



Research article

Occurrence of fungal microbial contamination in drinking water of megacity of Karachi (Pakistan) and their physico-chemical control

Faisal Hussain ^{a,*}, Iram-us Salam ^b, Farzana ^b, Zaibun-nisa Memon ^c, Muhammad Abdullah ^d, Ghulam Abbas ^e, Muhammad Akbar ^f, Alamdar Hussain ^f, Muhammad Majeed ^g, Kishwar Ali ^h, Haruna Musa Moda ^{i,**}

^a Department of Botany, Ghazi University, Dera Ghazi Khan, 32200, Pakistan

^b Department of Botany, Federal Urdu University of Arts, Sciences & Technology, Karachi, Pakistan

^c Department of Zoology, Shah Abdul Latif University Khairpur Mirs Sindh, Pakistan

^d Biodiversity Park, Director Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan

^e Department of Biotechnology, University of Agriculture Dera Ismail Khan-29111, Khyber Pakhtunkhwa, Pakistan

^f Department of Biological Sciences, University of Balistan, Skardu, Pakistan

^g Department of Botany, University of Gujrat, Hafiz Hayat Campus, Gujrat, 50700, Pakistan

^h College of General Education, University of Doha for Science and Technology, Al Tarafa, Jelajah Street, Duhail North, PO Box 24449, Doha, Qatar

ⁱ Senior Lecturer Occupational Safety Health and Environment, Manchester Metropolitan University, All Saints Building, Manchester, M15 6BH, United Kingdom

ARTICLE INFO

Keywords:

Fungi
Mycotoxicity
Water system
Mycodiversity

ABSTRACT

The water quality in Karachi (Pakistan) is uncertain due to the occurrence of fungi and other microorganisms. A total of twenty-five water samples were collected from public places, educational institutes, hospitals, water supply systems and surface water of the canal of Karachi (Pakistan). The different fungal species including *Acremonium* sp., *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. sulphureus*, *Cladosporium* sp., *Fusarium* sp., *Clonostachys (Gliocladium)* sp., *Macrophomina phaseolina*, *Mucor racemosus*, *Paecilomyces* sp. *Penicillium chrysogenum*, *P. citrinum*, *P. commune*, *P. expansum*, *Rhizoctonia* sp. and *Stachybotrys* sp. were isolated from these drinking water samples. However, the bacteria, microalgae and some other microorganisms were present in low concentrations. The reason for fungi infection and production of mycotoxicity depends upon various factors and the availability of their nutrients in filtration plants. The major threats to human health are fungal mycotoxicity which is responsible for carcinogenic and other lethal diseases. Mostly, the genus *Aspergillus* was dominated and isolated with a maximum of 88–98% of occurrence in the different samples of drinking water by the direct plate-spread method. For the control of fungi, various Physico-chemical coagulation treatments were used, but Potassium alum, clay pot, and hot water treatment disinfected effectively 69–70% removal of the fungi and its spore or mycelia from the water. In addition, it is concluded that drinking water purifications

* Corresponding author Department of Botany, Ghazi University, Dera Ghazi Khan, 32200, Pakistan.

** Corresponding author.

E-mail addresses: faisal.botanist2011@gmail.com (F. Hussain), iram.zulfqar@fuuast.edu.pk (I.-u. Salam), farzana.usman@fuuast.edu.pk (Farzana), zaiib.nisa@salu.edu.pk (Z.-n. Memon), abdullahfrw@iub.edu.pk (M. Abdullah), ghulam.abbas@gmail.com (G. Abbas), muhhammad.akbar@uobs.edu.pk (M. Akbar), alamdar.hussain@uobs.edu.pk (A. Hussain), m.majeed@uog.edu.pk (M. Majeed), kishwar.ali@udst.edu.qa (K. Ali), H.Modat@mmu.ac.uk (H.M. Moda).

<https://doi.org/10.1016/j.heliyon.2024.e28926>

Received 28 October 2023; Received in revised form 23 March 2024; Accepted 27 March 2024

Available online 31 March 2024

2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

such as chlorination, filtration and lime did not eliminate thermophilic fungal spores or mycelia including *Penicillium*, *Paecilomyces* and *Mucor* from the water.

1. Introduction

The water distribution system (pipeline) has been reported as biologically contaminated for several years by epidemiologists and pathologists in Asia and Europe [1,2]. The water is significantly affected by the number of fungi, bacteria, protozoa, actinomycetes and cyanobacteria and these are reported to change the adverse odour and taste of the drinking water distribution system [1–6]. The presence of fungi genera in drinking water not only changes the odour and taste but also causes some operational and technological difficulties [3]. Water contamination by fungal activities can produce mycotoxicity and allergy to human health [4,5]. It can be transmitted or inhaled during the sauna or showering. It may be ingested by different cavities such as cornea, skin and oral cavities into the human body and can be transmitted directly into the blood during surgical procedures [6].

It has been reported from African, Asian and European countries in several newspapers and social media last year that some genera of fungi and other microbes are reported in the distribution system of drinking water. Some fungi including water-borne are mutually associated with odour and taste problems and it also affects the different health-related and stomach problems [7–9]. The different genera of fungi are usually the source of toxins particularly antibiotic properties, mycotoxins, and some other secondary metabolites. The fungi genera which produce mycotoxins are known as toxigenic genera of fungi. It is reported by several researchers that 46 genera of fungi are responsible for producing mycotoxins, but the few dominant genera are *Aspergillus*, *Fusarium*, *Clonostachys (Gliocladium)*, *Penicillium*, *Stachybotrys*, etc., rapidly producing mycotoxicity in water. All 46 genera have not been studied for their mycotoxicity but a few dominant genera have been screened [10]. [7,11,12] found the features of the predominance fungi genera colonizing in water are *Acremonium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, *Mucor*, *Boeremia* and *Paecilomyces* in drinking water derived from lake water.

