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Yeast and Fungal contamination of Surface and Groundwater in Sohag Governorate, Egypt

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Abstract

One of the most prominent concerns for the water consumers is microbial contamination, which is linked to various sources and health related problems, mainly to immunocompromised individual. Therefore the present study was focused on screening of drinking water mycobiota. One hundred and eighty samples were collected from 30 sites in Sohag Governorate, Egypt. Temperature, pH, total dissolved solids (TDS) and electric conductivity (EC) were investigated for each tested sample. The population density of yeast and filamentous fungi were detected and identified using YPDA And PDA media at 28±2° C. ***Candida glabrata*** was the most common yeast species in surface water represented 60% and 88% in winter and 51% and 100% in summer. Whereas in groundwater occurred in 43% and 32% in winter and 31% and 41% in summer of total yeast count and total tested samples, respectively. On the other hand, *Aspergilllus* species were the most widespread filamentous fungi in tested water samples. It was isolated from surface water samples in 84% and 100% in winter and 79% and 100% in summer, while in groundwater they were recorded in 95% and 100% in winter and 93% and 100% in summer of total fungal count and total tested samples, respectively. ***A. flavus*** was represented by 43% and 100% in winter and 38% and 100% in summer from surface water and 47% and 82% in winter and 43% and 82% in summer from groundwater. ***A. niger*** ranked the second place with 16% and 63% in winter and 15% and 75% in summer from surface water and 30% and 91% in winter and 28% and 91% in summer from groundwater.

Key words: Surface water, ground water, yeast, filamentous fungi, water contamination.

Introduction

Water purity and quality are paramount importance to human health **(Tebbutt 1998).** Quality of the drinking water is a crucial demand as it is an essential substance upon which all life depends. Surface water sources only constitute 0.0067% of the total world water **(Liu *et al.* 2011)**. While the world groundwater attained about one hundred times more plentiful than surface water **(Alley 2003).**

In Egypt, more than 96% of all fresh water resources are supplied by the Nile River **(Abu-Zeid 1991).** While the water resources in study area are represented by the water from the Nile River and groundwater extraction. Groundwater provides about 85% of the potable water supply **(Abdel-Moneim 1992).** So, groundwater preservation and protection measures have been generally overlooked in the majority of the practices **(Shaibani 2008).**

Drinking water resources were contaminated by some fungi causing problems in taste and odor of water (**Ahmed *et al.* 2010).** Fungi are accounted as a significant cause of water pollution due to have the ability to survive after filtration **(Kirk *et al.* 2001 and Cabral and Pinto 2002).** Fungi have been reported as pollutant and contaminant of all water types, like raw water, treated water even distilled or bottled drinking water **(Hussain *et al.* 2011).** In addition, when present in certain matrixes, fungi may produce mycotoxins that can display overlapping toxicities to both vertebrates and invertebrates, also plant and microorganisms **(Bennett and Klich 2003)**.

To protect the consumers from waterborne diseases, drinking water utilities should be ensure completely free of pathogenic microorganisms **(Petrucio *et al.* 2005).** Therefore the objective of this work was to study the distribution, isolation and identification of mycobiota in surface and groundwater in Sohag governorate, Egypt.

Materials and Methods:

1. Study area :-

The study area (Sohag Governorate), represents a part of the Nile valley extending between latitude 26°05′58″ to 27°00′00″ N and longitude 31°10′38″ to 32°15′00″E **(Ahmed *et al.* 2014)**.

2. Samples collection :-

One hundred and eighty water samples were collected from 30 sites in Sohag Governorate, Egypt. These samples included, 8 sites from Nile river (surface water) and 22 wells represented groundwater. Six samples from each site, 3 samples during winter season (one sample monthly at each of December, January and February, 2017) and 3 at summer season (May, June and July in the same year) as stated in **Table (1)**.

All samples were collected in 1L sterilized plastic bottles (polyethylene) with tight covers. For sterilization, the sampling bottles were rinsed three

times with distilled water, then with HNO_3 1% and dried, then rinsed with sample before filling and covered, no air-bubbles in samples containers **(APHA. 1992).**

3. Determination of water properties :-

Temperature, hydrogen ion concentration (pH), total dissolved solids (TDS) and electric conductivity (EC) were determined in collection sites**.** All bottles were marked (date, time, samples location latitude and longitude then transferred to laboratory and stored in refrigerator at 4°C **(Trick *et al.* 2008).**

4. Isolation of yeast and filamentous fungi:-

Yeast extract peptone dextrose agar medium (YPDA) **(Ajello *et al*. 1963**) and potato dextrose agar medium (PDA) (**Difco 1985**) were used for isolation of yeast and filamentous fungi, respectively. Media were sterilized at 121ºC for 20 min. Chloramphenicol 100 mg/l was added to prevent bacterial contamination. Serial dilutions were performed for each sample. One ml of suitable dilution of each sample was put into three plates, and then medium was distributed into Petri dishes and let to solidify. Plates were incubated at 28ºC±2 for 5-7 days for fungi and 3 days for yeast. The developing colonies were counted, purified and microscopically examined for morphological characteristics.

5. Identification methods of yeast and filamentous fungal isolates :-

Yeast isolates were identified by morphological characteristics and biochemical testes (as indicated in **Barnett *et al.* 2007**) in Mycology Lab, Botany and Microbiology Department, Faculty of Science, Sohag University, Egypt. The identification of yeast isolates was also confirmed by Assuit University Mycology Center (AUMC), Egypt.

The filamentous fungi were identified based on macro- and microscopic characterization using different identification books **(Raper and Fennel 1965, Ellis 1971 & 1976, Moubasher 1993, Leslie and Summerell 2006, Dommsch *et al.* 2007, Pitt and Hocking 2009 and Ismail *et a*l. 2015)**

Table (1): Sites of samples collection in study area.

