representing about one third of total fungal CFUs (32.70 %). The common species of *Aspergillus* were *Aspergillus brasiliensis*, *A. flavus*, *A. niger*, *A. nidulans* and *A. sydowii* (7-12 of monthly samples). Among the 5 *Penicillium* species *P. aurantiogriseum* was the most common species being recovered from all samples followed by *P. solitum* which was recorded in 8 samples (Table 4). Additional prevalent species were represented by *Mucor circinelloides*, *Humicola fuscoatra*, *Cylindrocarpon lichenicola*, *Talaromyces purpurogenus*, *T. stipitatus*, *Acremonium hyalinulum*, *A. potronii* and *A. fusidioides*. The moderately isolated species comprised *Alternaria alternata*, *Chaetomium piluliferum*, *Galactomyces candidus*, *Scopulariopsis brevicaulis* and *Stachybotrys chartarum* as listed Table (4).

b- On DCPA agar

Thirteen species of *Aspergillus* were identified of which *A. flavus* was recovered from 11 samples constituting 10.56 % of total fungal CFUs. *Aspergillus niger*, *A. brasiliensis*, *A. sydowii* and *A. terreus* were also common but their counts were generally low. Of the five *Penicillium* species, *P. aurantiogriseum* was the most common as it was recovered from 9 samples accounting for 5.80 % of total fungal CFUs. The three *Mucor* species (*M. circinelloides*, *M. racemosus* and *M. hiemalis*) in addition to *Galactomyces candidus*, *Chaetomium piluliferum* and *Cylindrocarpon lichenicola* were common in mud samples.

Table 3: Total counts (CFU/ml in 12 months, %CFU), incidence (I, out of 12 samples) and occurrence remarks (OR) of fungal genera and species recovered from fresh water samples.

Taxa	Medium	DRBC				DCPA				Cellulose			
		CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>Absidia</i>		0.4	0.09	1	L	1	0.24	3	L				
<i>A. cylindrospora</i>		0.4	0.09	1	L								
<i>A. corymbifera</i>						1	0.24	3	L				
<i>Acremonium</i>		3.8	0.83	7	H	2	0.49	5	M	1.8	0.44	6	M
<i>A. furcatum</i>		0.6	0.13	1	L					0.2	0.05	1	L
<i>A. hyalinulum</i>		2.2	0.48	2	L	0.2	0.05	1	L				
<i>A. potronii</i>		0.2	0.04	1	L								
<i>Acremonium sp.</i>		0.8	0.17	3	L	1.6	0.39	5	M	1.2	0.32	5	M
<i>Acrostalagmus luteoalbus</i>		0.2	0.04	1	L								
<i>Alternaria alternata</i>		2	0.44	4	M	1	0.24	4	M	0.8	0.21	3	L
<i>Aspergillus</i>		120.2	26.15	12	H	95.82	23.36	11	H	86.2	23.06	12	H
<i>A. brasiliensis</i>		7.6	1.65	8	H	2.6	0.63	5	M	4.8	1.28	6	M
<i>A. candidus</i>										0.2	0.05	1	L
<i>A. carneus</i>						0.2	0.05	1	L				
<i>A. flavus</i>		47.4	10.31	9	H	48.42	11.81	10	H	42.8	11.45	9	H
<i>A. fumigatus</i>		2.8	0.61	3	L	1.8	0.44	3	L	2.4	0.64	3	L
<i>A. nidulans</i>		2.6	0.57	4	M	3	0.73	6	M	3.2	0.86	7	H
<i>A. latus</i>						0.2	0.05	1	L				
<i>A. niger</i>		30.8	6.70	11	H	15	3.66	9	H	17.4	4.65	9	H
<i>A. ochraceus</i>		3	0.65	5	M	0.6	0.15	2	L	0.2	0.05	1	L
<i>A. parasiticus</i>						0.2	0.05	1	L	0.2	0.05	1	L
<i>A. quadrilienatus</i>		0.8	0.17	3	L	1.4	0.34	3	L	0.6	0.16	1	L
<i>A. rugulosus</i>		0.2	0.04	1	L	0.2	0.05	1	L				
<i>A. sydowii</i>		12.4	2.70	8	H	16.2	3.95	8	H	5.8	1.55	6	M
<i>A. tamarii</i>		0.2	0.04	1	L					7.2	1.93	3	L
<i>A. terreus</i>		8.4	1.83	5	M	1	0.24	2	L	0.6	0.16	3	L
<i>A. unguis</i>		0.2	0.04	1	L								
<i>A. ustus</i>		1.2	0.26	1	L	0.6	0.15	2	L				
<i>A. versicolor</i>		2.6	0.57	6	M	4.4	1.07	5	M	0.8	0.21	2	L
<i>Cephalophora</i>		0.2	0.04	1	L	0.6	0.15	3	L				
<i>C. irregularis</i>		0.2	0.04	1	L	0.4	0.10	2	L				

