

Study of Drinking Water Contamination by Fungi in the Region of Albayda, Yemen

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ABSTRACT

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KEYWORDS

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Drinking water sources contain different kinds of biological pollutants, such as bacteria, viruses, protozoa, and fungi. Furthermore, some of biological pollutants may cause health problems. Albayda governorate has witnessed many diseases due to using water from open wells. As a result, the purpose of this report is to address the occurrence and frequency of fungi in drinking water in Albayda governorate, Yemen during the winter and summer seasons. 90 samples drinking water from 5 different sites were collected for isolation and identification of fungi. The results indicate that the most genera were dominant (*Aspergillus* spp., *Fusarium* sp., *Penicillium* sp.). In addition, some genera were observed in low frequency as *Exophiala* sp., *pythium*, *Phialophora* sp, *Bipolaris* sp, *Coccidioides* sp. Moreover, the results indicate that the most frequency of waterborne diseases in this governorate were Diarrhea, Illnesses of digestive system, and diseases of urology. Other diseases were also seen in low frequency, and the most notable category was over the category of susceptible age (15-45).

دراسة تلوث مياه الشرب بالفطريات في منطقة البيضاء ، اليمن

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المُستخلص

تحتوي مصادر مياه الشرب على أنواع مختلفة من الملوثات البيولوجية ، مثل البكتيريا ، الفيروسات ، الأوليات ، والفطريات. علاوة على ذلك ، قد تسبب بعض الملوثات البيولوجية مشاكل صحية. شهدت محافظة البيضاء العديد من الأمراض بسبب استخدام المياه من الآبار المفتوحة. ونتيجة لذلك ، فإن الغرض من هذا التقرير هو معالجة حدوث وتواتر الفطريات في مياه الشرب في محافظة البيضاء ، خلال فصلي الشتاء والصيف. تم جمع 90 عينة مياه للشرب من 5 مواقع مختلفة للعزل والتعرف على الفطريات. تشير النتائج إلى أن معظم الأجناس كانت هي السائدة (*Aspergillus* spp. ، *Fusarium* sp ، *Penicillium* sp). بالإضافة إلى ذلك ، لوحظت بعض الأجناس في تردد منخفض مثل *Exophiala* sp. ، *pythium* ، *Phialophora* sp ، *Bipolaris* sp ، *Coccidioides* sp على ذلك ، تشير النتائج إلى أن معظم الأمراض التي تنتقل عن طريق المياه في هذه المحافظة هي الإسهال وأمراض الجهاز الهضمي وأمراض المسالك البولية. كما شوهدت أمراض أخرى في التردد المنخفض ، وكانت الفئة الأبرز أكثر من الفئة العمرية الحساسة (15-45).

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الكلمات الدالة

الفطريات ومياه الشرب ، الرشاشيات ، فيوزاريوم. الجهاز الهضمي ، المسالك البولية ، اليمن.

Introduction

The occurrence of fungi in drinking water has received increased attention in the last decades. Fungi are now generally accepted as drinking water contaminants.

Outbreaks of disease attributable to drinking water are not common in the world, but they do still occur and can lead to serious acute, chronic, or sometimes fatal health consequences, particularly in sensitive and immune-compromised populations (Reynolds, *et al.*, 2008).

Contamination is not evenly distributed but rather affected by the number of pathogens in the source water, the age of the distribution system, the quality of the delivered water, and climatic events that can tax treatment plant operations (Al-gabr, *et al.*, 2014; Nichols, *et al.*, 2010; Salter, *et al.*, 2010). Many previous studies investigated the contamination of fungi from different countries as (Anaissie, *et al.*, 2003; Hageskal, *et al.*, 2007; Pereira, *et al.*, 2010; Al-gabr, *et al.*, 2014). The fungi can grow in water and cause many problems directly by inhalation the fragments of hypha and spore during shower. On other hands indirectly by changing the quality of water, pigments and odors (Paterson, *et al.*, 2006). Many species of fungi were identified from last studies. (Pereira, *et al.*, 2010) found the genera, *Cladosporium*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Pithomyces*, *Alternaria*, *Peacilomyces*, *Acremonium*, *Epicoccum*, and *Curvularia* while (Sammon, *et al.*, 2010) found that the *Aspergillus* was the most common genera. (Hageskal, *et al.*, 2006) noted that the *Penicillium*, *Aspergillus*, and *Trichoderma* were the dominated fungal flora. The drinking water in Albayda region is surface water, the depth of water wells range between (8- 50 m) and most of them are open wells, and probably contamination is very high. Many health problems appeared in different villages by consumption this water as illness of digestive system, kidney diseases, and skin diseases. Furthermore, recently this governorate and other regions in Yemen some waterborne diseases occurred and spread so that many of people died. Cholera and some fever

diseases are the most commonly occurred because of the consumption of contamination water. China's water monitoring system indicates that roughly 70 percent of the river water is unsafe for human consumption though many farmers in rural areas still rely on these sources for drinking water (World Bank, 2006). Water contamination with microorganisms is the main source of waterborne diseases like gastroenteritis, dysentery, diarrhea and viral hepatitis as complained by most of the respondents during questionnaire survey.