Surface water samples (Nile river)			
Sample No.	**Location**	**Longitude**	**latitude**
S_1	**Dar-EL-Saalm**	**31,56,22 E**	**26,12,51 N**
S2	**Dar-EL-Saalm**	**31,56,22 E**	**26,19,27 N**
S3	**EL-Balyna**	**31,52,45 E**	**26,17,27 N**
S4	**Gerga**	**31,53,31 E**	**26,21,13 N**
S5	**EL-Menshah**	**31,49,41 E**	**26,31,55 N**
S6	**Sakolta**	**31,41,40 E**	**26,44,11 N**
S7	**Maragha**	**31,37,58 E**	**26,38,00 N**
S8	**Tema**	**32,15,00 E**	**27,00,00 N**

Groundwater samples (Wells)				
Sample No	**Location**	**Depth (m)**	**Longitude**	**latitude**
G1	**Dar-EL-Saalm**	**42.0 m**	**31,58,14 E**	**26,19,54 N**
G2	**Gerga**	**18.0 m**	**31,52,31 E**	**26,21,17 N**
G3	**Dar-EL-Saalm**	**34.5 m**	**31.,57,14 E**	**26,21,01 N**
G4	**Gerga**	**17.0 m**	**31,50,19 E**	**26,20,01 N**
G5	**Gerga**	**36.0 m**	**31,53,39 E**	**26,22,13 N**
G6	**Gerga**	**15.0 m**	**31,50,27 E**	**26,40,07 N**
G7	**EL-Aoserat**	**31.0 m**	**31,49,48 E**	**26,47,09 N**
G8	**EL-Aoserat**	**48.0 m**	**31,48,51 E**	**26,47,54 N**
G9	**Tema**	**32.0 m**	**31.11.14 E**	**26,57,44 N**
G10	**Akhmem**	**11.0 m**	**31.,48,47 E**	**26,38,89 N**
G11	**Maragha**	**36.0 m**	**31,37,59 E**	**26,43,00 N**
G12	**Maragha**	**32.0 m**	**31,38,03 E**	**26,42,49 N**
G13	**Tahta**	**37.0 m**	**31,27.17 E**	**26,52.11 N**
G14	**Tahta**	**45.0 m**	**31,26.57 E**	**26,52.13 N**
G15	**Gehina,**	**29.5 m**	**31,31,40 E**	**26,41,11 N**
G16	**Sakolta**	**39.5 m**	**31,39,44 E**	**26,40.49 N**
G17	**Sakolta**	**36.5 m**	**31,39,44 E**	**26,41.02 N**
G18	**Sohag**	**31.0 m**	**31.38.12 E**	**26.39.08 N**
G19	**EL-Balyana**	**41.0 m**	**31,51,54 E**	**26,17,30 N**
G20	**Akhmem**	**47.0 m**	**31,47.85 E**	**26,38,51 N**
G21	**EL-Menshah**	**36.5 m**	**31,43,49 E**	**26,30,19 N**
G22	**EL-Menshah**	**32.0 m**	**31,44,03 E**	**26,31,46 N**

Results and Discussion

In Egypt, the Nile River and groundwater are considered the main water sources for human uses. In many parts of Egypt, the ground water is widely used for drinking and other domestic purposes **(Mamdouh *et al.* 2003).** Unfortunately, these sources of water could be contaminated chemically, physically or microbiologically and lead to health problems for human users. Therefore, to evaluate the quality of water must study their natural properties and estimate the extent of contamination and pollution.

1- Physicochemical properties :-

Temperature of drinking water does not effect on quality and there are no specific standers for it, but usually left to the individual taste of consumers. Data presented in Table **(2)** revealed that the temperature of samples ranged from 17.1-19.7 & 15.1-17.1^{o}C in winter and from 25.3-30.1 & 24.5 - 30.4^{o}C in summer in surface and groundwater samples, respectively. This result full agreed with that recorded by **Jayraman *et al.* (2003) and Sichel *et al.* (2007)** who reported that, water temperature differed with the collection time, depth, flow rate and influence of seasons.

pH of all tested samples was alkaline, in surface samples ranged from 8.0 -8.7, while in groundwater samples ranged from 7.1-8.1 as shown in Table **(2)**. This result was in relative agreement with **WHO (2011),** who reported that the optimum range of pH, is 6.5-8.5. Also, there is mild increase ratio in some surface water sites S_1 (Dar-EL-Saalm) and S_8 (Tema) (winter season). In respect, the higher pH induces the formation of toxic trihalomethanes **(Remia and Logaswamy 2010).**

Total dissolved solids (TDS), is consists of ions of inorganic salts which determine the saline behavior of the water stream and also a small amount of organic matter that may be dissolved in water **(Heydari and Bidgoli 2012).** A higher TDS causes adverse change in the taste of water and is also not good for metallic pipelines used for transportation of water inside homes **(Ramesh and Seetha 2013).** In general, TDS in groundwater samples was higher than the surface water. That TDS level of surface water samples ranged from 141 (S_3-ELBalyna) to 215 ppm (S_5-ELMenshah). In groundwater the TDS ranged from 201 (G_4-Gerga) to 765 ppm (G_{21}-ELNenshah). All surface tested samples were<600 ppm, while, in groundwater 4 samples (G_{18}-Sohag, G_{19}-EL-Balyana, G_{21}-EL-Menshah and G_{22}- EL-Menshah) were >600 ppm **(Table 2).** In spite of this relative increase in some samples the TDS content of surface and groundwater in the study area generally considered to be good for drink (<1000 ppm) according to **WHO (2011).**

The data recorded in Table **(2)** revealed that, the electric conductivity (EC) in surface water samples ranged from 271 (S_8-Tema) to 431 µS/cm, (S_5-EL-Menshah), while in groundwater samples, ranged from 316 (G_4-Gerga-EL-Koraan) to 1532 µS/cm (G_{21}-ELMenshah). EC is an indicator of the water mineralization degree and its increase is mainly related to effect of pollution in water resources **(Meena and Bhargava 2012)**.

2- Identification of mycobiota

a- Yeast identification :-

Data in Table **(3)** showed that the yeast isolates recorded from different surface water samples were 311 (winter) and 501 (summer), while from groundwater samples were 214 (winter) and 445 (summer) with total count of 812 in surface water and 659 in groundwater. The yeast species identified were *Candida albicans*, *C. glabrata*, *C. krusei*, *Saccharomyces cerevisiae*, *Pichia kudriavzevii* and *Rhodotorula mucilaginosa*.

Results in Tables **(4 and 5)** and Figs **(1a, 1b, 2a and 2b)** revealed that *Candida* was the most common yeast genus, represented by 3 species namely, *C. albicans*, *C. glabrata* and *C. krusei.* *C. glabrata* was the highly frequency of occurrence with high total count (60% in winter and 51% in summer) in surface water and 43% in winter and 31% in summer in groundwater, followed by *C. krusei* (22% in winter and 27% in summer) in the surface water and 32% in winter and 37% in summer in ground water samples. While, *Saccharomyces cerevisiae* was occurred in 6% in winter and 7% in summer in surface and 10% in winter and 13% in summer in groundwater samples. Whereas, *C. albicans* was isolated from surface water (1% in winter and 2% in summer) and from ground water (9% in winter and 10% in summer). *Pichia kudriavzeui* was detected in 8% in winter and 8% in summer from surface and 2% in winter and 5% in summer from ground water. Whilst, *Rhodotorula mucilaginosa* represented 3% in winter and 8% in summer in the surface and 3% in winter and 4% in summer in ground water samples.

