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Incidence of Terrestrial Fungi in Drinking Water Collected from Different Schools in Riyadh Region, Saudi Arabia

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Abstract: Forty five species belonging to nineteen genera of terrestrial fungi were recovered on glucose and cellulose Czapek´s media from one hundred and forty drinking water samples which, were collected from different kids (2 schools), primary (77 schools), mediatory (33 schools) and secondary (28 schools) schools from different regions in Riyadh city, Saudi Arabia. Cellulose Czapek´s (39 species related to 19 genera) was rich than glucose Czapek´s (37 species belonging to 15 genera) with the species diversity. Water samples collected from primary schools were the most contaminant with fungal populations as compared with other tested samples. Both *Aspergillus* and *Penicillium* contributed the broadest spectra of the isolated terrestrial fungal species where they were represented by eight and six species, respectively on the media of isolation. The most prevalent isolated species of terrestrial fungi on the two isolation media were; *Cladosporium cladosporoides*, *Trichoderma viride*, *Curvularia lunata*, *Aspergillus niger*, *Emericella nidulans*, *Mucor circinelloides* and *Rhizopus rhizopodiformis*. The majority of the isolated genera and species were almost similar on both of isolation media although several species were recorded in only one isolation medium.

Key words: Terrestrial fungi, drinking water, glucophilic, cellulose decomposer, Saudi Arabia

INTRODUCTION

Several researches were conducted dealing with the incidence and distribution of terrestrial fungi in different water resources in the world as reported by Malik and Eggins[1], Stewart and Walsh[2], Nasar and Munchi[3], Miyoshi *et al.*[4], Bettucci and Roquebert[5]. Also, frequent attention has been given for studying the occurrence of these terrestrial mycobiota suspended in freshwater samples especially from rainfall water in Saudi Arabia which nearly extended to most regions[6-9]. The origin of terrestrial fungi in freshwater habitats may be through living or dead animal or plant and soil or litter having been in contact with water as claimed by Park[10] or are likely to originate from air or washed with rain water according to Sparrow[11]. The drinking water in Saudi Arabia is mainly composed of 60% desalinized Sea water and 40% ground well water. Up till now, no attention has been given for studying the microbiological analysis of the drinking water especially in the different schools in Riyadh region, Saudi Arabia. So, it is interesting to know the possible contaminant of this water, which represents the artillery of life, with the terrestrial fungi.

MATERIALS AND METHODS

Collection of water samples: One hundred and forty drinking water samples were collected from different kids (2 schools), primary (77 schools), mediatory (33 schools) and secondary (28 schools) schools in Saudi Arabia and these schools represents regions of Soyeeh, Boyeeb, Salboukh, Manfoha, El-Shmeeshi, Milz and Haer in Riyadh city. These samples were collected and maintained in clean, sterile screwed glass bottles of 250 mL capacity each and only one drinking water sample was collected from each school. The collected samples were deposited in a refrigerator until use. It is known that 60% of the water component of these samples is desalinized Sea water, while the remaining percentage (40%) is well waters. The daily average of consumed water in Riyadh water is 1.137.804 m^3/day and the daily average for one person is 258 L/day. The samples were subjected to mycological investigations for the occurrence and distribution of terrestrial fungi. The temperature of these samples was measured directly at the time of sampling. Some physicochemical characteristics of these samples including pH, total soluble salts and organic matter contents were also estimated based on Jackson[12].

Isolation and recovery of terrestrial fungi: For the isolation and recovery of terrestrial fungi, one milliliter of each tested water sample was transferred aseptically into each of eight sterile petri-dishes (10 cm in diameter each) using sterile Menziess´s[13] dipper. Modified Czapek´s Dox agar medium in which glucose (10 g L^{-1}) or cellulose (20 g L^{-1}) was employed for the isolation of

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glucophilic and cellulose-decomposing fungi, respectively and 4 plates were used for each medium of isolation. Martin´s Rose Bengal (1/1500) was added as a bacteriostatic agent according to Smith and Dawson[14]. Glucose Czapek´s agar was poured onto 4 plates and cellulose Czapek´s agar medium onto the other four plates. Following the solidification of agar, petri-dishes were incubated at 28°C for two weeks at least and the developing colonies were counted, examined and identified and total counts were calculated per one mL water for each water sample.

