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Original Research Article

Relative Diversity of Filamentous Fungi and Yeasts in Groundwater and their Correlation to Fecal Pollution Indicators and Physicochemical Parameters

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A B S T R A C T

Groundwater could be chemically, physically, or microbiologically contaminated, each of which is linked to various sources and health related problems and consequences. This study is performed to evaluate (bacteriologically, mycologically and physicochemically) some private groundwater wells in Giza governorate, Egypt and to determine its suitability for drinking and human consumption according to the Egyptian standard methods for drinking water (2007). The objective of this study was to determine the occurrence and relative diversities of filamentous fungi and yeasts in groundwater samples as well as to investigate their correlation with the bacterial indicators of fecal pollution. The prevalence of fungi was investigated in parallel with fecal bacterial indicators in 60 groundwater samples from 20 wells in Giza governorate during summer season. Filamentous fungi and yeast were isolated from all examined samples. Results showed presence of some filamentous fungal species such as *Aspergilllus* spp., *Penicillium* spp., *Fusarium* spp. and *Mucor* spp., and showed presence of some yeasts such as *Saccharomyces cerevisiae* and *Candida* spp. in addition, bacterial indicators of pollution were detected with higher counts than limited standard counts. Most groundwater samples showed high concentrations of measured physicochemical parameters. Thus, groundwater could be considered as a possible transmission route for filamentous fungi and yeasts, and could constitute a potential health hazard, mainly to immunocompromised indivuals.

Keywords

Groundwater, filamentous fungi, fecal pollution, physicochemical parameters

Introduction

To protect consumers from waterborne diseases, drinking water utilities should ensure that the distributed water is completely free of pathogenic Micro-organisms. However, many drinking water utilities rely solely on monitoring indicator bacteria, such as total coliforms and *Escherichia coli* (*E. coli*), to ensure the microbiological quality of drinking water. Coliform bacteria are naturally present in the environment and may not cause disease but their presence is often used to indicate whether potentially harmful bacteria may be present in the water whereas fecal coliforms and *E. coli* originate exclusively from human and animal fecal waste (Rompré et al., 2002). Fungi are widely distributed in nature and can occur as unicellular yeast or filamentous and, multicellular molds. There are over 70,000 species of fungi. Fewer than 300 have been implicated in human diseases, and fewer than a dozen cause about 90% of all fungus infections. They are involved in different forms of diseases, including allergies to fungal antigens, production of toxins, or direct invasion of hosts. Several species of fungi are capable of infecting healthy hosts and causing diseases ranging from mucosal to life-threatening disseminated infections. In addition, there are increasingly numbers of severe fungal diseases by commensal or fully saprophytic species in immunocompromised hosts. These diseases are frequently associated with abrogated host immunity as a result of viral infections, mainly the human immunodeficiency virus, hematological and hormonal disorders, organ transplants, antibiotic usage, and more intensive and agressive medicals practices (Pereira et al., 2009). Fungal infections are difficult to treat since the agents are eukaryotes, as human cells. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments. Drinking water distribution systems are colonized by saprophytic heterotrophic microorganisms (such as bacteria, fungi, yeast) that grow on biodegradable organic matter. However, potentially pathogenic microorganism and microorganisms of fecal origin can also find favorable conditions and proliferate in these systems (Petrucio et al., 2005). It was witnessed an increased awareness about the potential for fungi to degrade complex natural and anthropogenic substances due to broad enzymatic capabilities (Yamaguchi et al., 2007), as well as reports of their possible pathogenicity towards susceptible humans, animals and plants. An increased occurrence of opportunistic systematic mycosis in immunocompromised patients has been reported. In addition, when present in certain matrixes, fungi may produce mycotoxins that can display overlapping toxicities to invertebrates, plants, and microorganisms (Bennett and Klich, 2003). In the present study, total and fecal coliforms, and total heterotrophic bacterial counts were estimated in parallel with counting of filamentous fungi and yeasts in groundwater, in addition to some physicochemical parameters.

Materials and Methods

Samples and Sampling

Twenty groundwater well samples were collected (Table 1) in clean and sterile polypropylene plastic bottles, which were previously soaked in 10% nitric acid solution and thoroughly rinsed several times with distilled water and finally with a portion of the water sample. These bottles were covered by aluminum foil, and sterilized in autoclave at 121ºC for 20 minutes. In all cases, well water pumps were opened for some little time before taking the samples. All samples were tightly sealed and immediately taken to the laboratory for analysis. The time between sampling and analysis was not more than 6 hours according to (APHA, 2005). Three runs of samples were collected (n=60).

