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OCCURRENCE OF FILAMENTOUS FUNGI FROM TAP AND RIVER WATER IN BAGHDAD - IRAQ

[1]Ammar Y. Alkhakany, [2]Jenan M. Khalaf, [1*]Abdulkarim J. Karim
[1]Unit of Zoonotic Diseases, 2 Department of Internal Medicine & Therapeutics, College of Veterinary Medicine / University of Baghdad, Baghdad – Iraq.
*Corresponding email: karimjafar59@yahoo.com

ABSTRACT
Occurrence of filaments fungi in water resources spoils water and makes it inappropriate for human or animal consumption. This study was designed to investigate the presence of different fungi in water. One hundred water samples were collected from tap and river during the period of September 2016 – February 2017. Samples were filtered through 0.45 millipore filter membrane and cultivated on the Sabouraud dextrose agar. Wide ranges of filamentous fungi from water were isolated like *Aspergillus spp.* (55%) including *A. fumigatus, A. niger, A. flavus, A. glaucocomplex* and *A. terrus* recorded 16%, 14%, 9%, 8.5% and 8% respectively, *Rizopus spp* (14%), *Pencillium spp* (14%), *Geotricum spp* (7%), *Alternaria spp* (6.5%), *Cludosporium spp* (5%), *Aurobasidium sp*p (5%), *Fusarium spp* (3.5 %), *philaphora spp* (1.5%) and Finally *Epicocum spp* (1%). We conclude that the occurrence of contamination of river water with numerous fungus species is more than tap water which reflect either the ineffectiveness of chlorine treatment, fungal resistance or contamination via destruction in water pipe line.

KEYWORDS: tap, river, water, fungal, contamination.

INTRODUCTION

Life couldn't be sustained, unless water is available, as well as safe drinking water is a basic human requirement (Alhassan & Kwakwa, 2014). The taste of water is confined to the way it's treated and quality of its source. However, taste of water doesn't always refer to safety (Mulamattathil *et al.,* 2014). The WHO (2011) recorded safeness of water for human consumption according to its priority for human health during consumption. This can avoid a wide range of drinking-water related diseases and promote public health status (Nichols *et al*., 2009; Wingender & Flemming, 2011).

Fungi are Known to occur ubiquitously in the soil, air and water and act as a source of water contamination. Researchers have declared that the presence of fungi in drinking water involved tap water and water distribution system and deteriorated its taste and odor (Goncalves *et al*., 2006; Pereira *et al*., 2010). Broad fungi species have been isolated from drinking water. Different genera of fungi are opportunistic human pathogen, *e.g. Alternaria spp, Rhizopus spp, Fusarium spp, Asperigillus spp* and are indebted in many nosocomial infections (De Hoog, 2000). These are incriminated in hypersensitivity, pneumonia, phaeohypo mycosis, onychomycosis, keratities, otitis, chronic hyper tropic sinusitis (Green *et al.*, 2003; Hageskal *et al*., 2009). Manikandan *et al*. (2011) reported that the *Fusarium spp* can cause several apportunistic mycosis such as subcutenous invasive mycosis predominantly in imunocompromised patient. Rhinocerebral zygomycosis, caused by *R. arrhizus* and *Rhizopus microspores*, occurs in those with poorly controlled diabetes. Fungi have been regarded as one of significant organism worldwide. Its implication in water includes variety of diseases, of these, 90% of all fungus infections refer to allergies to fungal antigens, direct invasion of hosts and production of toxins resulted in poisoning of human and animals (McGinnis, 1996; Russell *et al*., 2005). Exposure to small amounts of potential toxin for several years may have negative effect on immune system. One of its excrete metabolites is gliotoxin, a toxic antimicrobial materials (Egbuta, 2015) which affects the ability of phagocytes and loss of their protective functions. Gliotoxin is a virulence factor of *Aspergillus fumigatus* and immune suppressive mycotoxin (Sugui *et al.*, 2007). So, this study aim to detect the occurrence of fungus contamination in tap water and river water in Baghdad city in order to counteract their negative effect on human and animal health.

MATERIALS & METHODS

One hundred water samples were collected from tap and river water, 50 each, during six months, commenced from September 2016 till February 2017.

Collection of samples

Tap water samples, and similarly river water from Tigris, were collected in 250 ml sterile bottle with screw cup. Sodium thiosulphate 3% was added to tap water only. Before taking the samples, tap water was drained for 2 to 3 min to remove the accumulated dust and dirt. The procedure of water collection from Tigris River achieved from different locations at (0.5-1) meter depth and allowed to be filled slowly. All samples were tested immediately or within 8 hours after collecting and storing at 4°C.

Fungal isolation

From each sample, 100 ml of water was filtered through a sterial filter 0.45µm Millipore membrane. These

membranes were aseptically placed up on Sabouraud Dextrose Agar (SDA) with chloramphenicol prepared according to manufacturer's instruction.

Statistical Analysis

The Statistical Analysis System (SAS, 2012) program was applied to study the influence of difference factors. Chi-square test was utilized to compare significance between percentages in this study.