References used in the identification of terrestrial fungi: The identification of genera and species of terrestrial fungi was adopted by the following key references: De Vries[15], Gilman[16], Raper and Fennell[17], Simmons[18], Ames[19], Rifai[20], Ellis[21], Booth[22], Domsch and Gams[23].

RESULTS AND DISCUSSION

Physico-chemical characteristics of water samples: The physico-chemical characteristics of the investigated drinking water samples (Table 1) showed an ordinary odor and taste based on water qualities, the colour is less than 5 units and turbidity less than 2 units. pH value of the water samples ranged between 7.5 and 8.5 and it was found that the total soluble salts fluctuated between 250 and 500 mg L^{-1}. The Table 1 summarizes the minimal and maximal values of some evaluated physico-chemical characteristics of the tested drinking water samples.

It was found that none of the investigated physicochemical characteristics displays any role governing the incidence and distribution of the isolated terrestrial fungi from drinking water samples, which were collected from the different schools in Riyadh region in Saudi Arabia. In this respect and parallel with this finding, El-Nagdy *et al.*[6], El-Nagdy and Nasser[7], Ali and Nasser[8] found that pH value did not play any regular pattern governing the fungal occurrence and distribution in accumulated rainfall water and mud collected from sporadic regions in Saudi Arabia.

However, Ali and Nasser[8] concluded that rainfall water samples, which had a relatively high organic matter and low total soluble salts contents, were the richest in terrestrial fungi. The tested drinking water samples showed no great difference between them in their physicochemical characteristics evaluation. This is in agreement with the results obtained by El-Nagdy *et al.*[6], El-Nagdy and Nasser[7] during their studies on terrestrial fungi from accumulated rainfall water.

Table 1: Some physico-chemical characteristics of the tested drinking water samples.

Estimated characters	Obtained values
Colour	Less than 5
Turbidity	Less than 2
Taste	Ordinary
pH value	7.5-8.2
Conductivity (µcm cm^{-1})	390-750
Total soluble salts (mg L^{-1})	250-500
Mg^{+2}(mg L^{-1})	35-85
Ca^{+2} (mg L^{-1})	50-110
Na^{+} (mg L^{-1})	35-138
SO_4^{-} (mg L^{-1})	80-160
Cl^{-} (mg L^{-1})	46-110
Al^{+3} (mg L^{-1})	0-0.009
Fe^{+3} (mg L^{-1})	0-0.03
Cu^{+2} (mg L^{-1})	0-0.019
Zn^{+2} (mg L^{-1})	0-0.03
Mn^{+2} (mg L^{-1})	0-0.024
Cd^{+2} (mg L^{-1})	0
Cn^{+2} (mg L^{-1})	0
Hg^{+2} (mg L^{-1})	0
Se^{-2} (mg L^{-1})	0
Cr^{+2} (mg L^{-1})	0-0.002
No^{3+} (mg L^{-1})	1.1-3.3
No_2^{-2} (mg L^{-1})	0-0.0033
F^{-1} (mg L^{-1})	0.2-0.45
Pb^{+2} (mg L^{-1})	0-0.001

Terrestrial fungi: Forty-five species of terrestrial fungi concerning nineteen genera were identified and isolated from the tested water samples (one hundred and forty drinking water samples) on glucose and cellulose Czapek´s media, which were collected from different schools located in Riyadh region, Saudi Arabia. Water samples collected from primary schools were the richest with terrestrial fungal species than other tested samples of schools where the majority of samples yielded 3-5 species. Of the investigated drinking water samples 108 samples recorded the highest fungal contamination where the isolated fungal species ranged between 3-5 species in each sample. Eleven drinking water samples recorded no fungal occurrence and these were collected from secondary schools (8 samples), primary schools (2 samples) and mediatory schools (one sample). The rest of the collected water samples (21 samples) assumed a midway position between the previous samples (a moderate fungal occurrence) and recorded 1-2 species of terrestrial fungi and these samples represented kid schools (2 samples), secondary schools (10 samples), mediatory schools (6 schools) and primary schools (3 schools).