The fungal and bacterial control strategy of drinking water by suitable treatment has not been properly studied and the results are not prominent and unclear in Pakistan particularly in Karachi. The use of chemicals for disinfection is frequently used as a control strategy in drinking water plants for treatment. These treatments can initially or partially inactivate the contamination but later on, it may be recovered again in the water distributing system and the residual organisms are source of toxicity for human health. All fungi could not be deactivated or killed by chemical disinfectants because the nature of fungi is highly variable between genera and species [13]. According to Ref. [14], sand filtration may give better results as compared to chemical disinfectants. Sand filtration has been considered a good strategy to control and remove fungi from drinking water. The use of chlorination for disinfection is not sufficient and fit for water quality [15]. The chlorine treatment conveniently depends on pH and temperature (it also influences chlorine, as some bacteria and organisms grow better in warmer environments). But all spores, conidia, sclerotia or mycelia are reported not to be deactivated by the same treatment or constant temperature. Some fungal morphological structures and hyphal cells are capable of resistance against temperature. However, some fungal genera including *Penicillium* are reported to be extremely chlorine resistant. The demand for more chlorine can affect the other microbes which are environment-friendly or present in the soil where the pipe system is fixed which is made up of chlorine [16].

In Pakistan, water quality in most of the cities is decreasing quickly. The major cause of decreasing water quality is the ground water supply due to arsenic contamination. In Pakistan 20%–40% of hospitals are filled with people that are suffering from waterborne illness, according to United Nation International Children Emergency Fund (UNICEF). Fungal and bacterial diseases and infections represent about 80% (including diseases due to sanitation problems) of all diseases and are responsible for 33% of deaths [17]. [7] highlighted the drinking water quality, contamination sources, sanitation situation, and effects of unsafe drinking water on humans. There is immediate need to take protective measures and treatment technologies to overcome unhygienic condition of drinking water supplies in different areas of Pakistan particularly megacity of Karachi.

In previous studies, microbial contamination in drinking water distribution has been reported from Africa, Asia Europe and South America [1,2,35]. In the present study, it has been reported almost in all provinces and mega cities of Pakistan [7,17,34]. In recent study, the mega city of Pakistan (Karachi) has been focused only on fungal microbial contamination and explore the status of fungal microbial contamination in public places of mega city Karachi. However, other microbes including actinomycetes, bacteria, virus, protozoa, and cyanobacteria have also been detected [50–52]. UNICEF also reported that due to the presence of microbial diseases, 33% of deaths of children have been reported in Pakistan [17].

The main objectives of the present study were to explore and analyze the occurrence of fungi (Water-borne) mycotoxicity in drinking water and the assemblages of fungal composition in a different sampling of drinking water in public places, educational institutes, hospital, water supply system and surface water of the canal with percentage infection of fungal genera and to investigate the water treatment of fungi, in particular, physicochemical control.

2. Materials and methods

2.1. Study area and collection of water samples

A total of twenty-five samples (1.5 L of each sample) of drinking water were collected from public places, educational institutes, hospitals, water supply systems and surface water of the canal of the megacity of Karachi-Pakistan (Fig. 1) from July to Sept 2021. The

samples of drinking pipeline water were preserved and kept at room temperature at the Department of Biotechnology, Federal Urdu University of Arts, Science & Technology, Karachi (Pakistan).

2.2. Isolation of the fungal pathogens

For the isolation of fungal pathogens, a direct plate-spread method (Place the water sample on the solidified and dried surface of the agar medium and spread it uniformly using a spreader) with volumes of 1 mL of water was used. The 1 mL per sample was placed on Potato Dextrose Agar (PDA) medium plates containing Penicillin and Streptomycin anti-bacterial drops. The Petri plates were kept for incubation at 28 ± 2 °C for 5 d [11,18,19]. The measurement and observation of fungi in water samples were assessed by colony-forming units (CFU) per volume of drinking water collected sample [20]. The occurrence percentage was noted by the given formula:

$$\text{Occurrence \%} = \frac{\text{Total number of fungi occurrence in collected sample}}{\text{Total number of samples examined}} \times 100$$

Identification of Fungi:

The isolates of fungi were identified by their morphology and structure in culture. Fungi have mycelium (hyphae), the spores, the origin of the spores and asexual or sexual which are used in the identification. The isolates of fungi were identified by using 10 to 40× of magnifications under the compound electron microscope to explore the morphological characters such as conidia, spores, mycelia, hyphae, growth rate, colony texture and growth pattern on PDA medium containing plates [21]. The standard manuals of [22–25] and others were studied for the characteristics features and confirmation of different fungal species.

2.3. Physico-chemical test

The Physico-chemical test experiment was conducted in the Department of Biotechnology, Federal Urdu University of Arts, Science & Technology, Karachi-Pakistan. The different Physico-chemical treatment parameters including potassium alum, chlorination, lime, microfiltration, clay pot and boiling/hot water were utilized against isolates of fungi. After the treatment of different physicochemical coagulation, the fungi infection percentage was observed after the 2 h of exposure.

- Potassium Alum ($KAl(SO_4)_2$): One 1000 mL of collected samples were kept and 0.5 g potassium alum was added by using the method of [26]. After 2 h, water samples were collected for post-observation of pathogens.
- Chlorination (Cl_2): The concentration of chlorine 0.4 mg was added to 1000 mL of collected water samples [27] and it was further analyzed for isolation of pathogens after 2 h of time duration.