Physicochemical analysis

Table (2) represented the measured

physicochemical parameters and analysis methods used of collected groundwater samples. All chemicals used in the present study were purchased from BDH, Sigma, Aldrich and Merck.

Microbiological analysis

Bacterial fecal pollution indicators

Total coliforms, fecal coliforms and fecal streptococci, in addition to heterotrophic bacterial count, were detected according to (APHA, 2005). Membrane filtration (MF) technique was used to determine bacterial indicators in 100 ml sample.

Heterotrophic bacterial count (HBC)

Poured plate method was carried out for detection of HBC according to (APHA, 2005). Plate count agar (PCA) (Difco, USA) medium was used. A 23.5g of PCA was added to 1L distilled water and bout 10 ml of TTC (2,3,5-Triphenyl Tetrazolium Chloride) solution was added to medium after sterilization for the purpose of pigmentation of bacterial colonies with red color. The medium was heated to boiling with agitation and pH was adjusted at 7.2 before autoclaving for 15 min at 121°C, cooled to 45°C and poured into sterile Petri-plates. Inoculated plates were counted after incubation for 24 and 48 hours at 37 ºC and at 22 ºC, respectively. The numbers of organisms developed into colonies under these conditions were recorded as colony forming unit (cfu) per ml.

Total coliforms (TC)

The membrane filter (MF) technique was used to determine bacterial indicators in 100 ml sample. In this technique, a measured amount of water (usually 100 ml for drinking water) is passed through a membrane filter (pore size 0.45 μm) that traps bacteria on its surface. This membrane is then placed on a thin absorbent pad that has been saturated with a specific medium designed to permit growth and differentiation of the organisms being sought. m-Endo agar (Difco, USA) was used for enumeration of total coliform in water . Readymade m-Endo agar medium was prepared by adding 35 g to 1L distilled water. The medium was heated to boiling with agitation and pH was adjusted at 7.2, cooled to 45°C and poured into sterile Petri-plates, then filtrate 100 ml of water sample, the filter is placed on the surface of the media and incubated at 35ºC for 24 hours. Colonies having a red color with green metallic sheen were enumerated as total coliforms (cfu /100 ml).

Fecal coliforms (FC)

m-FC agar (Difco, USA) was used to enumerate fecal coliforms by using the membrane filter (MF) technique without prior enrichment. Readymade m–FC agar medium was prepared by adding 52 g to 1L distilled water containing 10 ml 1% rosolic acid in 0.2N NaOH. The medium was heated to boiling with agitation and pH was adjusted at 7.2, cooled to 45°C and poured into sterile Petri-plates and then heat to near boiling, then filtrate 100 ml of water sample, the filter is placed on the surface of the media and placed in a good tight closed plastic bags. Inoculated plates were incubated at 44.5ºC for 24 hours in water bath. Colonies having a blue color were enumerated as fecal coliforms (cfu /100 ml).

Fecal streptococci (FS)

m-Enterococcus agar (Difco, USA) was used to enumerate fecal streptococci by using the membrane filter (MF) technique. The medium was heated to boiling with agitation and pH was adjusted at 7.2 before autoclaving at 121°C for 15 h, cooled to 45°C and poured into sterile Petri-plates, then filtrate100 ml of water sample, the filter is placed on the surface of the media. Inoculated plates were incubated at 35°C for 48 h. Colonies with dark red to brown color were enumerated as fecal streptococci (cfu /100 ml).

Filamentous fungi (FF) and total yeasts

Potato dextrose (PD) agar (Difco, USA) and Rose Bengal (RB) agar Base (Himedia, India) were used for detection and enumeration of filamentous fungi and total yeasts, respectively. Media were prepared and autoclaved at 121ºC for 20 min. The media distributed into Petri dishes and let to solidify then 1ml of collected groundwater sample was distributed on the surface of agar plates and incubated at 25ºC for 5-7 days for PD and 3 days for RB. Filamentous fungi were identified on the basis of macroscopic and microscopic features. Served characteristics were recorded, (a) morphological characteristics such as septation of hypha; (b) formation and morphology of fruiting-bodies and conidiophores; (c) branching patterns and frequency of branching; and (d) size, form, color and ornamentation of the conidia (Zycha, 1963; Ainsworth, 1971; Booth, 1977; Klich and Pitt, 1992; Pitt, 2000). Yeast isolates (*Saccharomyces cerevisiae* and *Candida* spp.) were identified using Biolog® GEN III Microplates (Biolog, USA).

Statistical analysis

Correlations between both filamentous fungi and total yeasts counts, and both studied bacterial indicators of pollution (total coliforms, fecal coliforms and fecal streptococci) and measured physicochemical parameters of the collected groundwater samples. Paired samples test was used for calculation of correlation results using IBM® SPSS® (statistical package for the social sciences) program for windows 7.