Glucophilic fungi recovered on glucose Czapek´s agar medium. As indicated in Table 2, thirty-seven species related to fifteen terrestrial fungal genera were recovered from the investigated water samples on glucose Czapek´s medium. *Aspergillus* contributed the broadest spectrum of the isolated terrestrial fungal species on glucose

Table 2: Total counts (TC, calculated per mL water in each sample), number of cases of isolation (NCI, out of one hundred and forty samples) and occurrence remarks (OR) of terrestrial fungal genera and species on glucose- and cellulose-Czapek´s agar at 28ºC

Isolated genera and species of terrestrial fungi	Isolation medium: Glucose TC	Glucose NCI and OR	Sucrose TC	Sucrose NCI and OR
Alternaria	43	26 L	68	37 M
A. alternata (Fr.) Kreissler	36	18 L	52	31 L
A. tenuissima (Kunze: Fr.) Wiltshire	7	8 R	16	6 R
Aspergillus	340	102 H	263	84 H
A. carbonarius (Bainier) Thom	61	18 L	65	17 L
A. clavatus Desmatzieres	7	3 R	0	0
A. niger Van Tieghem	169	45 M	137	44 M
A. oryzae (Ahlb.) Cohn	8	3 R	0	0
A. sydowi (Bain. And Sart.) Thom and Church	27	6 R	46	13 R
A. tamarii Kita	15	11 R	0	0
A. terreus Thom	31	7 R	7	9 R
A. versicolor (Vuill.) Tiraboschi	19	8 R	8	6 R
Botryotrichum atrogriseum Van Beyma	0	0	31	27 L
Chaetomium	136	40 M	130	56 M
C. globosum Kuntze	56	14 R	49	26 L
C. olivaceum Cooke and Ellis	19	8 R	50	18 L
C. spirale Zoph	61	18 L	31	12 L
Cladosporium	167	64 M	152	87 H
C. cladosporioides (Fres.) de Vries	128	51 M	92	62 M
C. herbarum (Pers.) Link ex Gray	39	15 R	60	25 L
Curvularia lunata (Wakker) Boedijn	0	0	78	48 M
Drechslera specifera (Bain.) von Arx	19	11 R	68	34 L
Emericella nidulans (Eidam) Vuill.	72	38 M	18	10 R
Fusarium	79	45 M	90	72 H
F. acuminatum Ellis and Everh	0	0	17	13 R
F. moniliforme Sheldon	31	11 R	16	12 R
F. oxysporum Schlecht ex Fr.	18	15 R	22	18 L
F. solani (Mart.) Saccardo	30	19L	35	29L
Humicola grisea Traaen	23	14 R	40	32 L
Mucor	47	41 M	22	19 L
M. circinelloides Van Tiegh	42	38 M	18	16 R
M. hiemalis Wehmer	5	3 R	0	0
M. racemosus Fesenius	0	0	4	3 R
Myrothecium verrucaria (Alb. and Sch.) Dit. Ex St.	0	0	56	27 L
Paecilomyces variotii Bainier	0	0	41	18 L
Penicillium	180	81 H	163	71 H
P. chrysogenum Thom	54	32 L	42	24 L
P. citrinum Thom	21	8 R	30	11 R
P. funiculosum Thom	12	5 R	14	6 R
P. oxalicum Currie and Thom	40	18 L	44	16 R
P. purpurogenum Stoll	31	17 L	33	20 L
P. variabile Sopp	22	6 R	0	0
Phoma	51	17 L	102	56 M
P. glomerata (Corda) Wollen Weber and Hochafel	51	17 L	56	24 L
P. humicola Gilman and Abbott	0	0	46	32 L
Rhizopus	58	51 M	39	34 L
R. rhizopodiformis (Cohn) Zopf	42	36 M	39	34 L
R. stolonifer (Ehrenb.) Lind	16	15 R	0	0
Stachybotrys	14	6 R	36	15 R
S. atra Mather and Sankhla	0	0	6	2 R
S. chatarum (Ehrenb. Fr.) Hughes	14	6 R	30	13 R
Trichoderma	56	32 L	91	63 M
T. harzianum Rifai	35	18 L	20	9 R
T. viride Pers: Fr.	21	14 R	71	54 M
Ulocladium	21	14 R	42	28 L
U. atrum Preuss	18	14 R	31	22 L
U. botrytis Preuss	3	1 R	11	6 R
Number of genera	15		19	
Number of species	37		39	

Where, H = high occurrence (> 70 water samples out of 140); M = moderate occurrence (between 35 and 70 samples); L = low occurrence (between 17 and 34 samples); R = rare occurrence (< 17 samples).