Due to contamination of water, infectious disease, such as typhoid fever, cholera, campylobacteriosis, shigellosis, *Escherichia coli* diarrhea, amoebiasis, helminthiasis and others may occur (Collee, *et al.*, 1996). Symptomatic manifestation of some of these diseases is acute diarrhea, one of the causes of morbidity and mortality in the world, especially in children. From public health's part, water supply should be tested regularly to confirm that they meet the hygienic measures of the WHO for safe water (Bukhari, 2004).

This study is the first in the region to investigate the occurrence and frequency of fungi and bacteria in drinking water from 5 cites in Albayda governorate during the summer and winter seasons. Also this study investigates the quality of water, and study the relationship between contamination of drinking water and health problems as kidney and skin diseases.

Materials and Methods

Description of study area and water sampling

Albayda region is located at (14° 28' 0" North, 48° 8' 0" East) south west of Yemen and it is 2250 meter high above sea level (Figure 1). It has a population of (577,963) distributing on 21 directorates. For the study, a total of (5) different sites were selected in such a way adequately representing the area (Theynaam, Alswadih, Mukiras, Affar, and Rada'a). Some samples were collected from local water treatment plants for evolution of biological pollution before and after treatment. 90 water samples were collected from the study area during

the summer and winter seasons. Water samples were collected in one litter clean polyethylene bottles. Before samples were collected the bottles were sterilized by hypochlorite sodium (1%) and washed using the seam water several times before taking the samples.



Figure 1 The map show the location of the study area (Albayda, Yemen)

Isolation of Fungi:

For fungal Isolation of the pathogens from drinking water sampling the direct plate-spread method which requires water volumes of 0.1 ml (**Kanzler, et al., 2007; Pereira, et al., 2010**) was used. It has been carried out according to methods of plant pathogens sterile will and plated on Sabroud Dextrose Agar (SDA) media (**Kanzler, et al., 2007; Sammon, et al., 2010**) .

Petri dishes were incubated for 7 days at room temperature of 25 c.

Identification of Fungi

For identification of fungi, a microscope was used to identify the pathogens. All fungi were re-isolated on PDA media, and phenotypically identified to the species level (**Iesli and Summerell, 2006; Pitt and Hocking, 2009; Watanabe, 2010; Guarro, et al., 2012; Campbell, et al., 2012**). The characteristics of spores, hypha, and colony color were identified as they appear in media. The lacto- Fuchsin staining technique was used under microscopic light as well.

Determination of the quality of drinking water:

For this propose the HANNA instrument was used to analyze the physical characteristics, such as pH, TDS, and EC. Other parameters were determined by Central lab of Yemen standardization metrology and quality control organization in Sana'a using spectrophotometer in Table 1

Table 1. The level of parameter in samples collected from different locations in Albayda governorate

Sample	Ca	Mg	Na	K	Hco ₃	So ₄
1	3.2	1.2	2.5	0.07	1.7	2.7
2	7.8	4.8	3.4	0.08	3.3	7.4
3	6.4	1.2	2.3	0.15	2	3.8
4	3.5	2	1.6	0.04	2.7	1.7
5	14	8	5.3	0.18	0.7	11.5
6	5.7	4.2	3.5	0.15	1.3	9.2
7	3	2.8	1.3	0.1	2.7	3.2
8	4.1	4	2	0.1	7.7	13.2
9	17.6	15	12.6	0.13	2.7	2.8
10	12.8	9.6	8.4	0.2	1.7	3.3
11	5	5	2.8	0.05	4	11.8
12	11.6	10.4	5.4	0.08	3.3	0.9
13	2.6	2	1.6	0.07	2.3	4
14	4	4.3	1	0.03	2	1.7
15	4.1	1.2	1.7	0.07	3.3	1.7

Statistical Analysis

Analysis of variance was conducted for all parameters using the IBM SPSS statistics software program, version 24. Means of the media culture SDA at CFU/0.1 ml were compared using T-tests ($p \leq 0.05$). The correlation between the fungi and the different water parameters was also determined.