Some yeast species isolated from study area were a serious indicator for water contamination by pathogenic microbes. These results were in harmony with that recorded by **Paterson *et al.* (2009)** who reported that the most problematic mycobiota species are *Candida* spp. (especially *C. albicans*). Also, **Aronson and Soltani (1976)** and **Gomez-Lopez *et al.* (2005)** recorded that the members of the genus *Candida* and *Rhodotorula* are considered as waterborne pathogens. *Candida* is the most and *Rhodotorula* is rare opportunistic pathogenic yeasts associated with variety of human diseases. **Halaby *et al.* (2001)** reported that, In healthy adults, yeast species are usually commensal organisms in harmony with their host organisms but, in immunocompromised individuals, they may cause disorder especially *Candida* species which may cause candidiasis.

Table (2): Physicochemical parameters of surface and ground water samples.

Samples No	Temperature	pH	TDS ppm	EC µS/cm
Surface water (Nile river)				
S_1	17.3-25.3	8.4-8.7	168-170	312-341
S2	19.1-29.3	8.0-8.1	205-209	348-411
S3	19.2-25.7	8.0-8.0	141-158	297-321
S4	17.1-28.7	8.3-8.4	146-158	279-319
S5	17.8-29.7	8.1-8.1	215-215	401-431
S6	18.8-27.1	8.1-8.2	149-162	316-324
S7	19.7-29.6	8.2-8.2	162-168	284332
S8	17.1-30.1	8.4-8.6	199-209	271348
Groundwater (Wells)				
G1	15.6-27.8	7.7-7.9	219-249	387-497
G2	16.1-29.1	7.5-7.6	243-274	401-516
G3	15.9-26.7	7.5-7.6	399-419	711-842
G4	16.2-28.8	7.9-8.0	201-250	316-449
G5	16.4-30.4	7.6-7.6	234-299	479-603
G6	16.2-29.7	7.7-7.8	216-276	413-541
G7	16.1-28.8	7.8-7.8	218-289	417-566
G8	15.8-27.6	7.6-7.7	269-301	434-589
G9	15.6-27.1	7.6-7.6	241-267	389-519
G10	17.1-31.1	7.4-7.4	252-291	423-569
G11	15.4-27.4	7.5-7.6	273-314	489-612
G12	16.3-30.4	7.5-7.6	241-291	412-578
G13	15.1-24.5	8.0-8.0	219-249	416-497
G14	16.3-30.2	7.4-7.5	314-399	667-806
G15	15.8-28.2	8.1-8.1	243-274	419-516
G16	15.7-22.9	7.7-7.9	371-419	691-832
G17	15.8-27.5	8.0-8.0	211-250	387-499
G18	15.4-29.1	7.8-7.9	561-649	1119-1294
G19	15.1-27.6	7.9-8.0	547-619	1167-1237
G20	15.1-27.2	7.1-7.3	314-281	417-541
G21	15.9-29.6	7.7-7.8	711-765	1429-1532
G22	16.4-30.2	7.5-7.6	626-694	1211-1392

Table (3): Yeast genera and species isolated from surface and groundwater samples on YPDA medium at 30±2°C.

Yeast genera and species	Colonies counts					
	Surface water			Groundwater		
	W	S	Total	W	S	Total
Candida	257	403	660	181	34	530
C. albicans (C.P. Robin) Berkhout	02	10	12	20	45	65
C. glabrata (H.W. Anderson) S.A. Mey. & Yarrow .	187	257	444	92	14	232
C. krusei (Castell.) Berkhout	68	136	204	69	16	233
***Pichia** kudriavzevii* Boidin, pignal &Besson	25	38	63	04	24	28
***Rhodotorula** mucilaginosa* (A. Jörgensen.) F.C. Harrison	10	24	34	07	16	23
***Saccharomyces** cerevisiae* Meyen ex E.C. Hansen	19	36	55	22	56	78
Total count	311	501	812	214	44	659

W: winter S: summer

Table (4): Yeast genera and species isolated from surface water samples using YPDA medium at 30±2°C for 3 days.

S_1				S_2				S_3				S_4				S_5				S_6				S_7				S_8				Total					
W		S		W		S		W		S		W		S		W		S		W		S		W		S		W		S		W		S			
TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	T.C.	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI		
26	3	50	3	31	3	48	3	74	3	101	3	61	3	82	3	04	1	09	2	22	3	43	3	13	2	27	3	26	3	43	3	257	21	H	403	23	H
ND	---	02	1	ND	---	ND	---	ND	---	03	1	ND	---	ND	---	ND	---	ND	---	ND	---	02	1	ND	---	ND	---	02	1	03	1	02	1	R	10	4	L
21	3	32	3	27	3	39	3	57	3	71	3	44	3	52	3	ND	---	01	1	20	3	32	3	07	2	13	2	11	2	17	2	187	19	H	257	20	H
05	1	16	3	04	1	09	2	17	3	27	3	17	3	30	3	04	1	08	1	02	1	09	2	06	1	14	2	13	2	23	3	68	13	H	136	19	H
ND	---	ND	---	07	2	12	3	06	2	08	2	09	2	13	2	ND	---	ND	---	03	1	04	1	ND	---	ND	---	ND	---	01	1	25	7	M	38	9	M
03	1	05	2	02	1	03	1	ND	---	ND	---	ND	---	03	1	05	1	08	2	ND	---	02	1	ND	---	ND	---	ND	---	03	1	10	3	L	24	8	M
05	1	08	2	03	1	05	2	ND	---	02	1	06	2	09	2	ND	---	02	1	ND	---	ND	---	ND	---	02	1	05	2	08	2	19	6	M	36	11	M
34	3	63	3	43	3	68	3	80	3	111	3	76	3	107	3	09	2	19	3	25	3	49	3	13	3	29	3	31	3	55	3	311	23		501	24	

W: Winter season, S: Summer season, TC: Total count, NCI: Number of cases of isolation, ND: Not detected YPDA: Yeast extract peptone dextrose agar medium.