Taxa	Medium	DRBC				DCPA				Cellulose			
		CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>C. tropica</i>						0.2	0.05	1	L				
<i>Chaetomium</i>		0.4	0.09	1	L	4.2	1.3	9	H	5.2	1.39	6	M
<i>C. cunicolorum</i>						0.2	0.05	1	L				
<i>C. globosum</i>						0.2	0.05	1	L				
<i>C. piluliferum</i>		0.4	0.09	1	L	3.8	0.93	9	H	5.2	1.39	6	M
<i>Chrysosporium queenslandicum</i>		0.2	0.04	1	L	0.4	0.10	2	L				
<i>Cladosporium</i>		52	11.31	7	H	14.2	3.46	4	M	20	5.35	5	M
<i>C. cladosporioides</i>		34	7.40	5	M	10.4	2.54	4	M	12	3.21	5	M
<i>C. herbarum</i>		3.4	0.74	5	M	1.2	0.29	4	M	1.6	0.43	4	M
<i>C. oxysporum</i>		0.6	0.13	2	L	0.2	0.05	1	L				
<i>C. sphaerospermum</i>		14	3.05	5	M	2.4	0.59	3	L	6.2	1.66	5	M
<i>C. tunuissimum</i>										0.2	0.05	1	L
<i>Clonostachys rosea</i>						0.2	0.05	1	L				
<i>Cochliobolus spicifer</i>						0.2	0.05	1	L				
<i>Curvularia lunata</i>		0.2	0.04	1	L	0.4	0.10	1	L	0.2	0.05	1	L
<i>Cylindrocarpon lichenicola</i>		2.8	0.61	3	L	2.6	0.63	3	L	5.8	1.55	2	L
<i>Cylindrocladiella parva</i>										0.2	0.05	1	L
<i>Emericellopsis minima</i>						0.4	0.10	2	L	1	0.27	4	M
<i>Epicoccum nigrum</i>		0.4	0.09	1	L	0.2	0.05	1	L				
<i>Exophiala spinifera</i>		0.2	0.04	1	L	0.2	0.05	1	L				
<i>Exserohilum rostrata</i>		0.4	0.09	2	L								
<i>Flagellospora penicillioides</i>						0.2	0.05	1	L				
<i>Fusarium</i>		12.2	2.65	7	H	6.6	1.61	6	M	6.8	1.82	9	H
<i>F. circinatum</i>		0.4	0.09	1	L								
<i>F. incarnatum</i>		1.8	0.39	3	L	0.8	0.20	2	L	0.4	0.11	2	L
<i>F. oxysporum</i>		1	0.22	2	L	0.2	0.05	1	L				
<i>F. phaseoli</i>										0.2	0.05	1	L
<i>F. proliferatum</i>		6.2	1.35	6	M	3.2	0.78	5	M	5.4	1.44	8	H
<i>F. solani</i>		0.8	0.17	4	M	0.8	0.20	2	L	0.4	0.11	3	L
<i>F. subglutinans</i>		0.6	0.13	2	L	0.4	0.10	1	L	0.2	0.05	1	L
<i>F. verticillioides</i>		1.4	0.30	3	L	1.2	0.29	3	L	0.4	0.11	2	L
<i>Galactomyces candidus</i>		82.2	17.89	8	H	76.2	18.58	9	H	0.2	0.05	1	L
<i>Gliomastix luzulae</i>		0.2	0.04	1	L	0.6	0.15	3	L				