RESULTS

Out of 100 water samples examined for microbiological analysis, Table (1) showed high significant variation (P<0.01) of molds recovery at 46% and 82% from tap and river water, respectively. Diagnosis of these molds depends upon the characteristic features including frontal, reverse and staining with lacto phenol cotton blue as described by Mandell and Kauffman (2007). Opportunistic mixed contaminated pathogens including *Aspergillus spp.* were the most predominant isolated fungi. *Aspergillus species* included *A. fumigates* (Fig. 1), *A. flavus* (Fig. 2), *A. glaucuscomplex* (Fig. 3), *A. niger* (Fig. 4), *A. terrus* (Fig. 5). In addition, *Pencillum spp* (Fig. 6), *Aurobasidum spp* (Fig. 7), *Epicoccum spp* (Fig 8*), Rhizopus spp* (Fig. 9), *Alterneria spp* (Fig. 10), *Fusarium spp* (Fig 11), *Cludosporium spp* (Fig. 12), *Phialphora spp* (Fig. 13) and *Geotricum spp* (Fig. 14) were also reported. *Alterneria spp, Fusarium spp, Cludosporium spp* and *Phialphora spp* hadnt been recovered from tap water, while the *Epicoccum spp* were not recovered from river water.

TABLE 1: The positive (%) water sample for mold from tap and river water

	Molds	Sources of water		Chi-Square
		Tap water (n=50)	River water (n=50)	
1.	*A. fumigatus*	5 (10%)	10 (20%)	0.001 **
2.	*A. niger*	4 (8%)	12 (24%)	0.001 **
3.	*A. flavus*	4 (8%)	6 (12%)	0.001 **
4.	*A. glaucus complex*	4 (8%)	5 (10%)	0.001 **
5.	*A. terrus*	1 (2%)	8 (16%)	0.001 **
6.	*Pencillum spp.*	5 (10%)	11 (22%)	0.001 **
7.	*Geotricum spp.*	2 (4%)	4 (8%)	0.001 **
8.	*Alternaria spp.*	0	7 (14%)	0.001 **
9.	*Fusarium spp.*	0	3 (6%)	0.026 *
10.	*Aurobasidum spp.*	4 (8%)	4 (8%)	0.039 *
11.	*Epicoccum spp.*	2 (4%)	0	0.094 NS
12.	*Cludosporium spp.*	0	7 (14%)	0.001 **
13.	*Philaphora spp.*	0	1 (2%)	0.061 NS
14.	*Rhizopus spp.*	3 (6%)	13 (26%)	0.001 **
		23 (46%)	41 (82%)	0.01 **

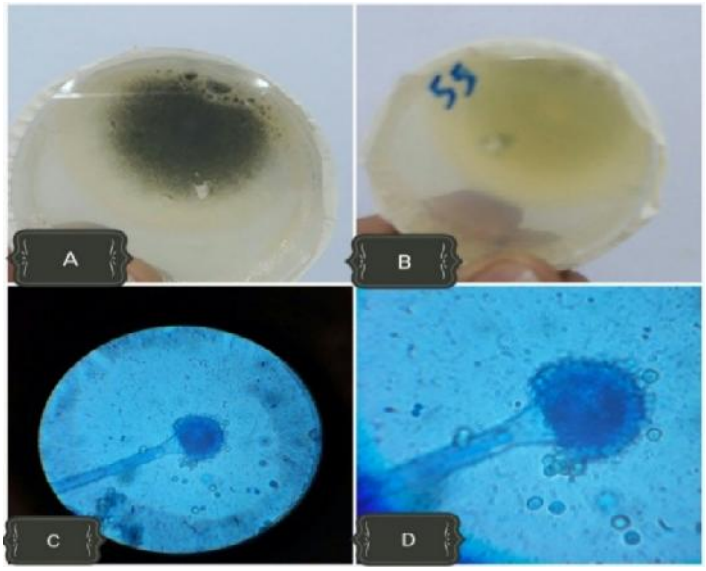

FIGURE 1: Macroscopical appearance of *Aspergillus fumigatus* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

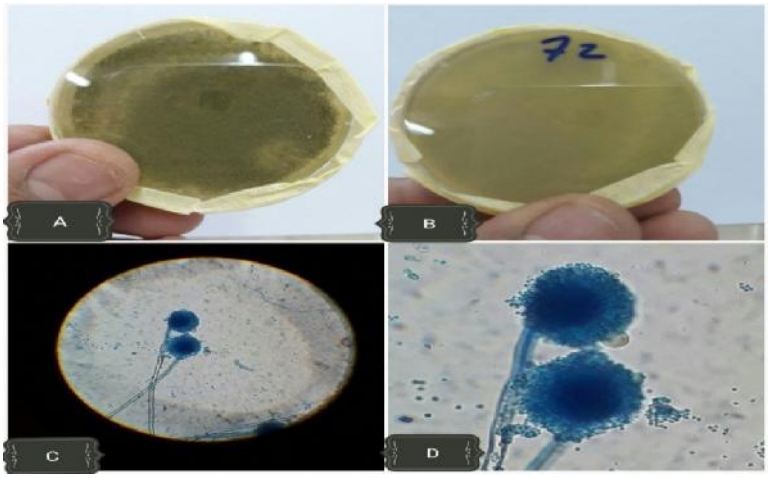

FIGURE 2: Macroscopical appearance of *Aspergillus flavus* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