*** Occurrence remarks:-**

H: High occurrence: 12-24 tested samples. M: Moderate occurrence: 6-11 tested samples. L: Low occurrence: 3-5 tested samples. R: Rare occurrence: Less than 3 tested samples.

Table (5): Yeast genera and species isolated from groundwater samples using YPDA medium at 30± 2°C for 3 days.

Samples	G_1			G_2			G_3			G_4			G_5			G_6			G_7		
Season	W		S	W		S	W		S	W		S	W		S	W		S	W		S
Yeast genera and species	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	NCI
Candida	03	1	07	ND	---	ND	ND	---	ND	23	3	40	01	1	06	10	3	21	ND	---	ND	---
C. albicans	03	1	04	ND	---	ND	ND	---	ND	ND	---	01	ND	---	01	04	1	06	ND	---	ND	---
C. glabrata	ND	---	ND	ND	---	ND	ND	---	ND	23	3	31	ND	---	ND	ND	---	ND	ND	---	ND	---
C. krusei	ND	---	03	ND	---	ND	ND	---	ND	03	1	08	05	1	2	06	2	15	ND	---	ND	---
Pichia kudriavzevii	ND	---	01	ND	---	ND	ND	---	ND	ND	---	02	ND	---	1	ND	---	ND	ND	---	ND	---
Rhodotorula mucilaginosa	ND	---	ND	ND	---	ND	ND	---	ND	ND	---	ND	ND	---	---	ND	---	ND	ND	---	ND	---
Saccharomyces cerevisiae	ND	---	01	ND	---	ND	ND	---	ND	ND	---	03	ND	---	ND	02	---	02	ND	---	ND	---
Total	03	1	09	00	---	00	00	---	00	26	3	45	01	1	06	10	3	23	00	---	00	---

Table (5): Continued

Samples	G_8			G_9			G_{10}			G_{11}			G_{12}			G_{13}			G_{14}			G_{15}		
Season	W		S	W		S	W		S	W		S	W		S	W		S	W		S	W		S
Yeast genera and species	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	NCI
Candida	26	3	42	19	3	39	ND	---	ND	05	1	12	ND	---	ND	03	1	14	ND	---	ND	ND	---	ND	---
C. albicans	N D	---	N D	03	1	05	N D	---	N D	N D	---	N D	N D	---	N D	N D	---	02	N D	---	N D	N D	---	N D	---
C. glabrata	19	2	27	N D	---	N D	N D	---	N D	05	1	08	N D	---	N D	N D	---	01	N D	---	N D	N D	---	N D	---
C. krusei	07	2	15	16	2	34	N D	---	N D	N D	---	04	N D	---	N D	03	1	11	N D	---	N D	N D	---	N D	---
Pichia kudriavzevii	N D	---	03	N D	---	N D	N D	---	N D	03	---	1	N D	---	1	N D	---	04	N D	---	N D	N D	---	N D	---
Rhodotorula mucilaginosa	N D	---	N D	N D	---	01	N D	---	N D	07	2	13	N D	---	2	N D	---	N D	N D	---	N D	N D	---	N D	---
Saccharomyces cerevisiae	05	2	08	09	2	17	N D	---	N D	N D	---	02	N D	---	1	N D	---	02	N D	---	N D	N D	---	N D	---
Total	31	3	53	28	3	57	N D	---	N D	12	3	30	N D	---	3	03	2	20	N D	---	N D	N D	---	N D	---

Table (5): Continued

Samples	G_{16}				G_{17}				G_{18}				G_{19}				G_{20}				G_{21}				G_{22}				Total													
Season	W		S		W		S		W		S		W		S		W		S		W		S		W		S		W		S											
Yeast genera and species	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI									
Candida	29	3	50	3	02	1	06	2	13	3	23	3	16	3	30	3	10	2	24	3	05	1	28	3	17	3	30	3	04	1	24	3	112	27	185	43						
C. albicans	12	2	16	3	ND	---	01	1	---	---	03	1	ND	---	01	1	ND	---	03	1	02	1	ND	---	04	---	01	---	ND	---	01	---	20	5	45	15						
C. glabrata	ND	---	02	1	ND	---	ND	---	13	2	19	2	05	2	07	2	17	3	26	3	ND	---	02	1	21	3	03	1	17	3	26	3	92	15	140	20						
C. krusei	17	3	32	3	02	1	05	2	ND	---	01	1	11	2	21	3	ND	---	03	1	01	1	03	2	ND	---	04	1	69	16	164	M									
Pichia kudriavzevii	ND	---	01	1	ND	---	02	1	04	2	07	2	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	01	---	04	1	24	R	11	L								
Rhodotorula mucilaginosa	ND	---	02	1	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	07	2	16	R	4	R								
Saccharomyces cerevisiae	ND	---	02	1	ND	---	01	1	ND	---	ND	---	08	2	14	3	ND	---	02	1	02	1	ND	---	ND	---	ND	---	22	6	56	R	17	M								
Total	29	3	55	3	02	1	09	3	17	3	30	3	24	3	42	3	17	3	32	3	07	1	25	3	10	2	214	35	445	45												

W: Winter season S: Summer season C :Total count NCI : Number of cases of isolation. ND: Not detected YPDA: Yeast extract peptone dextrose agar

*** Occurrence remarks:**

(H) High occurrence: 33-66 (M) Moderate occurrence: 16-32 (L) Low occurrence: 8-15 (R) Rare occurrence: Less than 8 tested samples

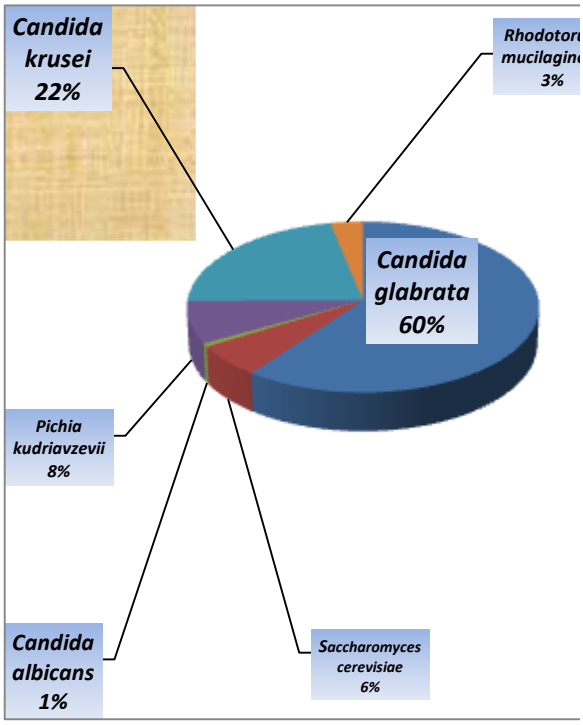

Fig (1a): Population density of yeast species in surface water samples (winter season)