Results and Discussion

The microbiological quality of ground water is an issue of major public and scientific awareness and when it is polluted, environmental as well as health issues arise. National and international legislation for microbiological pollution monitoring in ground water is confined to pathogenic indicator bacteria, i.e., total coliforms, fecal coliforms and fecal streptococci. However, data concerning the relationship between fecal pollution indicator bacteria and potential pathogens in waters are limited (Ashbolt, 2004). In Egypt, the groundwater is considered the third water source for irrigation and other human uses after the Nile River, and irrigation canals and drains. Thus, the ground water is considered as a secondary source to irrigate some agricultural areas in the Delta region, and as an essential source for some cultivated lands to which the Nile water is not reachable. In many parts of Egypt, the ground water is widely used for drinking and other domestic purposes (Mamdouh et al., 2003). Groundwater represents an important source of drinking water and its quality is currently threatened by a combination of microbiological and physicochemical contamination (Reid et al., 2003). However, groundwater could be chemically, physically, or microbiologically contaminated. Each of which is linked to various sources and health related problems and consequences. Two main factors determine the chemical and microbiological composition of water quality: artificial and natural contamination. Any microbiological or chemical analysis of water reveals the joint effects of both sources of contamination, and it is usually impossible to fully identify and separate these sources (Al-Khatib et al., 2003).

Physicochemical analysis

For the present study, the collected groundwater samples from twenty wells (*n*=60) at Giza governorate were analyzed for physical and chemical characteristics. The physicochemical parameters were given in Table (3). Obtained results showed that; ammonia content of samples ranged from 0.3 to 1.86 mg/l. The minimum value was found to be 0.3 mg/l at sample no. 8, whereas the maximum value 1.86 mg/l was observed at samples no. 2, 17 and 19. Results of ammonia in the examined groundwater samples were higher than the permissible limits, except sample no. 8 (>0.5 mg/l). The maximum value 9.82mg/l of nitrate was recorded at sample no.10, whereas the minimum 0.17 mg/l was noted at sample no.8. The nitrate contents of the samples were within the permissible limits (<45 mg/l). The sulphate values were found to be less in all groundwater samples. The minimum value 25.7 mg/l was observed at sample no. 7, whereas the maximum values 38.6 mg/l and 38.35 mg/l were observed at samples no. 13 and 20, respectively. All sulphate concentrations in the groundwater samples were within the permissible limits (<250 mg/l). The maximum value 1.02 mg/l of iron was recorded at sample no.18, whereas the minimum 0.219 mg/l was noted at sample no.17. Iron concentrations were higher than the permissible limits (>0.3 mg/l) except samples no. 15 and 18. Total dissolved solids (TDS) levels ranged from 341.88 to 560 mg/l. The minimum level was observed at sample no. 14, while the maximum level was observed at sample no. 19. All TDS results were within the permissible limits (<1000 mg/l).