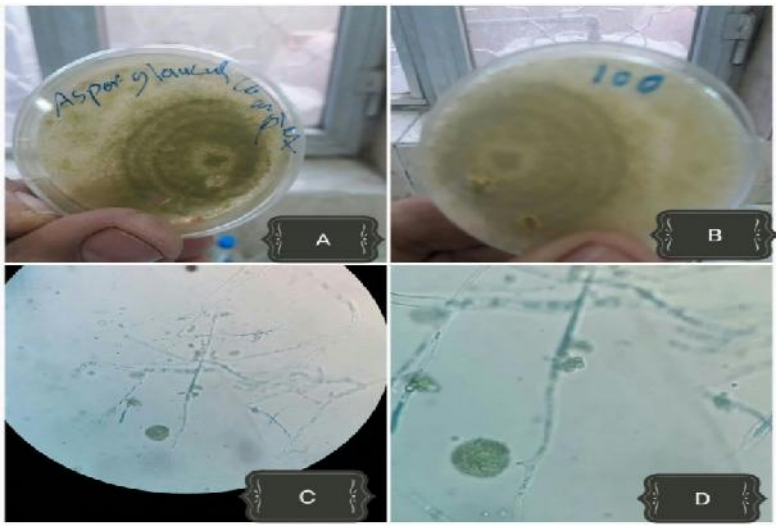

FIGURE 3: Macroscopical appearance of *Aspergillus glaucuscomplex* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

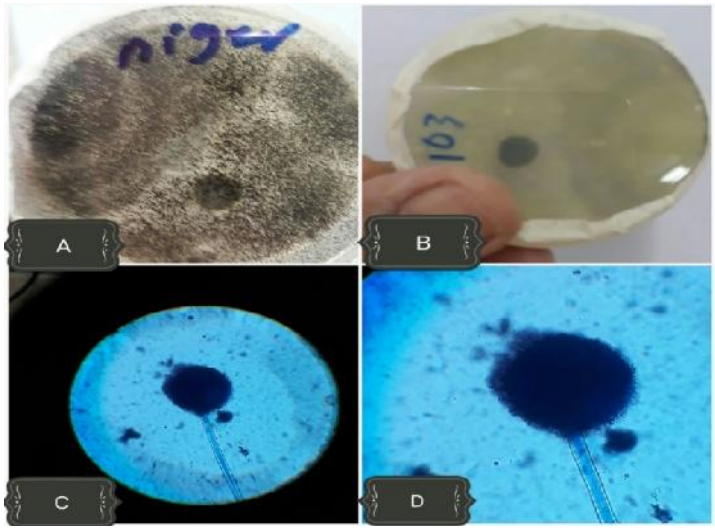

FIGURE 4: Macroscopical appearance of *Aspergillus niger* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

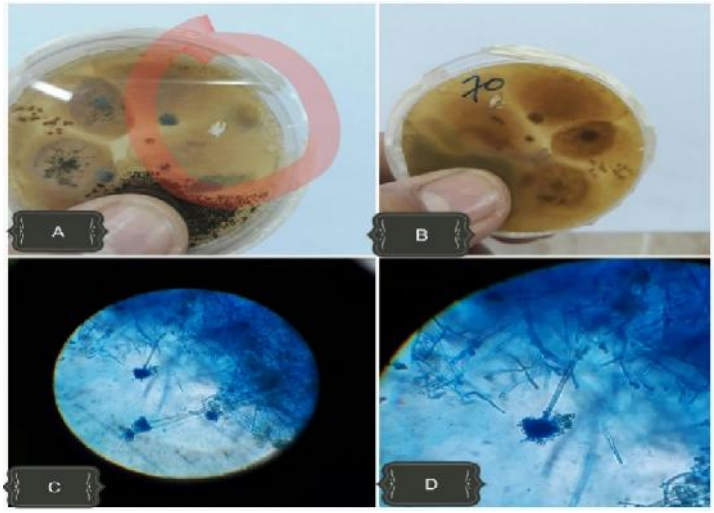

FIGURE 5: Macroscopical appearance of *Aspergillus terrus* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