Taxa	Medium	DRBC				DCPA				Cellulose			
		CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>Graphium penicillioides</i>													
<i>Humicola</i>		1.2	0.26	2	L	2	0.49	5	M	1	0.27	2	L
<i>H. fuscoatra</i>		1	0.22	2	L	1.6	0.39	3	L	0.8	0.21	1	L
<i>H. grisea</i>		0.2	0.04	1	L	0.4	0.10	2	L	0.2	0.05	1	L
<i>Lasiodiplodia theobromae</i>						0.2	0.05	1	L				
<i>Microascus manginii</i>										0.2	0.05	1	L
<i>Mucor</i>		17.8	3.87	11	H	23.2	5.66	5	M	30.8	8.24	10	H
<i>M. circinelloides</i>		12.4	2.70	11	H	16	3.90	5	M	22.4	5.99	8	H
<i>M. hiemalis</i>		1.8	0.39	6	M	1.8	0.44	4	M	4.6	1.23	7	H
<i>M. racemosus</i>		3.6	0.78	7	H	5.4	1.32	4	M	3.8	1.02	9	H
<i>Mycogone rosea</i>		0.6	0.13	2	L								
<i>Myrothecium verrucaria</i>		0.4	0.09	1	L	0.2	0.05	1	L	0.2	0.05	1	L
<i>Pacilomyces variotii</i>		0.8	0.17	2	L	0.6	0.15	2	L				
<i>Paraconiothyrium estuarinum</i>		0.2	0.04	1	L								
<i>Penicillium</i>		50	10.88	11	H	59	14.38	10	H	187.6	50.19	9	H
<i>P. arenicola</i>						0.2	0.05	1	L				
<i>P. aurantiogriseum</i>		37.2	8.09	10	H	45	10.97	10	H	161.4	43.18	9	H
<i>P. chrysogenum</i>		2.4	0.52	5	M	0.4	0.10	2	L	1.2	0.32	1	L
<i>P. citrinum</i>		0.6	0.13	3	L	0.4	0.10	2	L				
<i>P. colei</i>						0.2	0.05	1	L				
<i>P. digitatum</i>		0.2	0.04	1	L	0.2	0.05	1	L				
<i>P. italicum</i>		0.2	0.04	1	L								
<i>P. lividum</i>		0.4	0.09	1	L								
<i>P. oxalicum</i>		2.4	0.52	4	M					0.2	0.05	1	L
<i>P. restrictum</i>		0.4	0.09	1	L	1.8	0.44	3	L				
<i>P. olivicolor</i>		0.6	0.13	1	L	0.2	0.05	1	L				
<i>P. simplicissimum</i>		0.2	0.04	1	L								
<i>P. solitum</i>		5.4	1.17	8	H	10.8	2.63	8	H	24.8	6.63	8	H
<i>Pleurostoma richardisae</i>						0.2	0.05	1	L				
<i>Phoma</i>		1.4	0.30	4	M	1.2	0.29	4	M				
<i>P. herbarum</i>		0.4	0.09	2	L								
<i>P. lelveillei</i>		0.4	0.09	2	L	0.4	0.10	1	L				
<i>Phoma sp.</i>		0.6	0.13	2	L	0.8	0.20	3	L				

Taxa	Medium	DRBC				DCPA				Cellulose			
		CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>Pseudallescheria boydii</i>		0.2	0.04	1	L					0.4	0.11	2	L
<i>Purpureocillium lilacinum</i>						0.2	0.05	1	L	0.2	0.05	1	L
<i>Rhinochadiella aquaspersa</i>						0.2	0.05	1	L				
<i>Sarocladium</i>		3.2	0.70	3	L	2	0.49	7	H	4.2	1.1	5	M
<i>S. kiliense</i>		0.2	0.04	1	L	0.8	0.20	2	L	0.6	0.16	1	L
<i>S. strictum</i>		3	0.65	2	L	1.2	0.29	5	M	3.6	0.96	4	M
<i>Scopulariopsis</i>		1	0.22	2	L	11.4	2.78	9	H	7.8	2.09	7	H
<i>S. asperula</i>										0.4	0.11	1	L
<i>S. brevicaulis</i>		0.4	0.09	2	L	8.4	2.05	7	H	6	1.61	5	M
<i>S. brumptii</i>						0.8	0.20	3	L	0.8	0.21	3	L
<i>S. candida</i>		0.2	0.04	1	L	1	0.24	4	M	0.2	0.05	1	L
<i>S. koningii</i>		0.4	0.09	1	L	1	0.24	4	M	0.4	0.11	1	L
<i>Sphaerosporium lignatile</i>						0.2	0.05	1	L				
<i>Stachybotrys chartarum</i>		0.2	0.04	1	L	0.2	0.05	1	L	0.8	0.21	4	M
<i>Stemphylium vesicarium</i>		0.6	0.13	2	L	0.2	0.05	1	L	0.4	0.11	2	L
<i>Talaromyces</i>		3.8	0.83	8	H	2.4	0.59	8	H	0.2	0.05	1	L
<i>T. duclauxii</i>		1.4	0.30	6	M	1	0.24	4	M				
<i>T. helicus</i>						0.2	0.05	1	L				
<i>T. purpurogenus</i>		1.4	0.30	5	M	0.2	0.05	1	L	0.2	0.05	1	L
<i>T. stipitatus</i>		1	0.22	3	L	1	0.24	5	M				
<i>Thermoascus aurantiacus</i>						0.4	0.10	1	L				
<i>Trichoderma</i>		4	0.87	10	H	3.2	0.78	6	M	3.8	1.02	10	H
<i>T. atroviride</i>										0.2	0.05	1	L
<i>T. harzianum</i>		3.8	0.83	10	H	2.8	0.68	6	M	3	0.80	9	H
<i>T. kononjii</i>		0.2	0.04	1	L	0.4	0.10	2	L	0.2	0.05	1	L
<i>T. parceramosum</i>										0.4	0.11	2	L
<i>Verticillium</i> sp.						1.2	0.29	4	M	0.4	0.11	2	L
Red yeasts		1.2	0.26	3	L	1.8	0.44	3	L	1.2	0.32	2	L
White yeasts		84.8	18.45	10	H	89	21.70	8	H	4.2	1.12	4	M
Sterile mycelium		10.4	2.26	8	H	4	0.98	5	M	2.6	0.70	4	M