The minimum value 56.0 mg/l of chloride was observed at samples no. 11, whereas the maximum value 138.0 mg/l was noted at sample no.18. The values were found to be higher in sample no 18 than samples no 20 and 9, but still within the permissible limits at both regions (<250 mg/l). Total hardness ($CaCO_3$) values ranged from 80 to 482 mg/l. The maximum value was observed at sample no. 9, while the minimum value was observed at sample no. 8. Total hardness concentrations in all groundwater samples were within the permissible limits (<500 mg/l). Biological Oxygen Demand (BOD) values at all groundwater samples ranged from 0.0 to 1.0. Chemical Oxygen Demand (COD) values for samples 8, 9, 18 and 20 were 0. The maximum value (22) was observed at sample no. 15, while the minimum value (8) was observed at sample no. 1. In addition, Hydrogen ion concentration (pH) values in the present study showed neutrality (around 7.0) in all groundwater samples, which complies with the permissible limits (6.5-8.5). Ammonia range was considered to be in high concentration due to the anaerobic conditions that prevailed in the landfill which in return contributed to nitrate reduction towards ammonia gas phase. The high nitrates were the indicative of high pollution load. Excessive levels of nitrate in drinking water may cause serious illness and sometimes death. Nitrates have the potential to cause shortness of breath, "blue babies" syndrome in infant diuresis, an increase in starchy deposits and haemorrhaging at the spleen. The concentration of nitrogenous compounds indicates the occurrence of extensive anaerobic bacterial activities. It was reported that groundwater was contaminated from nitrate fertilizers and manures used in agriculture. Furthermore, nitrate is used by microorganisms as food resources. In addition, high nitrate levels are often accompanied by bacterial and pesticide contamination (Aydin, 2007). Sulphate is the common ion present in water. It can produce bitter taste at high concentrations. Sulphate originates from sedimentary rocks and igneous rocks. In the present study, the sulphate contents were quite below the permissible limits. Our study is coincided with the studies of Thirumathal and Sivakumar, (2003). Water containing iron does not show deleterious effect on human health, its presence in drinking water is not desirable for various reasons. Excessive iron content makes the water turbid, discolored and imparts an astringent taste to water Iron is biologically an important element. It is essential to all organisms and present in hemoglobin system. A stringent taste is detectable by some persons at levels above 1 mg/l (Remia and Logaswamy, 2010). In our study, the iron contents were slightly higher than the permissible limits. The high concentration may be due to dumping of wastes around the bore wells. TDS represents the amount of inorganic substances (salts and minerals). High TDS is commonly objectionable or offensive to taste. A higher concentration of TDS usually serves as no health threat to humans until the values exceed 10,000 mg/l (Aydin, 2007). Chloride concentration in water indicates presence of organic waste particularly of animal origin**.** Increase in chloride concentration on discharge of municipal and industrial waste has been reported. Chloride in water may react with sodium to form sodium chloride. Since sodium chloride has the salty taste, it can be deduced that chloride in water impacts a salty taste in the water (Priyanka et al., 2010). In the present study, the chloride values were lower than the permissible limits in all groundwater samples. Hardness is an important parameter in decreasing the toxic effect of poisonous element. Total hardness ($CaCO_3$) was found to be high above the permissible limit. Hardness has no adverse effect on human health and water above hardness of 200 mg/l may cause scale deposition in the water distribution system and more soap consumption. Soft water below hardness less than 100mg/l is more corrosive for water pipes (Remia and Logaswamy, 2010). Chemical oxygen demand (COD), a non-conventional pollutant, is sometimes used to characterize the global concentration of organic pollutants. COD can be used to provide data on the existence of organic substances that can only be oxidized by aerobic biological processes. Although this parameter was not considered directly as a risk tracer. According to obtained results, Evens et al. (2004), recorded that high concentration of COD was measured (59–112 mg/l) in the groundwater (well water) in France. Biological oxygen demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to decline (Evens et al., 2004).

The pH has no direct adverse effect on health, but at the same time alters the taste of water. Higher pH reduces the germinal potentiality of chlorine and induces the formation of toxic trihalomethanes (Remia and Logaswamy, 2010). In the present study, the pH values showed almost neutral condition in all samples. Temperature of drinking water is often not a major concern to consumers especially in terms of drinking water quality. The quality of water with respect to temperature is usually left to the individual taste and preference and there are no set guidelines for drinking water temperature .In the present study, temperature varied from 22 to 34 °C. The variation in the groundwater temperature may be due to different timing of collection and influence of seasons (Jayraman et al., 2003). The electrical conductivity (EC) of aqueous solution is ability to carry an electrical current. The current is conducted in solution by the movement of ions. \

The ions in solution are formed by dissociation of inorganic compounds. For this reason, the measurement of conductivity gives a good indicator of the concentration of dissolved salts in water. In the present study EC values were in the permissible limits (Priyanka et al., 2010). It is estimated that high turbidity may constitute health risk through protection of microorganisms from treatment and stimulation of microbial growth. Turbidity is the reflection of the total suspended matter to which it is inversely related on one hand and is an indication of clay and inert particles (Nkansah et al., 2011).

Bacterial pollution indicators

Results in Table (4) show the average counts of total bacterial counts at 37°C and 22°C, bacterial indicators (total coliforms, fecal coliforms and fecal streptococci), total fungi and total yeasts in groundwater samples collected from Giza governorate. Most of groundwater wells showed higher total bacterial counts (TBC) at both 37°C and 22°C than 50 cfu/ml. Only two groundwater wells showed no total coliforms (TC) counts, however, counts of total coliforms in other groundwater wells ranged from 4 to 46 cfu/100 ml. Also, fecal coliforms (FC) average counts behaved such as those of total coliforms counts, since only two groundwater wells were free of fecal coliforms, while other wells showed fecal coliforms counts ranged from 1 to 25 cfu/100 ml. In addition, fecal streptococci (FS) average counts were less than total and fecal coliforms in all groundwater wells, since three wells showed absence of fecal streptococci, while other wells showed average counts ranged from 1 to 12 cfu/100 ml. Filamentous fungi (FF) were detected in all studied groundwater wells. Egyptian standards for drinking water have no limit for total fungal count. Average counts of total fungi in studied groundwater samples ranged from 4 to 119 cfu/100 ml.