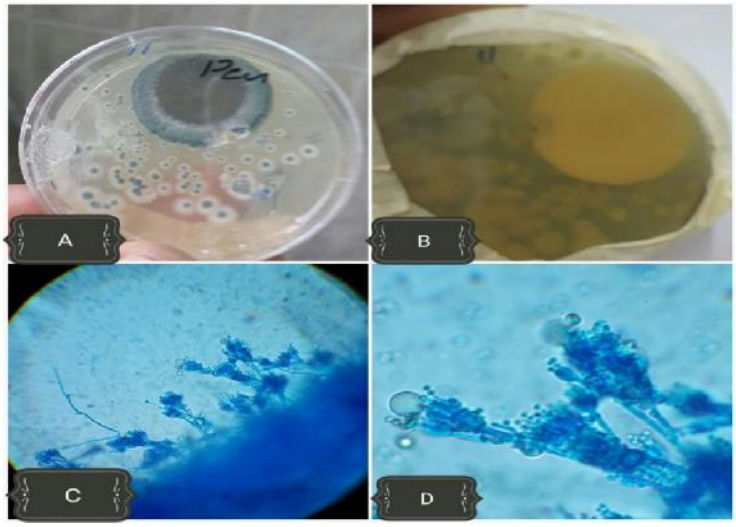

FIGURE 6: Macroscopical appearance of *Penicillum spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60 xs), (D) zoom in (x1.4).

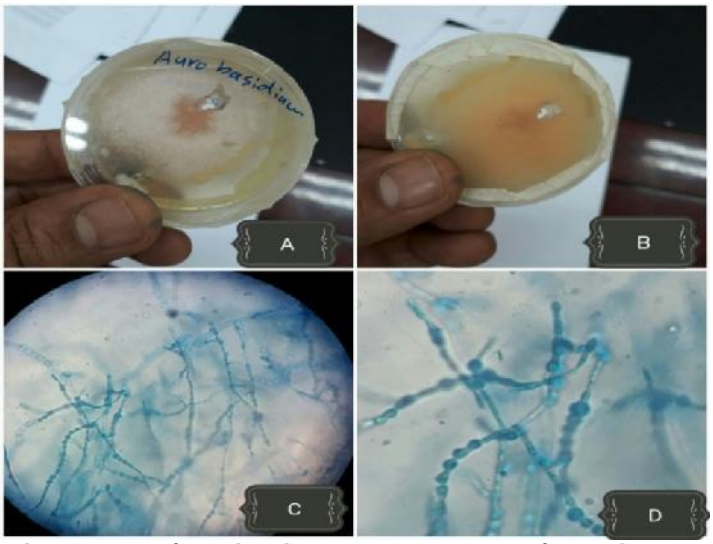

FIGURE 7: Macroscopical appearance of *Aurobasidium spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

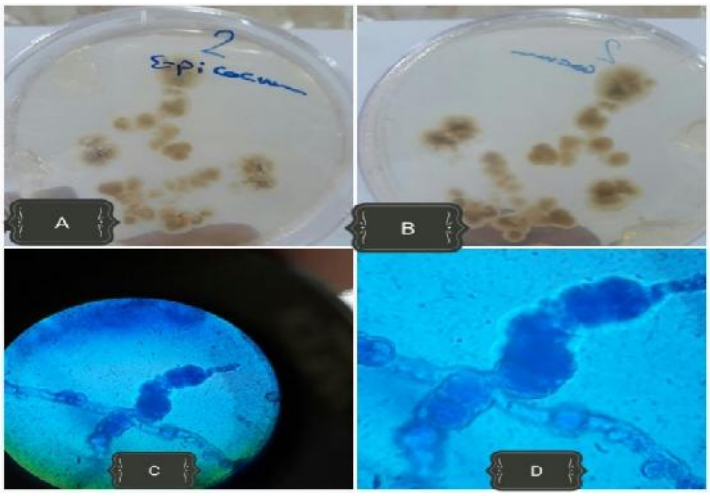

FIGURE 8: Macroscopical appearance of *Epicoccum spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

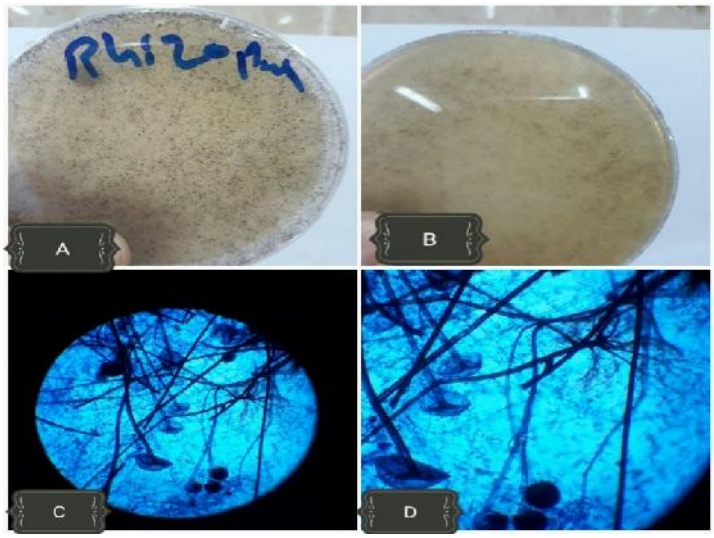

FIGURE 9: Macroscopical appearance of *Rhizopus spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