c- OnCellulose Czapek's agar

Since cellulose is a more complex organic substrate, the total number of fungal species isolated on cellulose Czapek's agar (48 species belonging to 22 genera) was markedly lower than those obtained on DRBC (76 species related to 35 genera) and DCPA (72 species attributed to 37 genera). Similarly the total number of colonies obtained on the two media containing dicoloran (1091200 and 1055000 CFUs) was markedly greater than that on Cellulose Czapek's agar (772400 CFUs/ml in all samples). The counts of unidentified yeasts and sterile mycelia were also higher on both DRBC and DCPA than on Cellulose Czapek's agar as shown in table (4). Moreover, the following fungal species were collected on DRBC but they were completely missed on the two remaining medium types: *Absidia cylindrospora*, *Acremonium fusidioides*, *Aspergillus aegyptiacus*, *A. carneus*, *A. puniceus*, *A. terricola*, *A. ustus*, *Chaetomium uniseriatum*, *Fusarium incarnatum*, *Gliomastix luzulae*, *Hormiactis candida*, *Pleurostoma richardsiae*, *Pseudallescheria fimeti*, *Purpureocillium lilacinum*, *Talaromyces purperogenus* and black yeasts. Few number of fungal species appeared on Cellulose Czapek's agar but not on DRBC and DCPA these were *Acremonium zonatum*, *Aspergillus unguis*, *Fusarium chlamydosporum*, *Phoma leveillei* and *Sporothrix* sp.

Similar findings on diversity of fungi in submerged mud were reported from different countries in Africa and Asia. *Aspergillus* and *Penicillium* were common in submerged mud of Aswan High Dam Lake, Egypt (El-Hissy *et al.*, 1990), *Acremonium*, *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Ulocladium* in mud-flats of Tigris Edges in Baghdad (Abdulla, 2008), *Penicillium*, *Fusarium*, *Aspergillus*, *Trichoderma* and *Talaromyces* in wetland sediments along the Changjiang River, China (Wu *et al.*, 2013), and *Penicillium* and *Mucor* in sandy loam soil samples collected from a fresh water swamp area in Obrikom, Ogbia Egbema Ndoni Local Government Area of Rivers State, Nigeria (Dirisu, 2015). In Southern India, Babu *et al.* (2010) identified *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. terricola* from water sediments. In Iraq

Farkha and Ahmadi (2011) found that the most dominant terrestrial fungal species isolated from submerged mud were belonging to *Alternaria*, *Aspergillus*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus*. In Pakistan, Leghari *et al.* (2016) performed mycological analysis of the mud and water of Hanna Lake in Quetta district during 2015, using baiting techniques. *Fusarium solani* was the only species isolated consistently from mud and water throughout the year. On the other hand, *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. versicolor*, *A. candidus*, *A. nidulans*, *A. ustus*, *A. wentii*, *A. kanagawaensis*, *A. restrictus*, *Alternaria alternata*, *Fusarium solani*, *Penicillium funiculosum*, *P. brevicompactum*, *P. raistrickii*, *P. bilaiae*, *Phoma* sp. and *Rhizopus stolonifer* were isolated from the upper surface of sediments at four stations in the Suq-Alshuyukh marshes in Thi-Qar Governorate, Iraq (Al-Jawhari, 2015). Moubasher *et al.* (2018) surveyed the mycobiota of mud from Lakes of Wadi Al-Natron and Ibrahimiya Canal, Egypt. They found that the most commonly encountered genera were *Aspergillus* (24 species), *Cladosporium* (9) and *Penicillium* (15), while less frequent genera included *Absidia* (1), *Acremonium* (10), *Alternaria* (2), *Microascus* (5), *Mucor* (2), *Sarocladium* (2), *Scopulariopsis* (5) and *Syncephalastrum* (1). The genera *Curvularia* (4 species), *Fusarium* (4), *Talaromyces* (5) and *Trichoderma* (3) were moderately frequent on DRBC and DG18.

Molecular identification of some fungal isolates

Nine fungal isolates from water and submerged mud were sent to Solgent Company, South Korea for sequencing of rDNA. Results are shown in table (4) and Figure (5). These fungi were generally rare in the present study.

The phylogenetic tree comprised 9 subclades which enabled satisfactory identification of 7 different fungal strains. Each of *Acremonium zonatum*, *Acrostalagmus luteoalbus* and *Exophiala spinifera* showed 100 % similarity with respective strains in the Genbank. *Chaetomium uniseriatum*, *Neurospora tetraspora*, *Hormiactis candida*, *Paraconiothyrium estuarinum* and *Penicillium colei* showed 99 % similarity. The remaining strain (*Fusarium phaseoli*) exhibited 97 % similarity.