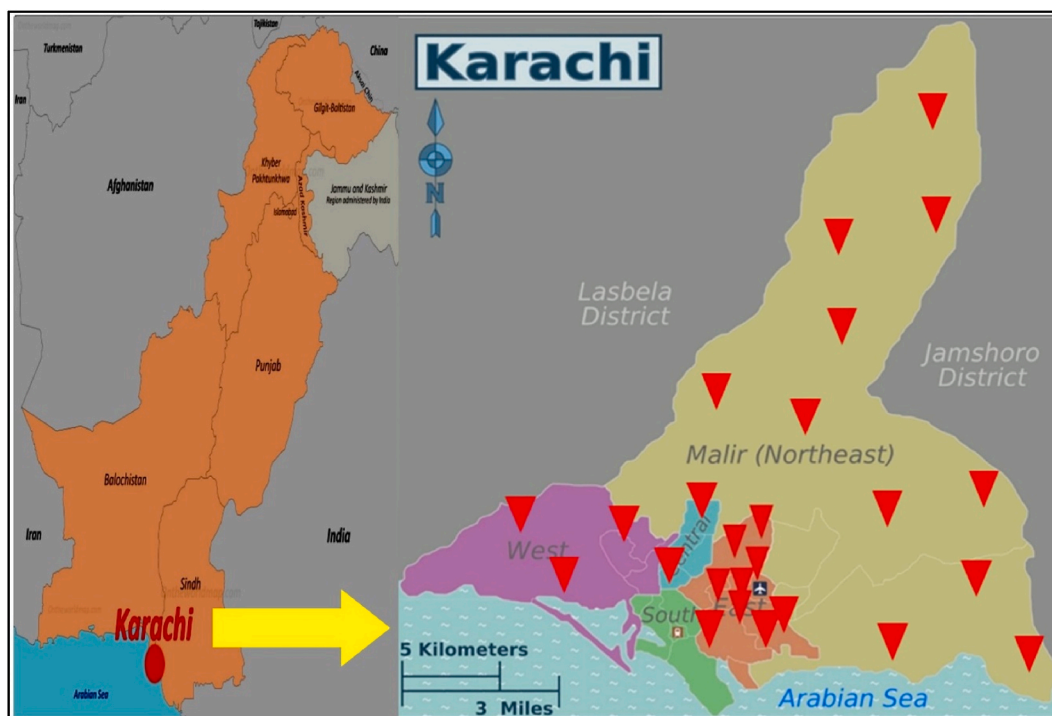


Fig. 1. Showing the sampling study map of Karachi, Pakistan.

- c. **Lime (CaCO₃):** In water treatment, lime is used for softening water. But it can be also used for the disinfection of pathogens. However, calcium hydroxide can also be used for softening water. CaCO₃ saves the usage of coagulants and settling time. The results of [28] suggested that CaCO₃ requires a lesser amount of coagulants and also shortens the settling time. The concentration of lime 40 mg was added to 1000 mL of collected water samples by using the method of [29] and post observations were noted after 2 h.
- d. **Microfiltration:** A thin layer of mesh which is capable of separating substances was used in water treatment. The size of the mesh was 0.007 μm which was used to remove silk, sand or microorganisms, mycelia and other substances [30].
- e. **Clay Pot:** The 7-Litre circular egg-like oval shapes of the clay pots were used for the observation of collected water samples after keeping them for 2 h.
- f. **Boiling/hot Water:** This is the very basic and simplest method of disinfection. Temperatures of collected water are raised to their boiling point (100 °C) for 10 min and keep it for 2 h at room temperature.

2.4. Statistical analysis

Data were analyzed and subjected to analysis of variance (ANOVA) depending upon the experimental design according to Ref. [31]. The follow up of ANOVA included Least Significant Difference (LSD), Duncan’s multiple range test was also used to compare the treatment means. Analysis of variance (ANOVA) was followed by the standard error of the difference between means.

3. Results

A total of twelve (12) genera and seventeen (17) species were isolated and identified from drinking pipe water samples (Table 1). In the present study, the genus *Aspergillus* was dominated as compared to all other genera of fungi. A maximum of 88–98% of *Aspergillus flavus*, 33–77% of *A. sulphureus* and 28–70% of *A. fumigatus* contamination were observed in the samples of drinking water, respectively. However, a minimum of 8–23% of *Paecilomyces* sp. and 14–33% of *R. solani* occurrence were detected from all drinking water samples (Fig. 2).

The samples of surface water of canal and drinking water supply system were contaminated 42.35% and 34.18%, respectively as

Table 1
Presence of fungi from different collected samples of drinking water.

Name of fungi	A					B					C					D					E				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<i>Acremonium</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Alternaria alternata</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Aspergillus flavus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>A. fumigatus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>A. sulphureus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Cladosporium</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Fusarium</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Clonostachys (Gliocladium)</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Macrophomina phaseolina</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Mucor racemosus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paecilomyces</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Penicillium chrysogenum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>P. citrinum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>P. commune</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>P. expansum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rhizoctonia</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Stachybotrys</i> sp.	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

A= Surface water of the canal, B= Drinking water of public places, C= Drinking water of educational institutes, D= Drinking water of hospital and E= Drinking water of water supply system

■ = Presence and ■ = Absence.

A = Surface water of the canal, B = Drinking water of public places, C = Drinking water of educational institutes, D = Drinking water of hospital and E = Drinking water of water supply system = Presence and = Absence.

compared to other collected sample sites. The minimum 16.18% contamination and microbial activity were recorded from drinking pipeline water samples of educational institutes (Table 2).

Physico-chemical Control:

The highest 7.7–10.1% and 7.3–9.3% infection was re-isolated from those samples of water which were treated with limestone (CaCO₃) and chlorination, respectively (Fig. 3). However, the least 6.6–8.2% infection was found in potassium alum, clay pots, and boiling/hot water after treatment (Table 3).

Six Physico-chemical treatments (potassium alum, chlorination, lime, microfiltration, clay pot and boiling/hot water) were tested against the isolated fungi of drinking water samples.

A maximum of 70% mean inhibition was recorded in potassium alum and 69% observed in boiling/hot water and clay pot, respectively. However, the minimum 59 and 64 mean inhibition percentage was observed from lime and chlorination treated water samples (Fig. 4). The genus *Aspergillus*, *Mucor*, *Paecilomyces* and *Penicillium* were regrown again in lab conditions after Physico-chemical treatments due to their thermophilic nature.

ANOVA result of occurrence (%) of different fungi isolated from samples of drinking water. All samples collected from different sites including surface water of canal, drinking water of public places, drinking water of educational institutes, drinking water of hospitals and drinking water of water supply system showed most significant differences among different sites (Table 4). All sites have been detected with significant occurrence of fungi.

Table 5 shows the ANOVA result of infection (%) of different fungi after different treatments of Physico-chemical control after 72 h of exposure. Almost all treatments of physico-chemical control including Potassium Alum, Chlorination, Microfiltration, Clay pot and Boiling/Hot water showed non-significant differences among different treatments as compared to control except Lime (Table 4). All treatments showed minor infection % after different treatments of Physico-chemical control after 72 h of exposure.