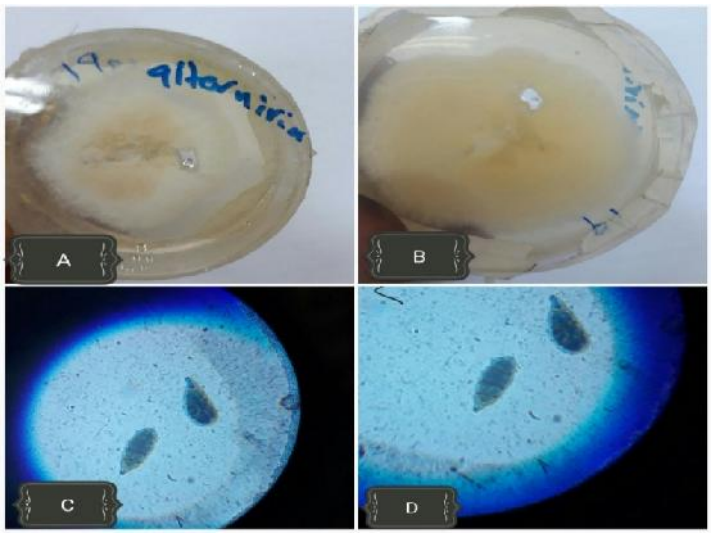

FIGURE 10: Macroscopical appearance of *Alterneria spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

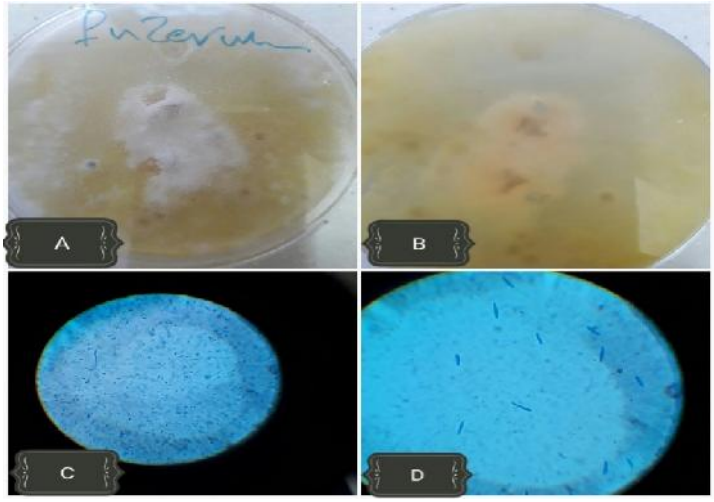

FIGURE 11: Macroscopical appearance of *Fusarium spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

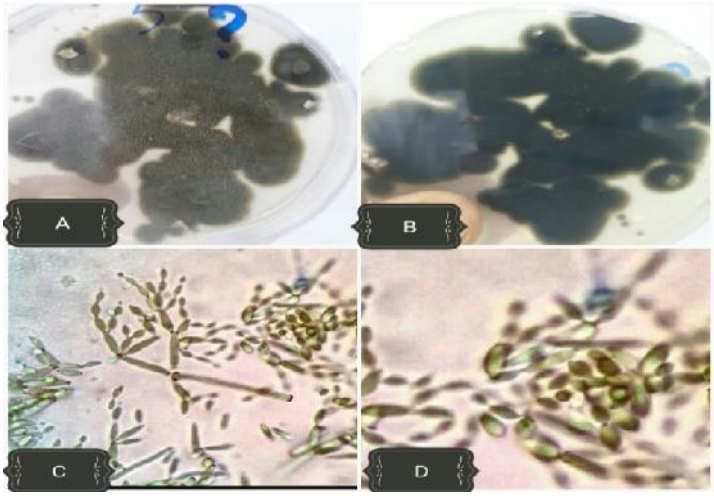

FIGURE 12: Macroscopical appearance of *Cludosporium spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

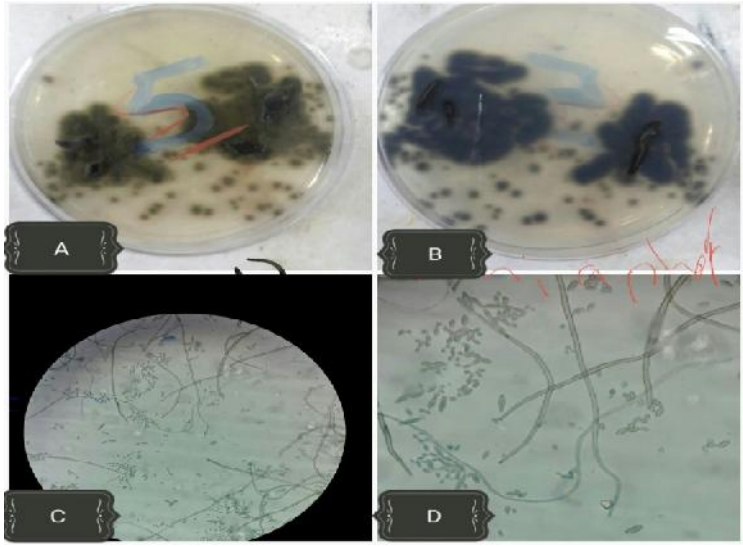

FIGURE 13: Macroscopical appearance of *Phialphora spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