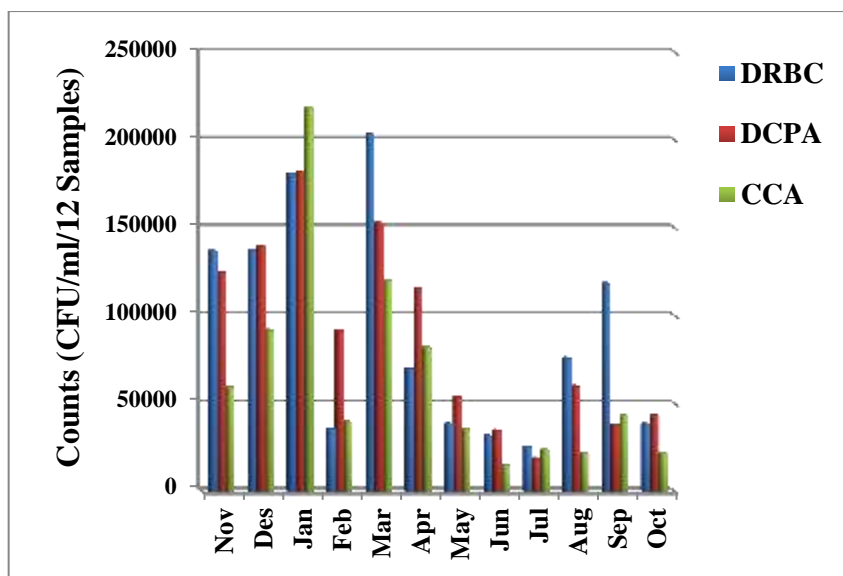


Figure 3: Monthly variations in the counts of terrestrial fungi in submerged mud samples

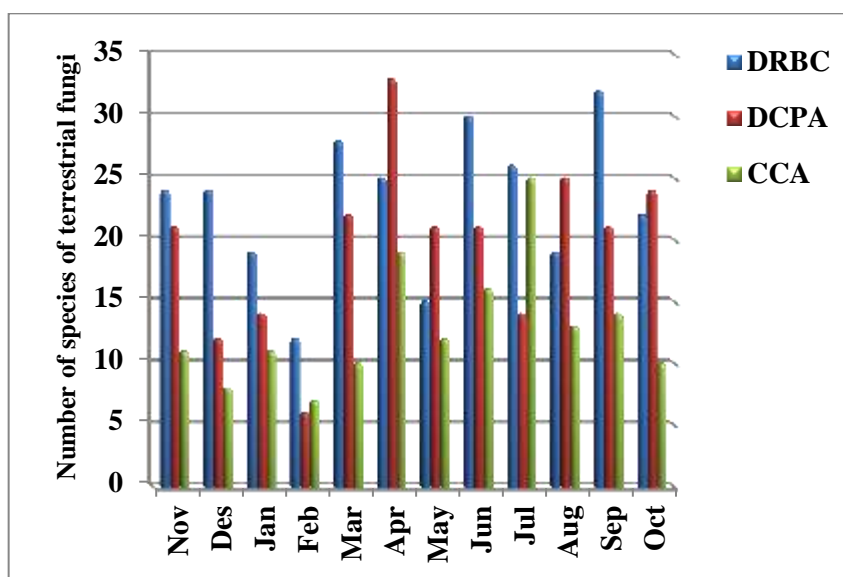


Figure 4: Monthly variation in the number of species of terrestrial fungi in submerged mud samples.

Table 4: Fungal species Identified by rDNA sequencing

AUMMC No.	Accession GenBank number	Identification	Source	Accession No. of related strains	Similarity %
7271	MK061547	<i>Fusarium phaseoli</i>	Water	AB587014	97
7272	MK061548	<i>Penicillium colei</i>	Water	NR137611 ^T	99
7273	MK061546	<i>Paraconiothyrium estuarinum</i>	Water	NR137669 ^T	99
7274	MK061549	<i>Exophiala spinifera</i>	Water	KF881966 ^T	100
7275	MK061550	<i>Acrostalagmus luteoalbus</i>	Water	KT824244	100
7276	MK061551	<i>Hormiactis candida</i>	Mud	MH481320	99
7277	MK061552	<i>Neurospora tetraspora</i>	Mud	NR077163 ^T	98
7278	MK043068	<i>Acremonium zonatum</i>	Mud	KT968535	100
7279	MK061553	<i>Chaetomium uniseriatum</i>	Mud	KP336751 ^T	99