Czapek´s agar where it was represented by eight species. However, several genera were isolated and represented by only one species. Both *Aspergillus* and *Penicillium* were recovered from the collected water samples in high frequency of occurrence (102 and 81 samples, respectively) matching 340 and 180 isolates, respectively. *Aspergillus* was represented by *A. niger* which moderately occurred (45 water samples) and *A. carbonarius* which was of low occurrence (18 water samples), whereas *A. clavtaus*, *A. oryzae*, *A. sydowi*, *A. terreus*, *A. versicolor*, *A. tamarii* and *A. flavus* were rarely occurred (3-15 water samples, each). *Penicillium* was represented by six species of which *P. chrysogenum*, *P. oxalicum* and *P. purpurogenum* were of low occurrence (17-32 water samples, each) constituting 54, 40 and 31 isolates, respectively. The remaining three species; *P. funiculosum*, *P. variabile* and *P. citrinum* were encountered and recovered rarely (5-8 water samples, each) matching 12, 22 and 21 isolates, respectively. *Cladosporium* was ranked as of moderate frequency of occurrence and it was isolated from 64 water samples. This genus was represented by *C. cladosporoides* (moderate occurrence, 51 samples, 128 isolates) and *C. herbarum* (rare occurrence, 15 samples, 39 isolates). *Rhizopus* was also arranged as of moderate frequency of occurrence where it was recovered from 51 drinking water samples. It was also represented by two species; *R. rhizopodiformis* (moderate occurrence, 36 samples, 42 isolates) and *R. stolonifer* (rare occurrence, 15 samples, 16 isolates). *Fusarium* came next and it was of moderate occurrence (45 water samples). This genus included three isolated species on glucose Czapek´s agar of which *F. solani* was of low occurrence (19 water samples; 30 isolates) while *F. oxysporum* and *F. moniliforme* 15 and 11 water samples, 18 and 31 isolates, respectively. *Mucor* was of moderate occurrence and it was represented by two species namely; *M. circinelloides* (moderate occurrence, 38 samples, 42 isolates) and *M. hiemalis* (rare occurrence, 3 samples, 5 isolates). *Chaetomium* was also isolated as of moderate frequency of occurrence (40 samples out of 140 water samples). This genus included three species; *C. spirale* (low occurrence, 18 samples, 61 isolates), *C. globosum* and *C. olivaceum* (rare occurrence each, 14 and 8 samples, 56 and 19 isolates, respectively). *Emericella* (*E. nidulans*) was assessed as of moderate frequency of occurrence (38 water samples, 72 isolates). *Trichoderma* was of low frequency of occurrence (32 water samples) and it was included two species namely; *T. harzianum* (low occurrence, 18 samples, 35 isolates) and *T. viride* (rarely occurred, 14 water samples, 21 isolates). Also, both *Alternaria* (*A. alternata*, low occurrence, 18 samples and *A. tenuissima*, rare occurrence, 8 samples, 7 isolates) and *Phoma* (*P. glomerata*) were ranked and recovered as of low frequency of occurrence (26 and 17 water samples, respectively).

The rest of other isolated terrestrial fungal genera were *Ulocladium* (*U. atrum* and *U. botrytis*) and *Drechslera* (*D. specifera*) which were recorded as of rare frequency of occurrence (6-14 samples out of 140 tested water samples, each). General outlook on the recovered and explored terrestrial fungal genera and species indicate that these fungi were almost recorded, but with various incidences and total numbers, in previous studies in Saudi Arabia as represented in substrata such as soil, mud, rain water and ferns[6-8,24,25]. Most of the isolated genera and species of terrestrial fungi (Table 2) are more or less similar to the mycobiota which were recovered from different water and mud habitats in other regions in the world as reported by Hudson[26], Sundaram[27], Abdel-Kader *et al.*[28], Tan *et al.*[29], El-Hissy *et al.*[30], Moharram *et al.*[31], Bettucci *et al.*[32], Bettucci and Roquebert[33]. Several terrestrial fungi were also reported to be recovered in England from Windermere Lake[34], Yately Lake[35] as well as from freshwater foam in India[36] and from the Sea of Japan[37].

Cellulose decomposing fungi isolated on cellulose Czapek´s agar medium: The data presented in Table 2 indicate that thirty-nine species appertaining to nineteen genera of terrestrial fungi were isolated and recovered on cellulose Czapek´s medium.