Results and Discussion

Quality of Water

The quality of water in Albayda region showed into (table 1) is that of surface water. The average of the

well depth ranges between (8- 50 m) and most of them are without covering so that the air and soil pollutions have effects on the quality of water and on species of fungi. Furthermore, with the rainfall many types of fungus genera penetrate the layers of soil moving into the water of those wells.

On the other hand, the wastewater has its effect on the quality of water because wastewater has infliction to the soil directly and it is used for vegetables irrigation without any treatment.

The results indicate that the levels of water parameter are of high concentration in some samples and very low in the other ones. As a result, the parameter of water has negative side on humans' health and may cause some diseases on humans and animals.

Table 2 The level of parameter(EC, PH, and TDS) in the samples collected from different locations

Parameter	EC	PH	TDS (mg/L)
Samples			
1	0.68	7.5	486
2	1.6	7.4	436
3	1	7.5	421
4	0.7	7.6	607
5	2.7	7.2	557
6	1.33	8	577
7	0.7	7.8	546
8	4.3	7.6	564
9	0.9	7.5	325
10	1.25	7.5	587
11	2.65	7.2	415
12	0.6	7.7	423
13	0.9	7.4	589
14	0.5	7.5	601
15	0.68	7.6	455

Table 1 shows the significance of the concentration of water from different sites. Also results indicate that the concentration of calcium is ranged between 2.6-17.6 mg/L. So some of the samples have more high level comparing to the control samples. The magnesium is ranged between 1.2- 10.4 mg/L.

The parameter of water may have an effect on water quality and allow microorganism and protozoa to increase growth and activation. Furthermore, consumption of this water may affect humans' health and cause many disease which are showed in Table 3. The results in Table 3 show the most popular diseases according to the health office in Albayda governorate. It is noted that the category of susceptible age is (15-45) and show cases of (16571,12036) between female and male respectively. Furthermore, the most diseases showed are Diarrhea, illnesses of digestive system, and diseases of urology in average (32132, 16755, 17017 respectively). Meanwhile, some waterborne pathogens are known to maintain viability inside amoebae or nematodes. The well-documented case of *Legionella* replication within amoebae is only one example of a bacterial pathogen that can be amplified inside the vacuoles of protozoa and then benefit from the protection of a resistant structure that favors its transport and persistence through water systems (Bichai, et al., 2008).

On the other hand, Stomach cancer and liver cancer now represent China's 4th and 6th leading causes of death and, in combination with other digestive tract cancers (e.g. esophageal), account for 11 percent of all fatalities and nearly one million deaths annually (World Health Organization, 2002). Researchers have found connections between water quality and acute water-borne diseases such as typhoid (Cutler and Miller, 2005) and diarrhea (Jalan and Ravalion, 2003), and access to cleaner water may lower infant mortality (Galiani, et al., 2005).

Table 3 The diseases in Albayda governorate caused by the contamination of water according to the statistics of the health office

Diseases	Less one year		1-4		5-14		15-45		45 more		Total	
	M	F	M	F	M	F	M	F	M	F	M	F
Food poisoning bacterial	0	0	5	4	14	36	17	11	8	6	44	57
Diarrhea	2411	1988	5476	4698	4389	3981	2136	2317	2718	2018	17130	15002
Malaria	0	0	21	8	15	17	22	14	36	54	94	93
Schistosomiasis	0	0	5	3	10	8	13	17	11	5	39	33
Illnesses digestive system	522	529	535	524	877	975	3632	5134	2099	1928	7665	9090
Tooth decay	0	0	315	454	348	547	1716	2147	547	794	2926	3942
Illnesses of teeth	1	0	13	23	54	46	119	263	200	398	387	730
Diseases of the liver	1	1	27	19	16	6	25	11	8	4	77	41
Diseases of urology	96	72	228	284	823	1009	4327	6619	2056	1503	7530	9487
Renal failure of acute and chronic	0	0	0	0	2	0	29	38	136	98	167	136
Total	3031	2590	6625	6017	6548	6625	12036	16571	7819	6808	36059	38611