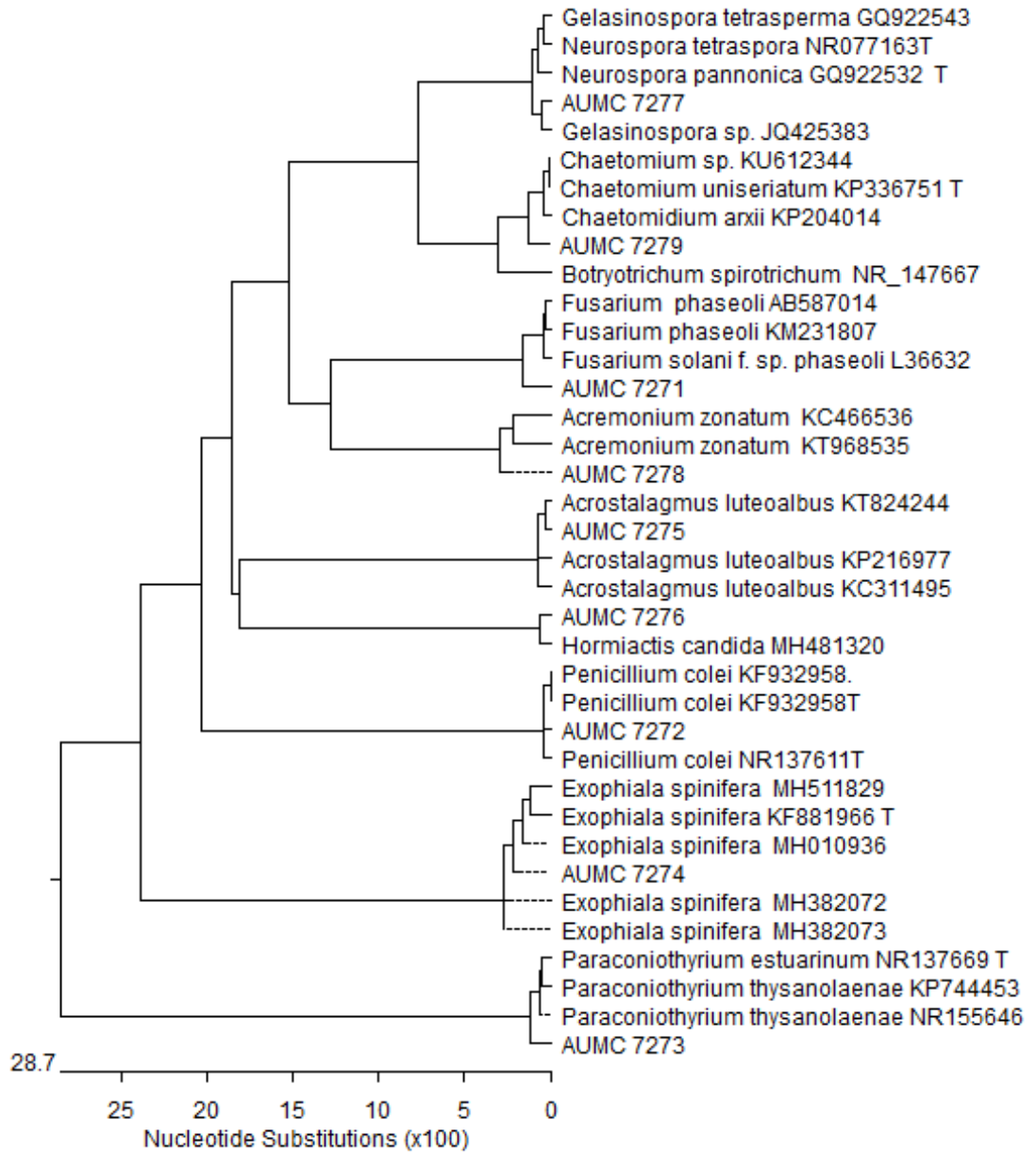


Figure 5: Phylogenetic tree based on ITS sequences of rDNA from fungi isolated in the present study (given AUMMC Numbers) aligned with related fungal strains accessed from the GenBank.

Table 4: Total count (CFU/g in 12 monthes, %CFU), incidence (I) and occurrence remarks (OR) of fungal genera and speciesrecovered from submerged mud.