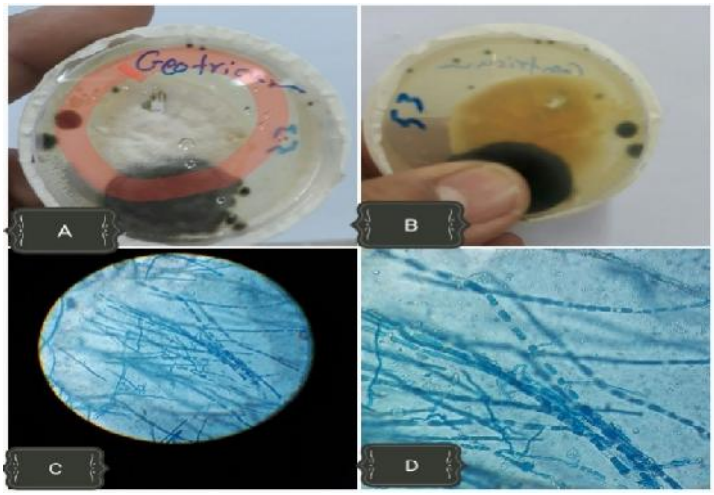

FIGURE 14: Macroscopical appearance of *Geotricum spp* on SDA at 25°C for 5-7 days. (A) frontal, (B) reverse, (C) stained with lactophenol cotton blue (60x), (D) zoom in (x1.4).

DISCUSSION

Fourteen different fungal species were recorded by this study (Table 1). Percentage of contamination of tap water (46%) differs significantly ($p<0.01$) from its occurrence in river water (82%). This indicates that some fungal species are prone to survive water treatment following introduction into the provided system. Coinciding our findings, Hageskal *et al*. (2006) found that the diversity of species isolated from private homes and hospitals was lower than that obtained from raw water samples. Also, our percentages for tap and river water contamination were very closely to Hageskal *et al*. (2007) in Norway who recorded 81% and 69% in row water and treated water, respectively. In Brazil, Goncalves *et al*. (2008) recorded 21.3% mold contamination in drinking water. Many authors confirmed the distribution of fungi in water supply regularly (Stevens *et al*., 2003; Hageskal *et al*., 2009; Kanzler *et al*., 2007; Yamaguchi *et al*., 2007; Richardson and Richardson 2015). Shaker and Sharif (2012) in Iraq isolated fungi in all stage of water treatment and raw water, which reached 41.27 cfu/ 100 ml of raw water, and these number were dropped to 4.88 cfu/ 100 ml in tap water, because the tap water treated by chlorine. In Norway, recovery of fungi in water leads to bad taste and odor (Hageskal *et al*., 2006). This might be attributed to the damage and corrosion of lining wall coatings of drinking water reservoirs (Emde *et al*., 1992). Furthermore, these fungi can produce toxin in water and can overlapping toxicities to consumer (Paterson *et al*., 1997). Our findings revealed that water can harbor different species of filaments fungi including Aspergillus spp, the most commonly isolated genus in water. Shaker and Sharif (2012) recorded 34.40% and 54.10% for the occurrence of Aspergillus spp in tap and raw water, respectively, and as low as 4.1% in mains municipal water (Sammon *et al*., 2010). However, many opportunistic human fungal pathogens are known to be waterborne (Göttlich *et al*., 2002; Kelley *et al*., 2003; Goncalves *et al*., 2006; Hageskal,*et al*., 2006; Hageskal *et al*., 2007; Kanzler *et al*., 2007; Yamaguchi,*et al*.,2007). Several studies referred to inhalation of water aerosols containing spores of those fungi to be the route for systemic infection in humans (Anaissie *et al*., 2002; Hapcioglu *et al*., 2005).The high occurrence of *A. fumigatus* in tap (5%) and river water (10%) in present study (Table 1) was in consistent with findings of Anaissie *et al*. (2002) who recorded the presence of *A. fumigatus* in air of patient's bathroom. They hypothesized that conidia didn't die by temperature and showering aerosol contain fungal spores are inhaled by patient. Warris *et al*. (2001) isolated filamentous fungi from 94% of all the water samples taken from a paediatric bone marrow transplantation unit. Other researchers proved the occurrence of *A. fumigatus* as one of the more commonly isolated genera in water (Arvanitidou *et al*., 1999 and 2000) and could be isolated at 7.2% from surface water in water system installation (Hageskal *et al*., 2006). The Aspergillus spp isolated from both tap and river water in this study include *A. fumigatus* (15%), *A. niger* (16%), *A. flavus* (10%), *A. glaucocomplex* (9%), *A. terrus* (9%) and this result was in consistent with finding of Arvanitidou *et al*. (2000) in Greece and with Warris *et al*. (2001) in Norway who isolated *A. fumigatus* in 60 % and 75 % from tap and main pipe, respectively and A. niger and A. terrus 1% for both. Fungal nosocomial infections are mostly attributedto Aspergillus. It is recorded in immune compromise patients (Richardson and Richardson, 2015). Out of 4.8 million adult people having asthma worldwide, 400.000 are estimated to have chronic pulmonary Aspergillosis (Denning *et al*., 2013). Warris *et al*. (2003) recognized that there are genotypic relatedness between clinical and water related isolation. Other Aspergillus species like *A. terrus* reported by the present study were in consistant with Warris *et al*. (2001) in Norway, Nazim *et al*. (2008) in Pakistan. In Virginia, Walsh (2003) recognized *A. terrus* as amphtrisen B resistant fungus that is incriminated in lethal infection. No study confined the isolation of *Aspergillus glucus* complex in drinking water as our findings did. Its word wide distribution brings the possibility of its isolation from soils, house dust, plants and dried food as well. Although it is rare, *Aspergillus glucus* complex has been implicated in ocular, cerebral, orofacial, cardiovascular and pulmonary infections,