Total yeast were observed and detected in all groundwater samples under study except wells no. 6 and 19. Average counts of total yeasts ranged from 3 to 211 cfu/100 ml. The main source of microbiological contamination is microorganisms from human or animal excreta, which reaches humans through contaminated groundwater from wastewater, landfills, or wastewater treatment stations, causing serious health problems. For example, according to the UN, diarrhea accounts for 80% of all diseases and over one third of deaths in developing countries, which are caused by the patients' consumption of contaminated water (Al-Khatib et al., 2003). Most of the gastrointestinal infections that may be transmitted through drinking water are transmitted via fecal–oral pathway. Hence, the effects of improvements in the quality of groundwater were felt on the combat against endemic diseases such as typhoid and cholera in adults, and diarrhea in children (Al-Khatib and Orabi, 2004).

The most commonly used indicators for bacteriological contamination are the coliforms: total and fecal coliforms and fecal streptococci. *E. coli* is a subgroup of fecal coliform group, and its presence indicates the fecal pollution of groundwater. Detection of bacterial indicators in drinking water signifies the presence of pathogenic organisms that are the source of water-borne diseases (Al-Khatib and Hassan, 2009). In rural areas, drinking water generally supplied groundwater through individual or community wells. The total viable bacterial count is used to estimate the total amount of bacteria in water and indicates the overall microbial status of the water (Aksu and Vural, 2004). Disease-causing microbes (pathogens) in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems. Fungi are natural inhabitants of soil and water and some species may behave as pathogens or opportunistic pathogens in man.

Although endogenous infection following colonization is the prevailing mode of fungal transmission, acquisition after contact with water may occur and result in outbreaks. With the increasing number of patients undergoing complex medical therapy, more unusual infectious disease processes have emerged. Fungal pathogens as agents of variety of infections are part of this trend and are found much more frequently as the cause of infections in immunocompromised patients (DEFRA, 2011).

Mycological survey

Table (5) and Figures (1&2) showed total counts, detected species and relative diversity of filamentous fungi (FF) and total yeasts of groundwater samples. Fourteen fungal species (eleven filamentous fungi and three total yeasts) were detected including; *Achlya debaryana*, *Asperigillus flavus*, *Asperigillus fumigatus*, *Asperigillus niger*, *Asperigillus terreus*, *Fusarium oxysporum*, *Mucor sativus*, *Paecilomyces varioti*, *Penicillium egyptiaccum*, *Penicillium notatum*, *Rhizopus nigricans* as filamentous fungi and *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata* as total yeasts. *Asperigillus* spp. showed the highest filamentous fungi relative diversity (29.8 %) followed by *Penicillium* spp. with relative diversity 8.9 % (Figure 1). *Saccharomyces cerevisiae* showed the highest relative diversity (54 %) in groundwater samples. Two species of Candida were detected including *C. albicans* and *C. glabrata* (Figure 2).

The health risks associated with the polluted water may cause health risk. Therefore, the detection and enumeration of indicator microorganisms in water is of great importance in assessing the presence of bacteria, viruses, fungi and parasites, although no consistent correlation has been found between numbers of indicators and pathogens (Yamaguchi et al., 2007). Reports determining qualitatively and quantitatively filamentous fungi and yeasts in groundwater and their relation with the standard pollution indicator bacteria are lacking. *C. albicans* yeasts and has been reported in fresh waters from several regions, although no consistent correlation has been found between numbers of indicators and pathogens (Arvanitidou et al., 2002).

Halaby et al., (2001) have shown that increased densities of *C. albicans* are usually associated with high densities of indicator bacteria. Only total and fecal coliforms were correlated with the counts of yeasts, but such an association was lacking for filamentous fungi as a total as well as for each mold separately. In healthy adults, *Candida* spp. are usually commensal organisms coexisting in harmony with its host organism but, in immunocompromised individuals, they may cause candidiasis. *Aspergillus fumigatus,* commonly isolated in our study, is the causal agent of pulmonary aspergillosis (Cabral and Pinto, 2002).

In recent years, more individuals with underlying chronic diseases and immunosuppression are exposed to potential pathogens such as filamentous fungi and yeasts being in an excess of risk of gastroenteritis, respiratory diseases, ear, eye and skin ailments. Therefore, almost all filamentous fungi and yeasts might be considered potentially pathogens and their quantification is practically identical to the determination of their total counts (Arvanitidou et al., 2002). That conducted with obtained results of the present study since, most frequent isolated fungi were *Penicillium* spp., *Aspergillus* spp., *Candida* spp. and *Saccharomyces cerevisiae*. Significantly higher counts of total and fecal coliforms and enterococci were found. Contamination with yeasts and filamentous fungi and their presence cannot always be predicted by the pollution indicator microorganisms, the extent to which the presence and density of fungi may be in fact valid indicators of pollution.