Taxa	Medium	DRBC				DCPA				Cellulose			
		CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>Absidia cylindrospora</i>		1200	0.11	1	L								
<i>Acremonium</i>		5200	0.48	7	H	3600	0.34	5	M	4000	0.52	6	M
<i>A. fusidioides</i>		800	0.07	2	L								
<i>A. hyalinulum</i>		1200	0.11	3	L					800	0.10	1	L
<i>A. potronii</i>		1200	0.11	3	L	2000	0.19	2	L	1200	0.16	3	L
<i>A. zonatum</i>										400	0.05	1	L
<i>Acremonium sp.</i>		2000	0.18	4	M	1600	0.15	3	L	1600	0.21	3	L
<i>Alternaria alternata</i>		5200	0.48	5	M	800	0.08	2	L	800	0.10	2	L
<i>Aspergillus</i>		356800	32.70	12	H	217000	20.57	12	H	151600	19.63	11	H
<i>A. aegyptiacus</i>		400	0.04	1	L								
<i>A. brasiliensis</i>		16200	1.48	11	H	9800	0.93	7	H	5600	0.73	6	M
<i>A. candidus</i>		2400	0.22	2	L	2400	0.23	1	L				
<i>A. carneus</i>		400	0.04	1	L								
<i>A. flavus</i>		161600	14.81	11	H	111400	10.56	11	H	84400	10.92	10	H
<i>A. fumigates</i>		2400	0.22	2	L	1600	0.15	3	L	1600	0.21	3	L
<i>A. nidulans</i>		32400	2.97	7	H	4800	0.45	4	M	26400	3.42	5	M
<i>A. niger</i>		54600	5.00	11	H	20200	1.91	8	H	14800	1.92	7	H
<i>A. ochraceus</i>		3200	0.29	4	M	800	0.08	2	L	400	0.05	1	L
<i>A. parasiticus</i>		10400	0.95	1	L	400	0.04	1	L				
<i>A. puniceus</i>		400	0.04	1	L								
<i>A. quadrilienatus</i>		800	0.07	1	L	800	0.08	1	L				
<i>A. sydowii</i>		44400	4.07	7	H	25600	2.43	6	M	2800	0.36	3	L
<i>A. tamaritii</i>		800	0.07	1	L	400	0.04	1	L				
<i>A. terreus</i>		15600	1.43	3	L	28000	2.65	6	M	12800	1.66	3	L
<i>A. terricola</i>		400	0.04	1	L								
<i>A. unguis</i>										400	0.05	1	L
<i>A. ustus</i>		800	0.07	2	L								
<i>A. versicolor</i>		10000	0.92	5	M	10800	1.02	5	M	2400	0.31	3	L
<i>Cephalophora irregularis</i>		800	0.07	2	L	1600	0.15	3	L				

Medium Taxa	DRBC				DCPA				Cellulose			
	CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>Chaetomium</i>	2800	0.26	5	M	19600	1.86	7	H	38000	4.92	5	M
<i>C. globosum</i>	400	0.04	1	L	400	0.04	1	L				
<i>C. piluliferum</i>	2000	0.18	5	M	19200	1.82	7	H	38000	4.92	5	M
<i>C. uniseriatum</i>	400	0.04	1	L								
<i>Chrysosporium</i>	1600	0.15	2	L	1200	0.11	2	L				
<i>C. keratinophilum</i>	800	0.07	1	L	800	0.08	2	L				
<i>C. queenslandicum</i>	800	0.07	1	L	400	0.04	2	L				
<i>Cladosporium</i>	20000	18.32	6	M	5200	0.49	4	M	18800	2.43	4	M
<i>C. cladosporioides</i>	12800	1.17	5	M	3200	0.30	4	M	18400	2.38	3	L
<i>C. herbarum</i>	6400	0.59	5	M					400	0.05	1	L
<i>C. sphaerospermum</i>	800	0.07	1	L	2000	0.19	2	L				
<i>Clonostachys rosea</i>					400	0.04	1	L	400	0.05	1	L
<i>Curvularia lunata</i>	1600	0.15	4	M	91200	8.64	6	M				
<i>Cylindrocarpon lichenicola</i>	74000	6.78	8	H	2000	0.19	4	M	94400	12.22	7	H
<i>Emericellopsis</i>	800	0.07	1	L	1200	0.11	1	L	1600	0.21	2	L
<i>E. tricola</i>	400	0.04	1	L					800	0.10	1	L
<i>E. minima</i>	400	0.04	1	L	1200	0.11	1	L	800	0.10	1	L
<i>Exserohilum rostratum</i>					400	0.04	1	L				
<i>Fusarium</i>	8000	0.73	6	M	13200	1.25	7	H	3200	0.41	5	M
<i>F. acutatum</i>					400	0.04	1	L				
<i>F. chlamyosporum</i>									400	0.05	1	L
<i>F. incarnatum</i>	800	0.07	2	L								
<i>F. nygamii</i>					1600	0.15	3	L				
<i>F. proliferatum</i>	2000	0.18	2	L	6000	0.57	4	M	2400	0.31	4	M
<i>F. solani</i>	5200	0.48	5	M	3200	0.30	5	M	400	0.05	1	L
<i>F. subglutinans</i>					1600	0.15	1	L				
<i>F. verticillioides</i>					400	0.04	1	L				
<i>Galactomyces candidus</i>	217600	19.94	5	M	201600	19.11	8	H				
<i>Gliomastix luzulae</i>	1600	0.15	3	L								
<i>Hormiactis candida</i>	400	0.04	1	L								
<i>Humicola</i>	23200	2.13	9	H	26800	2.54	7	H	10400	1.35	5	M