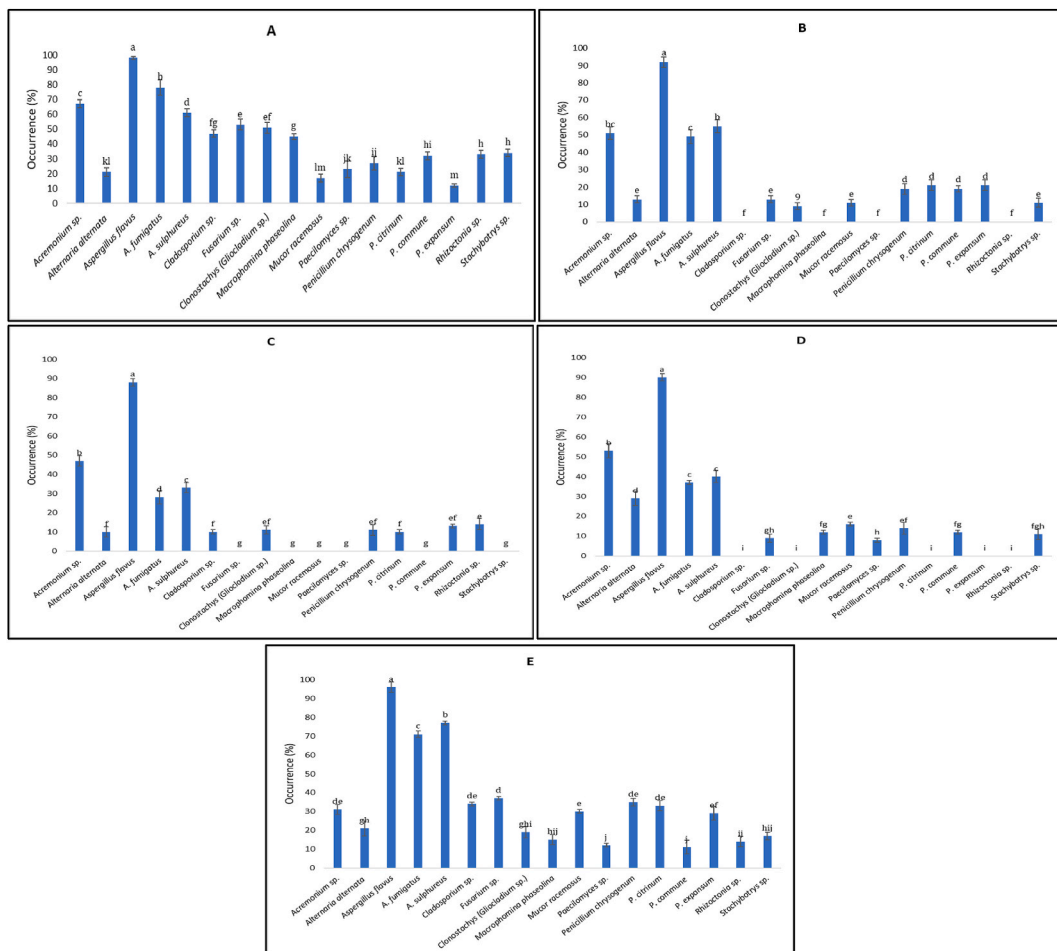


Fig. 2. Occurrence % with of different fungi isolated from collected samples of drinking water (A = Surface water of the canal, B = Drinking water of public places, C = Drinking water of educational institutes, D = Drinking water of hospital and E = Drinking water of water supply system).

Table 2

Mean Occurrence % with Standard Deviation of different fungi isolated from collected samples of drinking water.

Isolated Fungi	Occurrence % of fungi in drinking water samples				
	A	B	C	D	E
<i>Acremonium</i> sp.	67 ± 2.65	51 ± 3.61	47 ± 2.65	53 ± 3.61	31 ± 2.65
<i>Alternaria alternata</i>	21 ± 3.00	13 ± 2.00	10 ± 2.65	29 ± 3.61	21 ± 4.00
<i>Aspergillus flavus</i>	98 ± 1.00	92 ± 3.00	88 ± 2.00	90 ± 1.73	96 ± 2.65
<i>A. fumigatus</i>	78 ± 5.29	49 ± 4.00	28 ± 3.46	37 ± 1.00	71 ± 1.73
<i>A. sulphureus</i>	61 ± 2.65	55 ± 3.61	33 ± 2.65	40 ± 3.00	77 ± 1.00
<i>Cladosporium</i> sp.	47 ± 2.65	0 ± 0	10 ± 1.00	0 ± 0	34 ± 1.00
<i>Fusarium</i> sp.	53 ± 3.61	13 ± 2.00	0 ± 0	9 ± 2.00	37 ± 1.00
<i>Clonostachys (Gliocladium)</i> sp.	51 ± 3.61	9 ± 2.00	11 ± 2.00	0 ± 0	19 ± 3.00
<i>Macrophomina phaseolina</i>	45 ± 2.00	0 ± 0	0 ± 0	12 ± 1.00	15 ± 2.65
<i>Mucor racemosus</i>	17 ± 2.65	11 ± 2.00	0 ± 0	16 ± 1.00	30 ± 1.00
<i>Paecilomyces</i> sp.	23 ± 5.57	0 ± 0	0 ± 0	8 ± 1.00	12 ± 1.00
<i>Penicillium chrysogenum</i>	27 ± 4.58	19 ± 3.00	11 ± 2.65	14 ± 3.00	35 ± 2.00
<i>P. citrinum</i>	21 ± 2.65	21 ± 3.00	10 ± 1.00	0 ± 0	33 ± 2.65
<i>P. commune</i>	32 ± 2.65	19 ± 1.73	0 ± 0	12 ± 1.00	11 ± 3.61
<i>P. expansum</i>	12 ± 1.00	21 ± 3.00	13 ± 1.00	0 ± 0	29 ± 3.61
<i>Rhizoctonia</i> sp.	33 ± 2.65	0 ± 0	14 ± 3.00	0 ± 0	14 ± 2.65
<i>Stachybotrys</i> sp.	34 ± 2.65	11 ± 2.65	0 ± 0	11 ± 2.65	17 ± 2.00
Total Occurrence Percentage (%)	42.35%	22.59%	16.18%	19.47%	34.18%

A = Surface water of the canal, B = Drinking pipeline water of public places, C = Drinking pipeline water of educational institutes, D = Drinking pipeline water of hospital and E = Drinking pipeline water of water supply system.