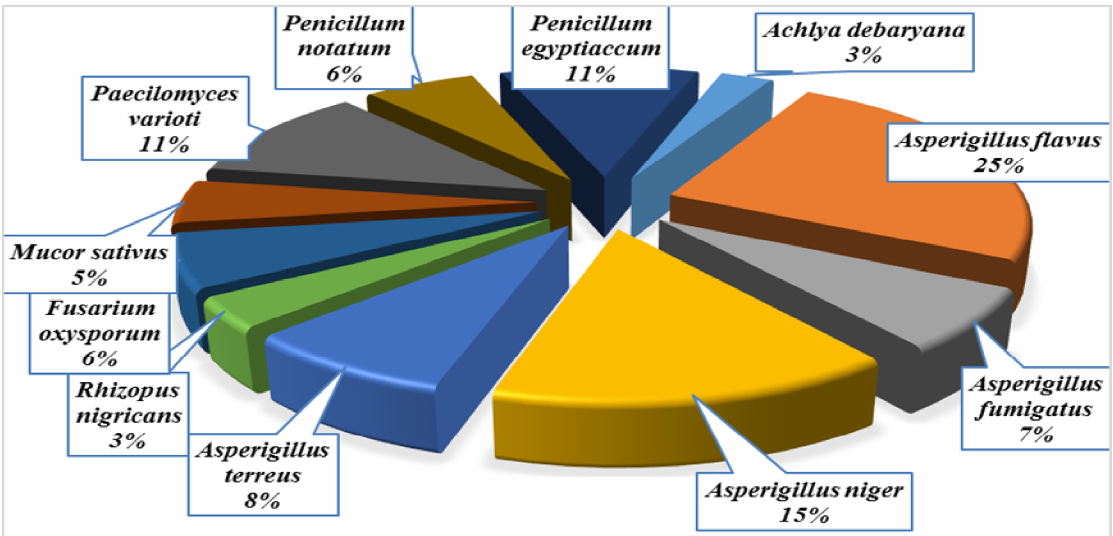

Figure.1 Relative diversity of filamentous fungal species in groundwater samples

Table.1 Sampling sites of groundwater wells in Giza governorate

Sample sites	Well names	Sample sites	Well names
1	Mazghouna	11	Meat Al Kaed
2	Abu Rakhowan	12	Nekla
3	AL Badrashin	13	Alreka
4	Abuel Nomers	14	Gerza
5	Tami	15	Kafr Ammar
6	Ezbet sherif	16	Al Maktafia
7	Kafr Hamido	17	Kafr Hegazy
8	Berush	18	Al Rahwey
9	EL Sheikh Zayed	19	Al Kata
10	Al Manaia	20	Galatma

Table.2 Physicochemical parameters and used analytical methods

Parameter	**Unit**	**Measurement method**
Electric conductivity (EC)	μs/cm	Conductivity method **(APHA, 2005)** using EC meter, Jenway, model 470.
pH	--	pH meter (WTW, Model pH, 315i).
Temperature	°C	Mercury thermometer, GH Zeal LTD, London, England.
Turbidity	NTU	Turbidimeter [10b] a portable Hannaturbidimeter (model: HI 93703)
Iron	mg/l	The phenanthroline method **(APHA, 2005)**.
Ammonia & Nitrate	mg/l	A colorimeter (Jenway 6510, England) at 410 nm
Chlorides	mg/l	Silver nitrate titrimetric method **(APHA, 2005)**.
Sulphate	mg/l	Turbidimetric method **(APHA, 2005)** using UV/Vis spectrophotometer, Unicam model UV4-200 (UK): at wave length 420 nm.
Total hardness ($CaCO_3$)	mg/l	EDTA Titrimetric Method **(APHA, 2005)**.
TDS	mg/l	APHA, 2005. Evaporation test method
COD	mg/l	Titrimetric method **(APHA, 2005)** using Spectrophotometer (Dr /20000) for use at 600 nm.
BOD	mgO_2/l	Winkler's iodometric method **(APHA, 2005)**.