Medium Taxa	DRBC				DCPA				Cellulose			
	CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>H. fuscoatra</i>	20400	1.87	7	H	25200	2.39	7	H	10000	1.29	5	M
<i>H. grisea</i>	2800	0.26	4	M	1600	0.15	2	L	400	0.05	1	L
<i>Lecanicillium nodulosum</i>	400	0.04	1	L	400	0.04	1	L	400	0.05	1	L
<i>Microascus manginii</i>	400	0.04	1	L	800	0.08	1	L				
<i>Mucor</i>	55200	5.06	10	H	172800	16.38	10	H	142000	18.38	10	H
<i>M. circinelloides</i>	36800	3.37	10	H	130400	12.36	10	H	101600	13.15	10	H
<i>M. hiemalis</i>	5600	0.51	6	M	13200	1.25	5	M	10800	1.40	5	M
<i>M. racemosus</i>	12800	1.17	7	H	29200	2.77	7	H	29600	3.83	7	H
<i>Mycogone rosea</i>					400	0.04	1	L				
<i>Neurospora tetraspora</i>					400	0.04	1	L				
<i>Paecilomyces variotii</i>	1600	0.15	3	L	1200	0.11	2	L				
<i>Penicillium</i>	128800	11.80	12	H	127600	12.09	11	H	252400	32.68	10	H
<i>P. aurantiogriseum</i>	88800	8.14	12	H	61200	5.80	9	H	237600	30.76	9	H
<i>P. chrysogenum</i>	1600	0.15	3	L	1600	0.15	3	L	8800	1.14	5	M
<i>P. citrinum</i>	5600	0.51	3	L	1200	0.11	1	L				
<i>P. solitum</i>	31200	2.86	8	H	60800	5.76	7	H	6000	0.78	2	L
<i>P. oxalicum</i>	1600	0.15	2	L	2800	0.27	4	M				
<i>Phialaphora bubakii</i>	400	0.04	1	L	400	0.04	1	L				
<i>Phoma</i>	2000	0.17	5	M	1200	0.11	2	L	800	0.10	2	L
<i>P. leveillei</i>									800	0.10	2	L
<i>Phoma</i> sp.	2000	0.17	5	M	1200	0.11	2	L				
<i>Pleurostoma richardsiae</i>	400	0.04	1	L								
<i>Pseudallescheria</i>	1600	0.15	2	L								
<i>P. boydii</i>	800	0.07	2	L	800	0.08	1	L				
<i>P. fimeti</i>	400	0.04	1	L								
<i>Purpureocillium lilacinum</i>	800	0.07	2	L								
<i>Sarocladium strictum</i>	800	0.07	2	L	1600	0.15	4	M	800	0.10	2	L
<i>Scopulariopsis</i>	3600	0.33	5	M	31600	3.00	8	H	32000	4.14	6	M
<i>S. brevicaulis</i>	2800	0.26	5	M	26000	2.46	6	M	28400	3.68	6	M
<i>S. brumptii</i>					2400	0.23	2	L	800	0.10	2	L
<i>S. candida</i>					2000	0.19	4	M	800	0.10	2	L

Taxa	DRBC				DCPA				Cellulose			
	CFU	%CFU	I	OR	CFU	%CFU	I	OR	CFU	%CFU	I	OR
<i>S. fusca</i>					400	0.04	1	L				
<i>S. knongii</i>	800	0.07	2	L	800	0.08	2	L	2000	0.26	2	L
<i>Stachybotrys</i>	2800	0.26	5	M	400	0.04	1	L	2400	0.31	4	M
<i>S. chartarum</i>	2800	0.26	5	M					1600	0.21	3	L
<i>S. havanensis</i>					400	0.04	1	L	800	0.10	1	L
<i>Sporothrix</i> sp.									1200	0.16	2	L
<i>Stemphylium botryosum</i>					400	0.04	1	L	800	0.10	2	L
<i>Talaromyces</i>	7200	0.66	8	H	4000	0.38	7	H				
<i>T. duclauxii</i>	1200	0.11	2	L	1200	0.11	3	L				
<i>T. islandicus</i>					800	0.08	1	L				
<i>T. purpureogenus</i>	1600	0.15	4	M								
<i>T. stipitatus</i>	4400	0.40	5	M	2000	0.19	3	L				
<i>Thermoascus aurantiacus</i>					1200	0.11	1	L				
<i>Trichoderma</i>	6400	0.59	4	M	2400	0.23	2	L	1600	0.21	2	L
<i>T. atroviride</i>	800	0.07	2	L	800	0.08	2	L				
<i>T. harzianum</i>	4400	0.40	4	M	1600	0.15	2	L	1200	0.16	2	L
<i>T. kononjii</i>	1200	0.11	2	L					400	0.05	1	L
<i>Verticillium</i> sp.					800	0.08	2	L				
Black yeast	400	0.04	1	L								
Red yeast	4400	0.40	3	L	12400	1.18	2	L	400	0.05	1	L
White yeast	144000	13.20	7	H	93600	8.87	4	M	5200	0.67	2	L
Sterile mycelium	9600	0.88	4	M	14000	1.33	5	M	4000	0.52	3	L

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