4. Discussion

Fungi in water distribution systems are well known and have gained importance recently [11,14,32,33]. It is unsurprising that fungi are isolated from the surface or underground raw water in reservoirs and distribution systems. Fungal contamination found in almost every environmental niche [13,34].

The occurrence of several fungal genera including *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* in a water distribution system supplied by groundwater in Recife—Pernambuco, Brazil was also reported by Ref. [35]. The various taxonomic studies of fungi have been reported that filamentous fungi genera are to be seen colonized in drinking water distribution systems including *Fusarium*, *Acremonium*, *Phialophora*, *Penicillium*, *Exophiala*, *Boeremia*, *Verticillium*, *Humicola*, *Paecilomyces*, *Aspergillus*, *Myrothecium*, *Cladosporium*, *Volutella*, *Chara*, *Geomyces*, *Mucor*, *Ochroconis*, *Phaeococcus*, *Conidiobolus*, *Tilletiopsis*, *Alternaria*, *Plectosporium* and *Sporocybe* [11,36–39]. *Paecilomyces lilacinus*, *Cladosporium cladosporoides*, *Aspergillus niger*, *A. ustus*, *A. fumigatus*, *A. versicolor*, *A. parasiticus*, *Geotrichum candidum* and *Penicillium rubrum* are pathogenic fungal species and risk to human health [22,40–42]. [6] reported that it depends on the removal process and effectiveness of water treatment strategy to minimize the degree of fungal contamination in the water distribution system. The presence of fungal microbial such as *Aspergillus versicolor*, *Penicillium frequentans*, *Cladosporium sphaerospermum* and *Aspergillus parasiticus* in bulk quantity in water is a cause of pathogenic to warm-blooded animals and human heaths. These fungi are also reported in other several species which occur in biocenosis, colonizing bird feathers, nests and droppings [40,41]. The dominant and common genera and fungal species are *Aspergillus flavus*, *A. fumigatus*, *Penicillium* sp., *Aspergillus niger* and *Cladosporium* sp., observed in the USA as well as it has been also reported from Germany in water distribution systems [11, 39]. The mold is also considered the main component of fungal microbial contamination in the drinking water systems as found in the term of frequency in abundant formation [11,43,44]. It is reported by Ref. [9] that those filamentous fungi particularly the genera *Aspergillus* and *Penicillium* are more complicated and dominated as compared to the yeast-like fungi or yeast in drinking water. Further research should therefore elaborate on the possible mechanisms affecting spore germination and growth within distribution systems. The effects of chlorine on fungal biofilms warrant particular attention since it remains undetermined how residual chlorine levels affect spore longevity and germination.

According to Ref. [9] the effect and treatment of chlorine is uncertain against fungal and other microbial complexes. It is very complicated to examine the degree of effectiveness against fungal genera. Therefore, the proper information and details about the treatment of chlorine against fungal colonies and spores are extremely scattered [45]. reported that in underground water supply systems including storage of water in underground reservoirs where ambient air is packed or prevented, the fungal microbial contamination is lower and insufficient as compared to open water systems. Furthermore, they suggested that water treatment and procedure of purification by chlorination are not sufficient or helpful to eliminate the fungal spores or fungal growth activity [36]. investigated that actinomycetes and fungi, including low concentrations of thermophilic strains, were present in raw water supplies and that they could pass through both sand filtration and disinfection and therefore can occur in drinking water. The disinfection of fungi and actinomycetes may not be prevented by the water distribution system.

Similarly [44], demonstrated that the number of microscopic fungi in water distribution systems may reach 100 CFU 10 mL. As the results of previous studies show, water treatment processes do not inhibit fungal presence in water systems. According to Ref. [6], it is caused by the growth of fungi as mycelial aggregations on water pipe surfaces to which chlorine access is difficult during treatment. Earlier studies by Ref. [46] showed that chlorine concentration 1.0–3.0 mg Cl₂ did not have fungicidal effects on yeast cells and