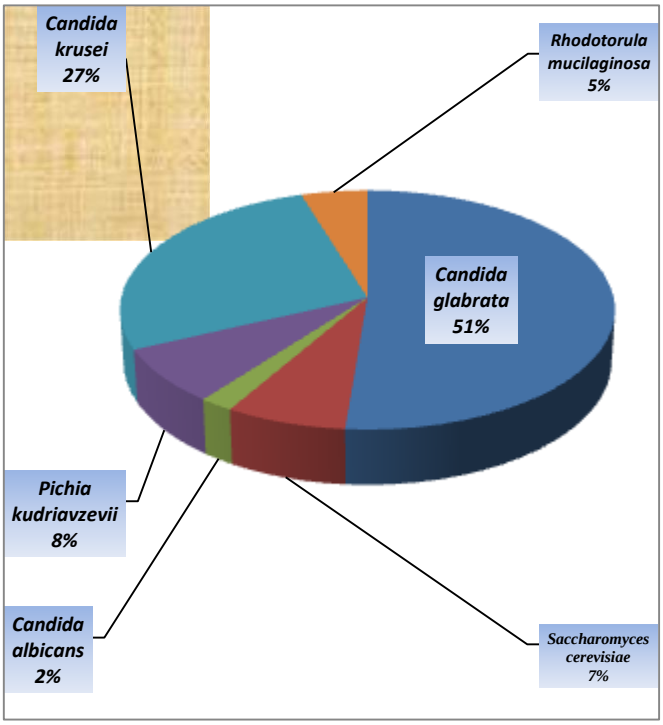

Fig (1b): Population density of yeast species in surface water samples (summer season)

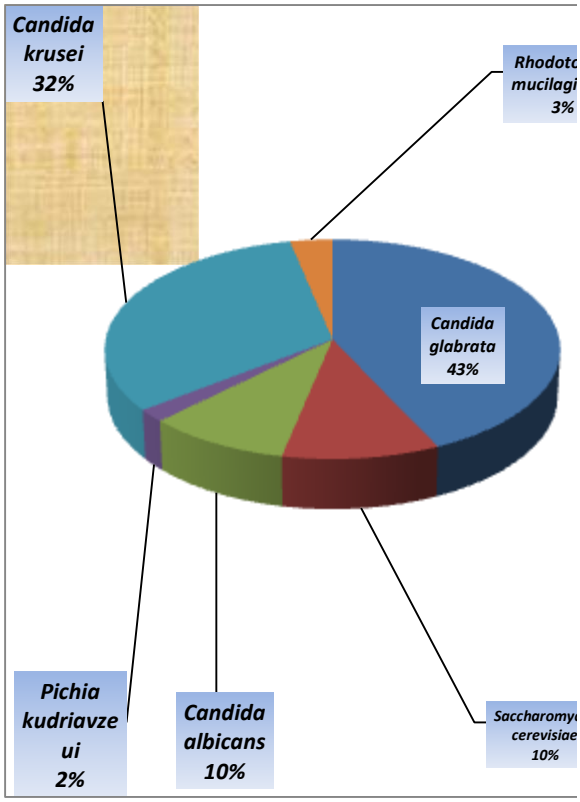

Fig (2a): Population density of yeast species in ground water samples (winter season)

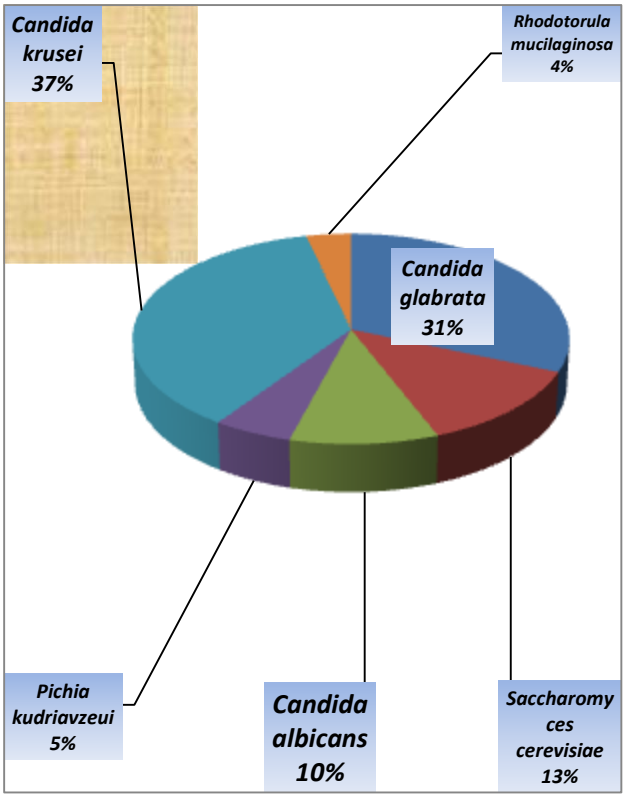

Fig (2a): Population density of yeast species in ground water samples (summer season).

b- Filamentous fungi

Data stated in Table (6) revealed that 21 fungal species and 3 species varieties belonging to 11 genera were isolated. 9 genera, 14 species and 2 species varieties from surface water while (9, 16 and 3) in groundwater. As general, the total count of filamentous fungi recorded in surface water were 367 (winter) and 741 isolates (summer), while in groundwater were 989 (winter) and 1817 (summer).

Aspergillus awamori, Acremonium alternatum, Humicola grisea, Neurospora crassa and *Talaromyces purpureogenus (Penicillium purpureogenum*) appeared in surface water and disappeared in groundwater. While, *A. avenasceus, A. foetidus, A. terreus* var. *africans, Acremonium rutilum, Cladosporium cladosporioides, Fusarium roseum* and *Gymnoascus aurantiacus* were detected in groundwater only.