Cladosporium was the leading of the recovered terrestrial fungal genera on cellulose Czapek´s agar and it was of high occurrence (87 out of 140 drinking water samples). This genus was represented by two species namely; *C. cladosporoides* (moderate occurrence, 62 samples, 92 isolates) and *C. herbarum* (low occurrence, 25 samples, 60 isolates). *Aspergillus* came next and it was also of high frequency of occurrence (84 samples out of 140 water samples). It was included five species of which; *A. niger* and *A. carbonarius* were of low occurrence (17-44 samples each, 137 and 65 isolates, respectively) whilst *A. sydowi*, *A. terreus* and *A. versicolor* (6-13 water samples, each, 46, 7 and 8 isolates, respectively). *Fusarium* was of high frequency of occurrence (72 water samples) and it was represented by four species. Two of these species (*F. solani* and *F. oxysporum*) were of low occurrence (29-18 water samples) while the other species (*F. acuminatum* and *F. moniliforme*) rarely occurred (13-12 samples). *Penicillium* also highly occurred in the investigated water samples (71 samples) and it was represented by five species. These were *P. chrysogenum*

and *P. purpurogenum* which regarded as of low occurrence (24 and 20 samples, 42 and 33 isolates, respectively) while *P. oxalicum*, *P. citrinum* and *P. funiculosum* were rarely occurred (11-24 samples each, 44, 30 and 14 isolates, respectively). *Trichoderma* was recovered in moderate frequency of occurrence and it was represented by two species. These species namely *T. viride*, which moderately encountered (54 samples, 71 isolates) and *T. harzianum,* which rarely occurred (9 samples, 20 isolates). *Phoma* was also considered as of moderate frequency of occurrence (56 samples out of 140 water samples). It was also included two species and these named; *P. humicola* and *P. glomerata* (low occurrence each; 54 and 32 samples, respectively) representing 56 and 46 isolates, respectively. *Chaetomium* came parallel to *Phoma* and it was of moderate occurrence where it was isolated from 56 water samples matching 130 isolates. This genus was represented by three species namely *C. spirale*, *C. olivaceum* and *C. globosum*, which were of low occurrence (12-26 samples, each) constituting 31, 50 and 49 isolates, respectively. *Curvularia* (*C. lunata*) was also isolated in moderate frequency of occurrence (48 water samples, 78 isolates). *Alternaria* was recovered from 37 water samples and it was of moderate occurrence. It was included *A. alternata* (low occurrence, 31 samples, 52 isolates) and *A. tenuissima* (rare occurrence, 6 samples, 16 isolates). Both of *Drechslera* (*D. specifera*, 34 water samples, 68 isolates) and *Rhizopus* (*R. rhizopodiformis*, 34 samples, 39 isolates) were recovered on cellulose Czapek´s in low frequency of occurrence. *Humicola* (*H. grisea*, 32 water samples, 40 isolates) was also recorded in low occurrence). *Ulocladium* was isolated in low frequency of occurrence (28 water samples) and it was included two species of which *U. atrum* was of low occurrence (22 samples, 31 isolates) and *U. botrytis* was of rare occurrence (6 samples, 11 isolates). *Myrothecium* (*M. verrucaria*, 27 samples, 56 isolates) was also regarded as of low frequency of occurrence. *Mucor* was of low frequency of occurrence where it was isolated from 19 water samples. It was represented by *M. circinelloides* and *M. racemosus* which were of rare occurrence (16 and 3 water samples, 18 and 4 isolates, respectively). *Paecilomyces* (*P. variotti*, 18 samples, 41 isolates) was also of low occurrence. Both *Stachybotrys* (*S. chatarum* and *S. atra*) and *Emericella* (*E. nidulans*) were of rare frequency of occurrence (10-15 water samples, each). The majority of the isolated genera and species of terrestrial fungi are, in almost, similar to those which were recovered on cellulose Czapek´s agar, but with different frequencies of occurrence and counts of isolation from various water resources in Saudi Arabia, as reported by El-Nagdy and Nasser[7] and Ali and Nasser[8]. Also, they were almost recorded in soil mud and water substrata in Saudi Arabia, Syria and Egypt[6,24,28,38,39]. It is of interest to recall that, several of the isolated species are famous for by its ability to decompose cellulose as noted and revealed by Tribe[40,41], Malik and Eggins[1], Walsh and Stewart[42], Stewart and Walsh[23], El-Nagdy and Nasser [7], Ali and Nasser[8].

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