particularly with immune compromised patients (Du *et al*., 2008). On the other hand, de Hoog *et al*. (2000) reported *Aspergillus niger* from tap (8 %) and river water (24%) as doubled as our records (Table 1), whereas Warris *et al*. (2001) in Oslo recorded it at 1% in tap water. Spores of A. niger commonly occur as secondary invader following bacterial otitis (Xavier *et al*., 2008), pulmonary infection in immunocompromised patients (Tarrand *et al*., 2003) and the production of oxalate crystals in clinical human specimens indicating that this genus act as common allergen and causes opportunistic invasive infection in hospitalized immunized patient (Oliveira *et al*., 2016).

Occurrence of Pencillum spp in tap water and river was 10% and 22% respectively (Table 1) lower than previous study (tap; 28.34%, river; 22.13%) conducted in Baghdad by Shaker and Sharif (2012). Our finding was similar with Sammon *et al*. (2010) in Australia who recorded (9.2%), and lower than that of Goncalves *et al*. (2006) in Norway and Oliveira *et al*. (2016) in Brazil who recorded contamination of tap water at 33 % and 25%, respectively. Warris *et al*. (2001) recorded Pencillum spp in tap water and shower water at 17.7% and 5.6%, respectively. Penicillum spp produce patulin and geosmin (Paterson, 2004). Patulin is a mycotoxin with statutory levels, worldwide, considered as bacterial quorum sensing inhibitor (Rasmussen *et al*., 2005). Nevertheless, geosmin is associated with earthy smells associated with problem waters. Penicillum spp can survive in water, and it causes several diseases like allergy, asthma or other respiratory problem (Schwab & Straus, 2004). Geotrichum species occur worldwide and commonly isolated from soil, water, air, and sewage, as well as in plants, cereals, and dairy products. It is also found in normal human flora and is isolated from sputum and digestive tract in humans and other mammals (De Hoog and Smith 2004) and incriminated in many systemic and cutaneous infection (De Hoog *et al*., 2000). Our results (tap water 4% and in river water 8%) differ from Shaker and Sharif (2012) who recorded 00.46% in raw water and nil in tap water. An explanation might refer to the colony becomes yeast-like or slimy optimal growth temperature at 15ºC and might be confused with yeasts (Kaur *et al*., 2008). Alternaria spp occured in this study in river water only (14%), and wasn't recoverd in tap water, differed from Shaker and fayadh (2012) in Baghdad whose records in raw water and tap water were 3.27% and 6.35%, respectively. Its occurrence in tap water was 1% in Norway (Warris *et al*., 2001) and 2.5% in Australia (Sammon *et al*., 2010). Cutaneous infections due to Alternaria species has been reported mainly from Mediterranean countries, particularly in injury farmers from France and Spain (Gene *et al*., 1995).

Fusarium spp occurred in river water in current study almost similar to that observed by Shaker and Sharif (2012). Its presence in tap water agreed with Warris *et al*. (2001) where it was nil and disagreed with Shaker and Sharif (2012), Sammon *et al*. (2010), Nazim *et al*. (2008) and Göttlich *et al*. (2002) who recorded 0.82%, 14.3%, 3.33% and 0.14%, respectively. In USA, De Lucca and Walsh (2015) isolated *F. solani* frequently from water and incriminate to produce water soluble T-2 toxin. O'Donnell *et al*. (2010) reported that *F. oxysporum* and *F. solani* are responsible for 80% of human Fusarium infection. They are able to establish biofilm on contact lens causing eye infections and on polyvinyl chloride pipes suggesting water plumbing systems as the main environmental reservoir for this infection (Chang *et al*., 2006; Short *et al*., 2011).