Statistical analysis

Table (6) shows the correlation results between filamentous fungi and total yeasts, and bacterial indicators of pollution (total coliforms, fecal coliforms and fecal streptococci) of studied groundwater samples. The obtained results showed that, filamentous fungi showed strong proportional correlation (0.727 and 0.885) with total coliforms and fecal coliforms average counts, respectively, while filamentous fungi showed weak proportional correlation (0.181) with fecal streptococci. In addition, total yeasts showed weak proportional correlation (0.065) with total coliforms average counts, and weak reverse correlation (-0.154 and -0.128) with fecal coliforms and fecal streptococci, respectively. Table (7) shows the correlation results between filamentous fungi and total yeasts counts, and some measured physicochemical parameters of the groundwater water samples. From the obtained statistical analysis, it can be observed that, filamentous fungi showed reverse correlation with all measured physicochemical parameters except with temperature and ammonia, the correlation was proportional. In addition, total yeasts showed reverse correlation with some measured physicochemical parameters (TDS, chloride, temperature, EC, nitrate, total hardness and iron) and showed proportional correlation with (BOD, COD, ammonia, sulphate, turbidity and pH).

Table.3 Physicochemical parameters of groundwater samples at Giza governorate

Parameter Sample no.	Ammonia (mg/l)	Nitrate (mg/l)	Sulphate (mg/l)	Iron (mg/l)	TDS (mg/l)	Chlorides (mg/l)	T. ardness (mg/l)	BOD	COD	pH	Temp. (°C)	EC (µs/cm)	Turbidity (NTU)
1	1.4	5.75	30.5	0.554	435.6	108	300	0.0	8.0	7.36	28	660	0.0
2	1.86	8.27	35.6	0.669	374.4	84	280	0.0	12.0	7.3	22	567	4.64
3	1.42	3.42	31.1	0.67	355.7	76	250	0.0	12.0	7.25	27	539	2.0
4	1.49	3.34	36.45	0.98	414.4	84	300	1.0	21.4	7.78	25	628	7.76
5	1.4	7.51	34.17	0.67	431.6	92	310	1.0	13.0	7.36	28	654	4.0
6	1.0	3.45	35.44	0.956	353.1	64	230	0.0	16.4	7.68	27	535	0.84
7	1.5	3.54	25.7	1.02	352.4	64	230	0.0	17.3	7.7	28	534	1.0
8	0.3	0.17	31.6	0.847	425	100	80	0.0	0.0	7.73	30	1022	0.0
9	0.8	2.84	34.86	0.658	449	125	482	0.0	0.0	7.62	32	628	5.9
10	0.69	9.82	26.1	0.556	448.8	92	314	1.0	16.3	7.76	28	680	4.55
11	0.89	2.47	32.9	0.566	413.8	56	292	1.0	19.3	7.73	29	627	8.1
12	1.5	9.77	30.06	0.569	369.6	80	240	0.0	12.4	7.36	23	560	0.0
13	1.74	1.59	38.6	0.904	413.8	84	272	1.0	19.8	7.79	28	627	8.81
14	1.35	3.32	34.33	0.713	341.8	56	204	0.0	14.9	7.79	22	518	8.0
15	1.36	3.45	35.12	0.254	534	121.7	321	0.0	22.0	7.7	28	516	19.9
16	1.0	0.97	27.8	0.451	556	121.7	347.2	0.0	16.3	7.43	32	1027	0.25
17	1.86	1.5	34.87	0.543	412.9	79	320	0.0	11.0	7.34	27	760	4.35
18	1.0	1.07	33.14	0.219	505	138	105	0.0	0.0	7.26	34	449	6.2
19	1.86	5.78	37.78	0.675	560	109	340	0.0	11.0	7.56	28	780	6.98
20	0.98	5.37	38.35	0.557	389	125	270	0.0	0.0	7.68	28	690	5.76
Egyptian standards	0.5	45	250	0.3	1000	250	500	-	-	6.5-8.5	-	-	0-1

Table.4 Average counts of total bacterial counts, bacterial indicators, total fungi and yeast in groundwater samples

Samples	TBC (cfu/ml)		TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	FF (cfu/100ml)	T. Yeasts (cfu/100ml)
	At 37°C	At 37°C					
1	52	61	6	3	6	22	31
2	70	79	9	1	5	9	60
3	148	167	46	25	12	97	15
4	90	112	12	7	4	56	20
5	118	125	32	16	8	78	19
6	94	108	17	8	6	64	0
7	115	119	21	10	1	89	84
8	50	57	0	0	0	7	3
9	16	25	0	0	0	4	12
10	63	72	7	2	6	8	19
11	152	170	29	12	4	96	180
12	58	72	14	6	1	77	37
13	60	69	4	1	2	9	130
14	64	80	18	4	3	29	211
15	84	120	15	5	4	20	75
16	103	135	18	9	1	47	5
17	126	148	37	13	6	89	92
18	60	81	10	23	0	119	56
19	75	94	20	19	5	77	0
20	70	85	40	21	3	97	16
Egyptian standards	≤ 50	≤ 50	≤ 2	≤ 1	≤ 1	-	-