Data in Tables (7 and 8) and illustrated Figure (3a, 3b, 4a and 4b) revealed that, in surface water the site S_8 (Tema) contains the highest fungal population density (73 out of 367 isolates) in winter and 138 out of 741 in summer and the lowest density was recorded in site S_1 (Dar-EL-Salam), 16 out of 367 isolates (winter) and 43 out of 741 (summer). On the other hand, in groundwater the highest density was observed in well G_{22} (EL-Menshah) with 114 out of 989 isolates (winter) and 194 out of 1817 (summer). While, the lowest density was recorded in well G_5 (Gerga) with 13 out of 989 isolates (winter) and 30 out of 1817 (summer).

Aspergillus was the most dominant genus isolated with the richest total count and frequencies of occurrence, from surface water samples (309 colonies and 23/24 of tested samples in winter) and 583 and 24/24 in summer. While from groundwater samples (943 colonies and 64/66 of tested samples in winter) and 1690 and 66/66 in summer. *Aspergillus* was represented by 5 species and 2 species varieties in surface water, and 6 and 3 in groundwater samples as shown in Tables (7 and 8).

A. flavus showed the highest total count of filamentous fungi in both tested water samples 158 and 469 colonies in winter and 281 and 782 in summer in surface and ground water respectively as listed in Tables (7 and 8). *A. niger* came the second rank represented by 58 out of 367 (winter) and 109 out of 741colonies (summer) in surface water, while in groundwater accounted 292 out of 989 (winter) and 512 out of 1817 (summer). With mentioning that, the total count of filamentous fungi was higher in groundwater than in surface water and in summer than in winter. They were accounted 1817 and 741 (summer) and 989 and 367 (winter) in ground and surface water, respectively (Table 9).

Katsouyannopoulos (2000) and Anaissie and Costa (2001) reported that, many species of fungi particularly *Aspergillus* species are found in water and can cause kidney problem, liver disorders, allergy, otitis and increase risk of invasive infections**.**

Also, **Cabral and Pinto (2002)** reported that water can be contaminating by different species of yeasts and filamentous fungi and *Aspergillus* species were common and the causal agent of pulmonary aspergillosis. **Abbott (2002)** stated that, some species like *A. flavus* able to produce a wide spectrum of aflatoxins and confirmed to be carcinogenic effects.

Some species isolated in this study, such as *Aspergillus*, *Cladosporium*, *Penicillium* and *Rhodotorula* can cause life-threatening disease for human **(Richardson 2005).** Also some of them are considered to produce mycotoxins as; trichothecenes produced by *Fusarium* strains and Ochratoxin are secondary metabolites of *Aspergilllus* and *Penicillium* strains (**Speijers and Van Egmond 1993).** Ochratoxin A, is the most toxic, has been shown to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic in all experimental animals tested (**WHO 1990).**

The two major *Aspergilllus* species that produce aflatoxin are *A. flavus* and *A. parasiticus* produce aflatoxins B_1, B_2, G_1 and G_2. The evaluation of epidemiological and laboratory results carried out by IARC (1987), who found that there is sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxin, which classified as Group1 carcinogens.

Conclusion

Microbiologically, all tested surface and groundwater samples were contaminated with different filamentous and yeast species. Some of them are potentially risk healthy hazard on human especially *Aspergillus flavus* and *A. parasiticus* which are aflatoxin-producers that considered as carcinogenic agents and have clinical signs of aflatoxicosis resulting in carcinoma of liver and acute hepatic failure and *A. niger* which causes aspergillosis. In addition to ***Candida albicans*** which causes candidiasis. Therefore to ensure the quality of water drinking, it is necessary to investigate the microbiological presence of pathogenic yeast and moulds in drinking water sources.

In light of the knowledge obtained from fungal water studies, and the insufficient epidemiological knowledge about health impacts, it seems a good idea to take precautionary measures for pathogenic fungal species and yeasts in the water environment and should be included in the Egyptian and international standards methods for drinking water quality.

Table (6): Fungal species from surface and groundwater samples on PDA medium at 30±2°C

Fungal genera and species	Colonies count					
	Surface			Ground		
	Winter	Summ	Total	Winter	Summer	Total
Acremonium W. Games	06	16	22	01	05	06
A. alternatum Link	06	16	22	ND	ND	---
A. rutilum W. Gams	ND	ND	---	01	05	06
Alternaria alternata (Fr.) Keissl.	05	24	29	16	32	48
Aspergillus P. Micheli ex Link	309	583	892	943	1690	2633
A. awamori Nakaz.	24	53	77	ND	ND	---
A. avenaceus G. Sm.	ND	ND	---	46	98	
A. flavus Link	158	281	439	469	782	1251
A. flavus* var. *columnaris Raper & Fennell	05	15	20	22	56	78
A. foetidus Thom & Raper	ND	ND	---	11	33	44
A. niger Van Tiegh	58	109	167	292	512	804
A. parasiticus Speare	07	21	28	01	05	06
A. terreus Thom	52	91	143	88	172	260
A. terreus* var. *africans Fennell & Raper	ND	ND	---	02	04	06
A. terreus* var. *aureus Thom & Raper	05	13	18	12	28	40
Cladosporium cladosporioides (Fresen.) G.A. de	ND	ND	---	04	11	15
Fusarium Link	19	42	61	16	39	55
F. oxysporum Sensu Smith & Swingle	08	19	27	01	03	04
F. roseum Link	ND	ND	---	09	18	27
F. solani (Mart.) Sacc.	11	23	34	06	18	247
Gymnoascus aurantiacus (Peck) Sacc.	ND	ND	---	02	08	10
Humicola grisea Traaen	02	09	11	ND	ND	---
Neurospora crassa Shear & B.O. Dodge	05	18	23	ND	ND	---
Scopulariopsis Bainier	04	12	16	06	25	31
S. candida Vuill.	ND	ND	---	02	08	10
S. brevicaulis Bainier	02	07	09	04	17	21
Sepedonium chrysospermum (Bull.) Fr.	04	12	16	01	07	08
Talaromyces purpureogenus Stoll	15	30	45	ND	ND	---
Total	367	741	1108	989	1817	2806

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able (8): Fungal genera and species isolated from 100 µl of groundwater samples using PDA medium at 30±2°C for 7 days.