Mark difference was observed in the recovery of Rhizopus spp from tap and raw water in this study, as it was higher than the findings of Shaker and Sharif (2012) who recorded 4.16% and 5,73% in raw water and tap water respectively. Warris *et al*. (2001) and Goncalves *et al*. (2006) recorded the occurrence of Rhizopus spp in tap water at 1% nd 2.94% respectively. Records of Aurobasidum spp in present study revealed lower occurrence compared with 32.40%, 37% and 18% recorded by Shaker and Sharif (2012), Oliveira *et al*. (2016) and Sammon *et al*. (2010) respectively. Niedoszytko *et al*. (2007) isolated this genus as airborne allergens, including grass, tree, cat and dog fur, associated to severity of asthma.

Ranges of other isolated fungi recorded by this study, *e.g*. Epicoccum spp, *Cladosporium spp* and Philaphora spp donot differ widely from the global records. The epicoccum spp isolated by Sammon *et al*. (2010) at 0.9% in main municipal water in australia, while *Epicoccum nigrum* was recorded by Aldred *et al*. (2005) in solid substrate fermentation system, and lastly, Suraiya & Azira, 2010) isolated this fungi from intramuscular abscess of an immunocompromised patients in Malaysia and the patients not response to Amphotricen-B. Spores of *Cladosporium spp* occur more abundantly worldwide than any other spore type and are the dominant airborne spores, especially in temperate climates and considered as a major source of fungal inhalant allergens (Bordo & Helbling, 2003). Shaker and Sharif (2012) isolated these fungi from river and tap water at 16.54% and 7.99% respectively. From tap water, Warris *et al*. (2001), Sammon *et al*. (2010), Oliveira *et al*. (2016) and Goncalves *et al*. (2006) isolated the fungi at 12.5%, 37.8%, 1.3% and 3.53%, repectively. Shaker and Sharif (2012) in Baghdad coinicided our finding regarding the absence of Philaphora spp in tap water. However, some of Philaphora spp can tolerate 37◦C, and have broad capacity to survive disinfection regimes (Philipps *et al*., 1999).

The present study reported significant increment in fungal contamination of river water than tap water. This may be attributed to pollution from air and surrounding environment. The high turbidity of river water due suspending soil and organic particles is positively related with fungal isolation in river water. This is in agreement with Shaker and Sharif (2012), Nagy and Olson (1982), Niemi *et al*. (1982) and Sammon *et al*. (2010). The significant occurrence of filaments fungi in tap water, although treated with chlorine (Yamaguchi *et al*., 2007; Hageskal *et al*., 2009) because fungi can survive and persist after treatment, or enter through repairs, replacement of pipes, obstruction of water piping and the presence of odor and pigments in water (Siqueira *et al*., 2011; Hussain *et al*., 2010) which provided favorable condition for establishment of fungi and growth of biofilm inside the water. Few countries have standards for the presence of fungi in drinking water. Sweden is the only country that implies fungal analysis in drinking water

since 1993. The limit for the occurrence of fungi in drinking water is 100cfu per 100 ml sample (Hageskal *et al*., 2009). The Norwegian drinking water regulation does not include micro fungi (Anon, 2001). Kelley *et al*. (2003) isolated filaments fungi from surface water in UK and USA. Likely, no standards for fungi contamination in drinking water in Iraq. However, few guidelines as to what is considered as normal or acceptable levels of molds recovered from water. Several factors control the length of active time of chlorine and ozone in water such as temperature. Fungi that survived chlorine treatment in descending order include Aspergillus spp, pencillum spp and Cladosporium spp. In similar studies, Shaker and Sharif (2012), Kanzler *et al*. (2007), Hinzelin *et al*. (1985) isolated Alternaria spp and Fusarium spp in low frequency. Chlorination is the most economical method of disinfecting water and has been adopted by commercial growers in modern country (Gleick, 2003; Ibrahim *et al*., 2015). As shown by this study, the predominant fungi isolated from Tigris river water were Aspergillus spp., Rhizopus spp. and pincillum spp. These genera can tolerate extreme environmental stress, low water availability and high temperatures (90°C), and can be recovered under appropriate conditions (McGee *et al*., 2006), which could enhance their ability to survive under various environmental conditions in Tigris river. Shaker and Sharif (2012) in Iraq and Liu *et al*. (2015) from Songhua river catchment in China supported our findings. Moreover, fungal distribution in river water depends on geographical location and that physical barriers have significant contributions to the fungal distribution (van der Gast *et al*., 2011; Wu *et al*., 2013). Water surface is already contaminated with fungi from air and surrounding environment as reported by Wu *et al*. (2013) who argued that elements like total nitrogen, total phosphorus, nitrate and pH have different effects in fungal community and influence both fungal biomass and community structure. Hageskal *et al*., (2006) explained mold recovery by the content of organic material and chemical conditions in the water.

Although no obvious variation was observed between global studies, variation of results may occur due to sample size, type of water analyses, research methods and cultivation and enumeration of fungi in water. Our conclusion indicates the occurrence of contamination of river water with numerous fungus species more than tap water which reflect either the ineffectiveness of chlorine treatment, fungal resistance or contamination via destruction in water pipe line.

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