Table.5 Total fungal count and relative density isolated from ground water samples

Fungal spp	Total count cfu/100 ml	R.D.* (%)
Filamentous fungi		
Achlya debaryana	30	1.5
Asperigillus flavus	271	13.4
Asperigillus fumigatus	78	3.8
Asperigillus niger	166	8.2
Asperigillus terreus	89	4.4
Rhizopus nigricans	38	1.9
Fusarium oxysporum	68	3.4
Mucor sativus	54	2.7
Paecilomyces varioti	119	5.9
Penicillium notatum	65	3.2
Penicillium egyptiaccum	116	5.7
Total yeasts		
Candida albicans	230	11.4
Candida glabrata	200	9.8
Saccharomyces cerevisiae	500	24.7
Total fungal count	**2024**	**100**

*R.D.: Relative Diversity.

Table.6 Correlation between filamentous fungi and yeasts, and bacterial indicators

	n	**Correlation**		
		Total coliforms	**Fecal coliforms**	**Fecal streptococci**
Filamentous fungi	20	0.727	0.885	0.181
Total yeasts	20	0.075	-0.154	-0.128

Table.7 Correlation between filamentous fungi and yeasts, and physicochemical parameters

	Correlation (*n*=20)												
	TDS	Chlor.	BOD	COD	Temp.	EC	Amm.	Nitrate	Sulph.	T. Hardness	Iron	Turb.	pH
Filamentous fungi	-0.081	-0.087	-0.082	-0.098	0.146	-0.231	0.161	-0.091	-0.002	-0.237	-0.153	-0.152	-0.366
Total yeasts	-0.295	-0.517	0.200	0.367	-0.303	-0.354	0.218	-0.222	0.106	-0.141	-0.017	0.400	0.298

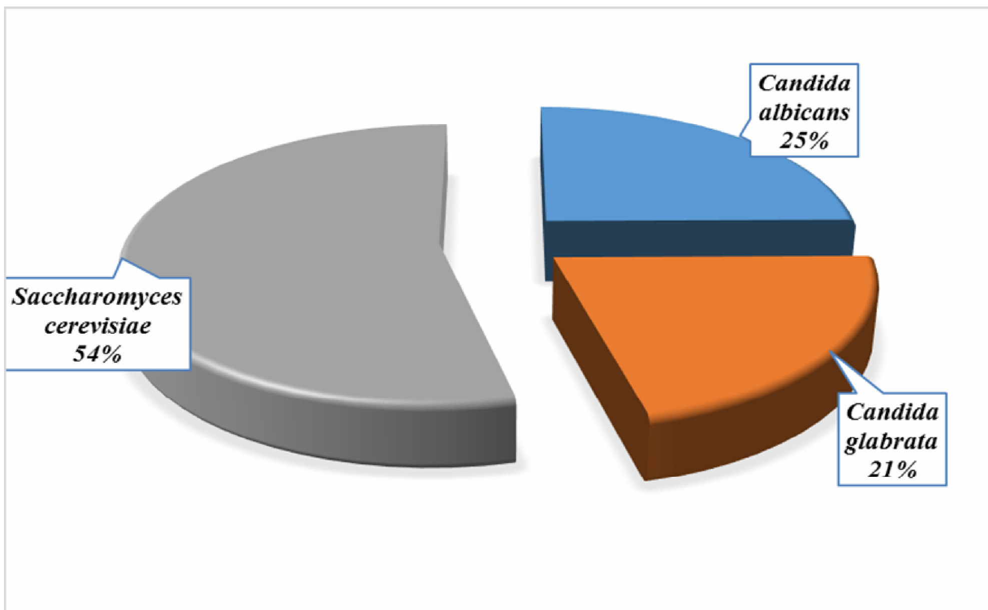

Figure.2 Relative diversity of total yeasts species in groundwater samples

In Conclusion, All examined groundwater samples were not suitable for drinking and human consumption according to the Egyptian standards for drinking water (2007) from the bacteriological view, since these wells showed higher counts of total viable bacterial counts and also showed the presence of bacterial indicators of pollution (total coliforms, fecal coliforms and fecal streptococci), which means the presence of fecal pollution sources around these wells. Groundwater samples showed presence of some filamentous fungal species and yeasts, and some of these microorganisms may cause some diseases for human, thus, the detection of common pathogenic fungal species and yeasts in the Egyptian water environment should be included in the Egyptian standards methods for drinking water quality. An action plan to protect these groundwater wells and the surrounding environment from sewage and industrial effluents should be devised.

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