Samples	G1			G2			G3			G4			G5			G6			G7		
Season	W		S	W		S	W		S	W		S	W		S	W		S	W		S
Yeast genera and species	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	TC	NCI	TC	NCI
Acremonium rutilum	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
Alternaria alternata	ND	----	ND	ND	----	ND	01	1	03	ND	1	74	ND	----	ND	ND	----	ND	02	1	05	2
Aspergillus	29	3	57	13	2	34	36	3	74	93	3	147	10	2	25	40	3	76	47	3	85	3
A. avenaceus	ND	----	ND	ND	----	03	ND	----	ND	46	3	69	ND	----	ND	ND	----	07	ND	----	ND	----
A. flavus	24	3	41	05	2	13	14	3	23	27	3	41	04	2	09	34	3	58	37	3	62	3
A. flavus var. *columnaris*	ND	----	ND	04	2	09	04	2	07	06	2	11	01	1	04	ND	----	ND	ND	----	ND	----
A. foetidus	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
A. niger	03	2	07	ND	----	ND	07	2	17	07	2	13	05	2	09	ND	----	ND	10	3	23	3
A. parasiticus	01	2	05	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
A. terreus	01	1	04	ND	----	ND	05	1	14	07	2	13	01	1	03	04	1	07	ND	----	ND	----
A .terreus var. *africans*	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	02	1	04	ND	----	ND	----
A. terreus var. *aureus*	ND	----	ND	04	2	09	06	2	13	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
Cladosporium cladosporioides	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
Fusarium	01	1	03	03	1	07	ND	…	ND	ND	…	ND	ND	…	ND	ND	…	ND	ND	…	ND	…
F. oxysporum	01	1	03	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
F. roseum	ND	----	ND	03	1	07	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
F. solani	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
Gymnoascus aurantiacus	ND	----	ND	ND	----	ND	ND	----	3	ND	----	ND	02	1	05	ND	----	ND	ND	----	ND	----
Scopulariopsis	ND	----	ND	ND	----	ND	02	1	05	ND	----	ND	ND	----	ND	02	1	07	ND	----	ND	----
S. candida	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
S. brevicaulis	ND	----	ND	ND	----	ND	02	1	05	ND	----	ND	ND	----	ND	02	1	07	ND	----	ND	----
Sepedonium chrysospermum	01	1	07	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	ND	----	ND	----
Total	31	3	72	16	3	41	39	3	85	93	3	147	13	3	30	42	3	83	49	3	90	3

Table (8): Continued

Samples	G8				G9				G10				G11				G12				G13				G14				G15			
Season	W		S		W		S		W		S		W		S		W		S		W		S		W		S		W		S	
Yeast genera and species	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Acremonium rutilum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Alternaria alternata	ND	----	ND	----	02	2	07	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Aspergillus	28	3	54	3	28	3	47	3	30	3	50	3	40	3	72	3	87	3	152	3	31	3	45	3	18	3	25	3	55	3	79	3
A. avenaceus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	06	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
A. flavus	ND	----	ND	----	10	3	16	2	12	3	19	3	ND	----	ND	----	52	3	89	3	16	3	19	3	ND	----	ND	----	38	3	48	3
A. flavus var. columnaris	ND	----	04	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	03	1	07	2
A. foetidus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	11	2	23	3	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
A. niger	19	3	29	3	15	3	24	3	18	3	31	3	29	3	43	3	26	3	47	3	15	3	26	3	14	3	19	3	11	2	17	3
A. parasiticus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
A. terreus	07	2	15	3	03	1	07	2	ND	----	ND	----	ND	----	ND	----	09	3	16	2	ND	----	ND	----	04	1	06	2	03	1	07	2
A .terreus var. africans	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
A. terreus var. aureus	02	1	06	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Cladosporium cladosporioides	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	04	2	09	2	ND	----	ND	----
Fusarium	ND	…	ND	…	ND	…	ND	…	03	2	6	2	ND	…	ND	…	ND	…	ND	…	06	2	11	2	03	1	08	2	ND	…	04	1
F. oxysporum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
F. roseum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	06	2	11	2	ND	----	ND	----	ND	----	ND	----
F. solani	ND	----	ND	----	ND	----	ND	----	03	2	06	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	03	1	08	2	ND	----	04	1
Gymnoascus aurantiacus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Sopulariopsis	ND	----	03	1	ND	----	ND	----	ND	----	02	1	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
S. candida	ND	----	03	1	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
S. brevicaulis	ND	----	ND	----	ND	----	ND	----	ND	----	02	1	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Sepedonium chrysospermum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----
Total	28	3	57	3	30	3	54	3	33	3	58	3	40	3	72	3	87	3	152	3	37	8	56	3	25	3	42	3	55	3	83	3

Table (8): Continued

Samples	G16				G17				G18				G19				G20				G21				G22				Total			
Season	W		S		W		S		W		S		W		S		W		S		W		S		W		S		W		S	
Yeast genera and species	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Acremonium rutilum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	01	1	05	2	ND	----	ND	----	ND	----	ND	----	01	1	05	2
Alternaria alternata	ND	----	ND	----	03	2	04	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	08	2	13	3	ND	----	ND	----	16	8	32	10
Aspergillus	39	3	78	3	14	3	23	3	62	3	103	3	34	3	82	3	28	3	54	3	66	3	118	3	114	3	194	3	943	64	1690	66
A. avenaceus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	13	2	----	ND	----	ND	----	46	3	98	10
A. flavus	21	3	36	3	ND	----	ND	----	20	3	31	3	20	3	48	3	15	3	27	3	45	3	78	3	75	3	124	3	469	52	782	51
A. flavus var. columnaris	04	1	11	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	03	1	ND	----	ND	----	ND	----	ND	----	22	9	56	15
A. foetidus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	05	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	11	2	33	5
A. niger	14	3	31	3	07	2	11	2	40	3	69	3	09	2	17	3	07	2	13	2	13	2	25	3	23	3	41	3	292	52	512	55
A. parasiticus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	01	1	05	2
A. terreus	ND	----	ND	----	07	2	12	2	02	1	03	1	05	2	12	2	06	2	11	2	08	2	13	2	16	3	29	3	88	26	172	32
A .terreus var. africans	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	02	1	04	2
A. terreus var. aureus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	12	5	28	6
Cladosporium cladosporioides	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	02	1	ND	----	ND	----	04	2	11	3
Fusarium	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	ND	…	16	7	39	10
F. oxysporum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	01	1	03	1
F. roseum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	09	3	18	4
F. solani	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	06	3	18	5
Gymnoascus aurantiacus	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	02	1	08	3
Scopulariopsis	ND	---	ND	---	02	1	05	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	04	2	17	5
S. candida	ND	----	ND	----	02	1	05	2	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	02	1	08	2
S. brevicaulis	ND	----	03	1	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	04	2	17	5
Sepedonium chrysospermum	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	ND	----	01	1	07	2
Total	39	--	81	3	19	3	32	3	62	3	103	3	34	3	82	3	29	3	59	3	74	3	144	3	114	3	194	3	989	66	1817	66

W: Winter season S: Summer season TC :Total count NCI : Number of cases of isolation. ND: Not-detected PDA: Potato dextrose agar

* Occurrence remarks in surface water.