M= male, F= female

Source of the table is statistics of the health office 2013

Identification of Fungi

After a two-week-incubation of the SDA media, the fungi was observed most dominant in water samples collected from the different sites in Albayda, as well as, the samples collected from the private water treatment plant as showed in (Table 4). Some of those genera are well-known causes of many diseases. Recently, cholera has spread very fast in Yemen including Albydaa and many people died. The results indicate that the genes of *Aspergillus* has the most occurrence and frequency more than others in different samples. The second fungi to appear was *Fusarium* spp. in samples then the genes of *Penicillium* spp. The other genera were occurring in samples but in low frequency. In general, the drinking water samples examined are found to be of high contaminations and are used

directly without any treatment to be more safe for utilization by humans or animals. This study come in line with the results of (Arvanitidou, et al., 2000; Hageskal, et al., 2006; Grabinska-Loniewska, et al., 2007) which found that fungal flora was dominated by species representing the genera *Penicillium* , *Trichoderma* , and *Aspergillus*. Also this study agrees with (Goncalves, et al., 2006) who noted that the dominant genera were *Phialophora* , *Exophiala* , *Acremonium* , *Penicillium*. The results also show episode the genera of *Cladosporium* sp. and *Penicillium* sp found in a recent study by (Cabral and pinto, 2000) when they studied samples of water collected in bottled mineral water. While (Kanzler, et al., 2007) found that the most frequently were *Cladosporium* sp. and *Penicillium* sp. These authors commented that

drinking water serves as a reservoir for fungi, and opportunist species thereof can cause infections in immune-suppressed individuals. additionally, this study agrees with the (Abdel-Hameed, et al., 2008) who reported fungi representing *Aspergillus flavus*, *A. parasiticus*, *Penicillium*, *Fusarium*, and *Trichoderma* appeared in samples collected from water environments. and (Anaissie, et al., 2003) noted that species of *Aspergillus* and *Penicillium* were the fungi most often found.

Table 4 The fungi isolated from the drinking water samples in Albayda governorate

Genera	Species
Aspergillus	A.flavus
	A.niger
	A. candidus
	A. fumigatus
	A. glaucus
Exophiala sp.	
Epidermophyton sp.	
Candida sp.	
Mucor sp.	
Fusarium	F. proliferatum
	F. spp.
Coccidioides sp.	
Penicillium spp.	
Cladosporium sp.	
Rhodotorula sp.	
Bipolaris sp.	
Pythium sp.	
Phialophora sp.	

The frequency of fungi from different sites showed below in (Fig. 2). The results indicated the frequency of fungi is of high level in all drinking water samples. Also show more frequency in samples collected from Alshroog and Alwady samples with an average of (150 cell/ 0.1 ml), and a lower frequency in samples collected from Theynaam1, Theynaam3, Almurbash, Mukirass, and Alshiekh (11, 21, 22, 29, and 28 cell/ 0.1ml respectively).

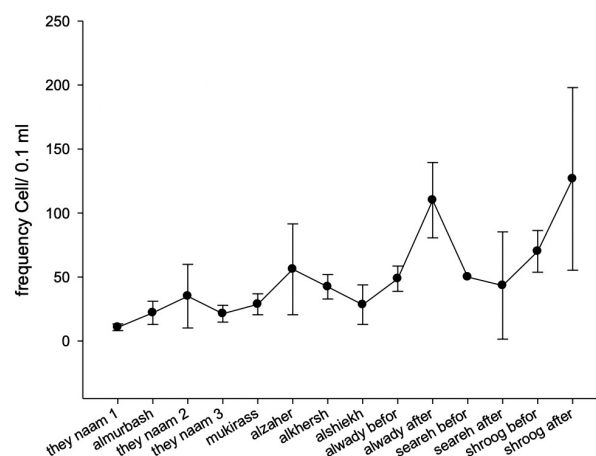


Figure 2 The frequency of fungi per 0.1ml from different sites in Albayda governorate

Statistical Analysis

The correlation analysis between the water parameters and fungi (using SPSS) show that frequency of fungi has a correlation with TDS at the level of $p \leq 0.05$, and at a level of $p \leq 0.01$ with all other parameters.

Conclusion

This study, which is the first report from Albayda governorate in Yemen, concluded that the drinking water samples possess a high frequency of fungi and showed many strains of fungi that are well known for the production of metabolites like mycotoxins. as well as some strains that may directly cause health problems.

In fact, the high frequency of fungi that was found in samples collected from the local water treatment plant as well as the known production of aflatoxins (causing health hazards for humans) by *Aspergillus* spp and *Fusarium* spp make our study significant. In addition, fungal contaminated water that passes through showerheads, taps, or toilet cisterns, mold spores and hyphal fragments may be aerosolized into indoor air. Such aerosols could be inhaled by human inhabitants and cause harmful effects. Moreover, It founded that many health problems such as Diarrhea, illnesses of digestive system, and diseases of urology were reported as caused by fungi in this region, because, like other governorates in Yemen, water is used directly without any treatment. In addition, the water which is used in this governorate is taken from the wells which are open like a surface water, and might be subjected to contamination by different microorganisms. Therefore, further research work is needed in order to characterize the contamination process of drinking water and to find out best disinfection processes to remove contaminants.

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