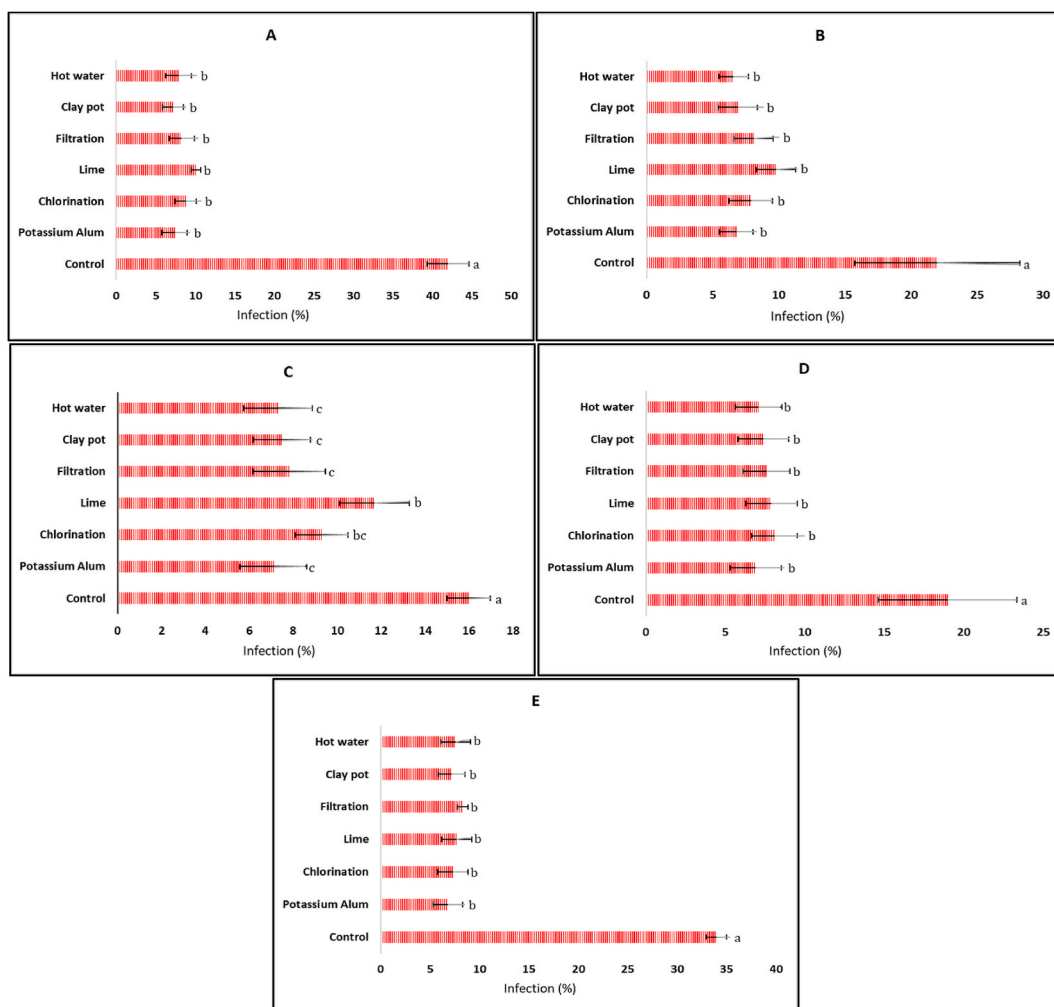


Fig. 3. Infection % of different fungi after different treatments of Physico-chemical control after 72 h of exposure (A = Surface water of the canal, B = Drinking water of public places, C = Drinking water of educational institutes, D = Drinking water of hospital and E = Drinking water of water supply system).

Table 3

Infection % of different fungi after different treatments of Physico-chemical control after 72 h of exposure with Mean and Standard Deviation.

Treatment	Fungi infection % at different treatments of Physico-chemical coagulation after 72 h of exposure				
	A	B	C	D	E
Control	42 ± 2.65	22 ± 6.24	16 ± 1.00	19 ± 4.36	34 ± 1.00
Potassium Alum	7.4 ± 1.59	6.8 ± 1.27	7.7 ± 1.53	8.2 ± 1.62	8.2 ± 1.52
Chlorination	8.8 ± 1.35	7.9 ± 1.65	9.3 ± 1.20	8.1 ± 1.45	7.3 ± 1.56
Lime (CaCO ₃)	10.1 ± 0.60	9.8 ± 1.50	11.7 ± 1.59	7.9 ± 1.65	7.7 ± 1.53
Microfiltration	8.3 ± 1.61	8.1 ± 1.45	7.8 ± 1.65	7.6 ± 1.47	8.3 ± 0.53
Clay pot	7.2 ± 1.35	6.9 ± 1.47	7.5 ± 1.30	7.4 ± 1.59	7.2 ± 1.35
Boiling/hot water	7.9 ± 1.65	6.6 ± 1.12	7.3 ± 1.56	7.1 ± 1.46	7.6 ± 1.48

A = Surface water of the canal, B = Drinking water of public places, C = Drinking water of educational institutes, D = Drinking water of hospital and E = Drinking water of water supply system.

conidia. According to Ref. [48] that, in drinking water treatment plants, fungal spores are more resistant to chlorine disinfection than bacteria and viruses, which can regrow in drinking water distribution systems and subsequently pose health threats to water consumers.

The results of [49] point to the garbage disposal facility (GDF)/contamination decreasing the water quality of the stream and possibly being responsible for Lifetime cancer risk (LCR). However, the ecological environment and human health will be at risk as

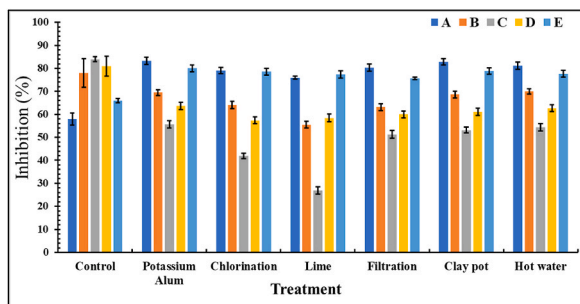


Fig. 4. Fungi inhibition % at different treatments of Physico-chemical control after 2 h of exposure (A = Surface water of canal, B = Drinking water of public places, C = Drinking water of educational institutes, D = Drinking water of hospitals and E = Drinking water of water supply system).

Table 4

F-ratio derived from ANOVA for occurrence (%) of different fungi isolated from collected samples of drinking water.

Samples	F-ratio	P-value	LSD _{0,05}
Surface water of canal	160.093	0.0000***	5.354
Drinking water of public places	309.007	0.0000***	4.104
Drinking water of educational institutes	440.112	0.0000***	3.117
Drinking water of hospitals	469.535	0.0000***	3.194
Drinking water of water supply system	155.206	0.0000***	5.667

Table 5

F-ratio derived from ANOVA for Infection % of different fungi after different treatments of Physico-chemical control after 72 h of exposure.

Treatments	F-ratio	P-value	LSD _{0,05}
Control	27.044	0.0000***	6.659
Potassium Alum	0.445	0.7736ns	2.747
Chlorination	0.908	0.4952ns	2.639
Lime (CaCO ₃)	4.057	0.0330*	2.59
Microfiltration	0.147	0.9600ns	2.55
Clay pot	0.075	0.9881ns	2.58
Boiling/Hot water	0.34	0.8448ns	2.658

long as the activity of the GDF lasts. Environmental monitoring to assess the effect of the GDF on the ecological system should be maintained reported by Ref. [49].

The previous study on the bacterial contamination of drinking water of Karachi was conducted by Refs. [50,51] and fungal mycotoxicity has not been reported in previous studies [50–52]. The study recommended that immediate actions be taken to ensure the supply of potable drinking water to the city residents of Karachi. The study did not examine the physical and chemical quality of drinking water, and they recommended that further studies are needed to assess both the physico-chemical and microbiological quality of the city’s drinking water supply and their impact on human health [50,51]. These study correlates with our present study. However, a large number of previous studies [6,9,28,32–36,43,46–52] closely related and confirm our present findings.