(H) High occurrence: 12-24 (M) Moderate occurrence: 6-11 (L) Low occurrence: 3-5 (R) Rare occurrence: Less 3

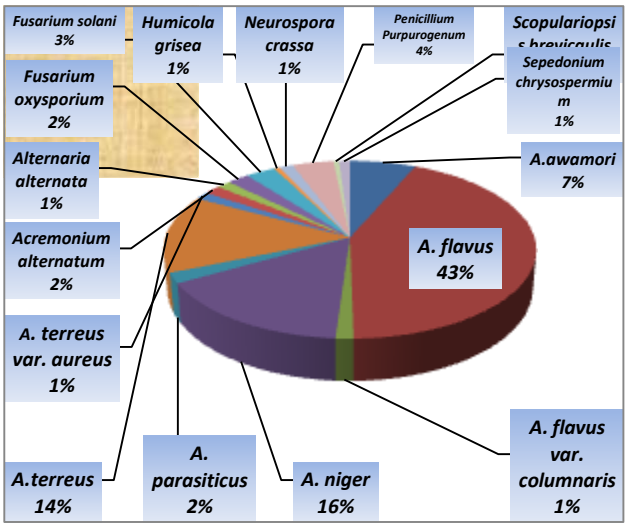

Fig (3a): Population diversity of filamentous fungal species in surface water samples at winter season

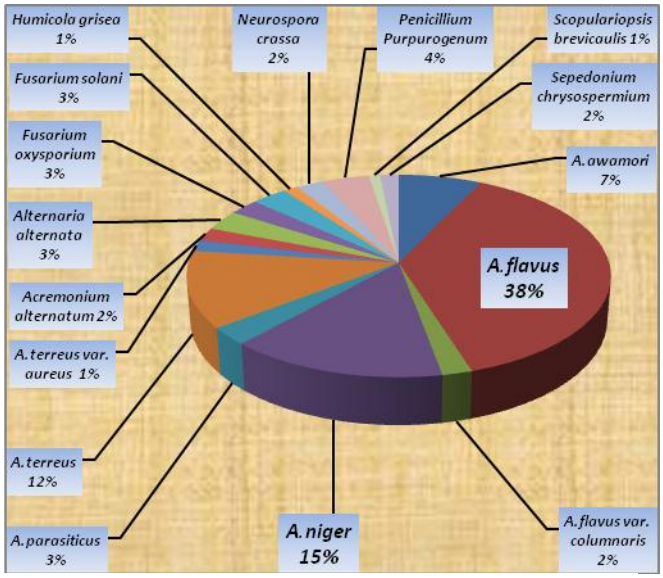

Fig (3b): Population diversity of filamentous fungal species in surface water samples at summer season

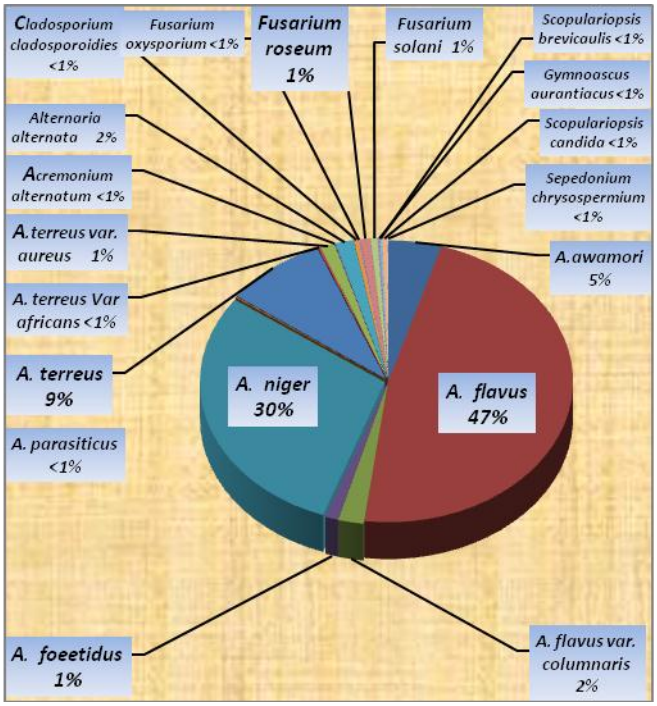

Fig (4a):Population diversity of filamentous fungal species in groundwater samples at winter season.

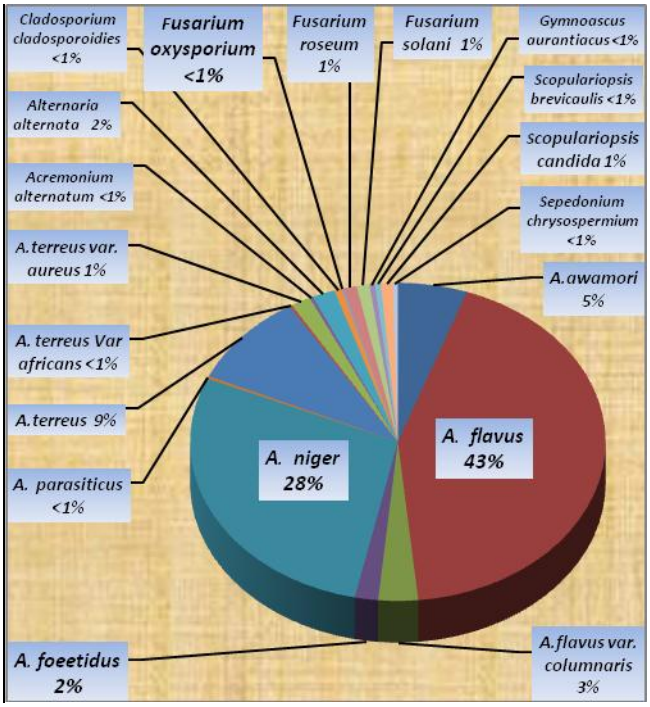

Fig (4b):Population diversity of filamentous fungal species in groundwater samples at summer season.

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