5. Conclusions

- > The occurrence of fungi genera in drinking water is not acceptable for people of the urban population. Drinking water contamination has been reported over the entire world.
- > The water quality is uncertain due to the occurrence of fungi and other microorganisms. In the present study, a total of twenty-five water samples were collected, and 17 different pathogenic filamentous fungi were isolated from these water samples.
- > However, the bacteria, microalgae, and some other microorganisms were present in low concentrations. The reason for fungi infection and production of mycotoxicity depends upon various factors including thermophilic and mesophilic conditions and the availability of nutrients that infiltrate plants.
- > The genus *Aspergillus* was dominated in all samples of drinking water collected from different sites. The major threat to human health is fungal mycotoxicity which is responsible for carcinogenic, Aspergillosis in the liver, allergic, epidemic diseases and some other serious lethal diseases.
- > Mostly, for the control of fungi, different physicochemical control treatments were used, but Potassium alum, clay pot, and hot water treatment disinfected effectively removing the fungi and its spore or mycelia from the water.

- > In addition, it is concluded that drinking pipeline water purifications such as chlorination, filtration and lime did not properly eliminate the fungal spores or mycelia from the water.

6. Patents

There is no patent resulting from the present work of this manuscript.

Funding

No funding has been received from any organization.

Data availability statement

It is certified that the raw data will be delivered on-demand. However, all generated data and analysis of parameters during this study are included in this article.

CRedit authorship contribution statement

Faisal Hussain: Supervision, Conceptualization. **Iram-us Salam:** Data curation, Conceptualization. **Farzana:** Investigation, Formal analysis. **Zaibun-nisa Memon:** Methodology, Formal analysis. **Muhammad Abdullah:** Resources, Project administration, Methodology. **Ghulam Abbas:** Formal analysis. **Muhammad Akbar:** Software, Resources, Data curation. **Alamdar Hussain:** Writing – original draft, Conceptualization. **Muhammad Majeed:** Writing – original draft, Validation. **Kishwar Ali:** Visualization, Resources, Methodology. **Haruna Musa Moda:** Supervision, Software, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Open Access funding provided by Qatar National Library.

References

- [1] J. Jachimová, J. Votruba, N.I. Vide, J.R. Anka, Identification of *Streptomyces* odour spectrum, *Folia Microbiol.* 47 (2002) 37.
- [2] E. Lanciotti, C. Santini, E.L. Pi, D. Burrini, Actinomycetes, cyanobacteria and algae causing tastes and odours in water of the River Arno used for the water supply of Florence, *J. Water Supply Res. Technol. - Aqua* 52 (2003) 489.
- [3] G. Izaguirre, R.W.D. Taylo, A guide to geosmin and MIB-producing cyanobacteria in the United States, *Water Sci. Technol.* 49 (2004) 19.
- [4] C. Klausen, N.O.G. Jorgensen, M. Burford, M. O'Dohue, Actinomycetes may also produce taste and odour, *Water* 31 (2004) 45–49.
- [5] B. Zaitlin, S.B. Watson, Actinomycetes in relation to taste and odour in drinking water: Myths, tenets and truths, *Water Res.* 40 (2006) 1741.
- [6] A. Grabińska-Loniewska, T. Konitłowicz-Kowalska, G. Wardzyński, K. Boryn, Occurrence of fungi in water distribution system, *Pol. J. Environ. Stud.* 16 (2007) 539–547.
- [7] M.K. Daud, M. Nafees, S. Ali, M. Rizwan, R.A. Bajwa, M.B. Shakoor, M.U. Arshad, S.A.S. Chatha, F. Deeba, W. Murad, I. Malook, S.J. Zhu, Drinking water quality status and contamination in Pakistan, *BioMed Res. Int.* (2017) 18, <https://doi.org/10.1155/2017/7908183>. Article ID 7908183.
- [8] E.E. Geldrich, *Microbial Quality of Water Supply in Distribution Systems*, Lewis Publishers, New York, 1995.
- [9] M.S. Doggett, Characterization of fungal biofilms within a municipal water distribution system, *Appl. Environ. Microbiol.* 66 (2000) 1249–1251.
- [10] B. Kendrick, *The Fifth Kingdom*, third ed., Focus Publishing, R. Paulins Company, New Bury Port, Massachusetts, USA, 2000.
- [11] E. Göttlich, W. Lubbe, B. Lange, S. Fiedler, I. Melchert, M. Reifenrath, H.C. Flemming, S. Hoog, Fungal flora in ground water-derived public drinking water, *Int. J. Hyg. Environ. Health* 205 (2002) 269–279.
- [12] H.B. Apcioglu, Y. YeGenoglu, Z. Erturan, Y. Nakipoglu, H. Issever, Heterotrophic bacteria and filamentous fungi isolated from a hospital water distribution system, *Indoor Built Environ.* 14 (2005) 483.
- [13] G. Kinsey, R. Paterson, J. Kelley, Filamentous fungi in water systems, in: D. Mara, N. Horan (Eds.), *Handbook of Water and Wastewater Microbiology*, Academic Press, London, UK, 2003.
- [14] J. Kelley, G. Kinsey, R. Paterson, D. Brayford, *Identification and Control of Fungi in Distribution Systems*, Awwa Research Foundation and American Water Works Association, Denver, CO, 2003.
- [15] L.A. Nagy, B.H. Olson, Occurrence and significance of bacteria, fungi and yeasts associated with distribution pipe surfaces, in: *Proceedings of the American Water Works Association, Water Quality Technical Conference*, American Water Works Association, Denver, CO, 1985, pp. 213–238.
- [16] J. Kelley, R. Paterson, G. Kinsey, R. Pitchers, H. Rossmoore, Identification, significance and control of fungi in water distribution systems, in: *Water Technology Conference Proceedings*. Public American Water Works Association, US, Denver, CO, 1997. November 9–12, 1997.
- [17] M.A. Tahir, M.A. Bhatti, A. Majeed, Survey of drinking water quality in the rural areas of rawalpindi district, *Pakistan Council for Research in Water Resources, Islamabad* (1994) 35–39.
- [18] F. Usman, M. Abid, F. Hussain, Soil-borne fungi associated with different vegetable crops in Sindh, Pakistan, *Pakistan Journal of Scientific and Industrial Research Series B: Biol. Sci.* 57 (2014) 140–147.
- [19] F. Hussain, *A Manual of Collection, Isolation Techniques and Identification of Fungi*, Scholar's Press, Germany, 2015.
- [20] D. Mara, N. Horan, *The Handbook of Water and Wastewater Microbiology*, first ed., Elsevier Academic Press, London, 2006.
- [21] I. Promputtha, R. Jeewon, S. Lumyong, E.H.C. McKenzie, K.D. Hyde, Ribosomal DNA fingerprinting in the identification of non-sporulating endophytes from *Magnolia liliifera* (Magnoliaceae), *Fungal Divers.* 20 (2005) 167–186.
- [22] K.H. Domsch, W. Gams, T.H. Anderson, *Compendium of Soil Fungi*, Academic Press, New York, 1980, p. 859.
- [23] K.H. Domsch, W. Gams, T.H. Anderson, *Compendium of Soil Fungi*, second ed., IHW-Verlag and Verlagsbuchhandlung, Eching, Germany, 2007.

- [24] J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, third ed., Springer Science plus Business Media, LLC, 233, Spring Street, New York, NY 10013, USA, 2009, p. 524.
- [25] R.A. Samson, C.M. Visagie, J. Houbraken, S.B. Hong, V. Hubka, C.H. Klaassen, G. Perrone, K.A. Seifert, A. Susca, J.B. Tanney, J. Varga, S. Kocsube, G. Szigeti, T. Yaguchi, J.C. Frisvad, Phylogeny, identification and nomenclature of the genus *Aspergillus*, *Stud. Mycol.* 78 (2014) 141–173.
- [26] K.N. Oo, K.S. Aung, M. Thida, W.W. Knine, M.M. Soe, T. Aye, Effectiveness of Potash Alum in decontaminating household water, *J. Diarrhoeal Dis. Res.* 11 (1993) 172–174.
- [27] Environmental Protection Agency (EPA), in: *Basic Information about Disinfectants in Drinking Water: Chloramine, Chlorine and Chlorine Dioxide*. USA, 2013. <https://www.epa.gov/dwstandardsregulations>.
- [28] R. Sudoh, M.S. Islam, K. Sazawa, T. Okazaki, N. Hata, S. Taguchi, H. Kuramitz, Removal of dissolved humic acid from water by coagulation method using polyaluminum chloride (PAC) with calcium carbonate as neutralizer and coagulant aid, *J. Environ. Chem. Eng.* 3 (2015) 770–774.
- [29] Central Public Health and Environmental Engineering Organization (CPHEEO), *Manual on Water Supply and Treatment*, Ministry of Urban Development and Poverty Alleviation, New Delhi, India, 1999, p. 777.
- [30] A.G. Fane, C.Y. Tang, V. Wang, *Membrane Technology for water: microfiltration, ultrafiltration, nanofiltration and reverse osmosis*, *Treatise on Water Science*, Chapter 4 (11) (2011) 301–335.
- [31] K.A. Gomez, A.A. Gomez, *Statistical Procedures for Agricultural Research*, second ed., Wiley, New York, 1984, p. 680.
- [32] G. Hageskal, P. Gaustad, B.T. Heier, I. Skaar, Occurrence of moulds in drinking water, *J. Appl. Microbiol.* 102 (2007) 774–780, <https://doi.org/10.1111/j.1365-2672.2006.03119>.
- [33] G. Hageskal, N. Lima, I. Skaar, The study of fungi in drinking water, *Mycol. Res.* 113 (2009) 165–172, <https://doi.org/10.1016/j.mycres.2008.10.002>.
- [34] T. Hussain, C.M. Ishtiaq, A. Hussain, T. Mahmood, K. Sultana, M. Ashraf, Incidence of fungi in water springs of samahni valley, district bhimber, azad kashmir, Pakistan, *Int. J. Biol.* 2 (2010) 94–101.
- [35] H.M. Oliveira, C. Santos, R.R. Paterson, N.B. Gusmão, N. Lima, Fungi from a groundwater-fed drinking water supply system in Brazil, *Int. J. Environ. Res. Publ. Health* 13 (2016) 304, <https://doi.org/10.3390/ijerph13030304>.
- [36] R.M. Niemi, S. Knuth, K. Lundstrom, Actinomycetes and fungi in surface waters and in potable water, *Appl. Environ. Microbiol.* 43 (1982) 378–388.
- [37] H. Hapcioglu, Y. Yegenoglu, Z. Erturan, Y. Nakipoglu, H. Issever, Heterotrophic bacteria and filamentous fungi isolated from a hospital water distribution system, *Indoor Built Environ.* 14 (2005) 483.
- [38] L.A. Nagy, B.H. Olson, The occurrence of filamentous fungi in drinking water distribution systems, *Can. J. Microbiol.* 28 (1982) 667–671.
- [39] W.D. Rosenzweig, W.O. Pipes, Fungi from potable water: Interaction with chlorine and engineering effects, *Water Sci. Technol.* 20 (1982) 153.
- [40] Z. Hubalek, Fungi associated with free-living birds in Czechoslovakia and Yugoslavia, *Acta Scientiarum Naturalium Academiae Scientiarum Bohemicae – Brno* 8 (1974) 1.
- [41] Z. Hubalek, Pathogenic microorganisms associated with free – living birds, *Acta Scientiarum Naturalium Academiae Scientiarum Bohemicae – Brno* 28 NS (1994) 1.
- [42] J. Pinowski, B. Pinowska, A. Haman, Fungi in bird's plumage and nests, *Int. Stud. Sparrows* 26 (1999) 3.
- [43] M. Arvanitidou, K. Kanellou, T.C. Constantinides, V. Katsouyannopoulos, The occurrence of fungi in hospital and community potable waters, *Lett. Appl. Microbiol.* 29 (1999) 81.
- [44] K. Lahti, Microbial quality of drinking water in some finish distribution systems, *Water Sci. Technol.* 27 (1993) 151.
- [45] A. Warris, A. Voss, T.G. Abrahamsen, P.E. Verweij, Contamination of hospital water with *Aspergillus fumigatus* and other molds, *Clin. Infect. Dis.* 34 (2002) 1159–1160.
- [46] W.D. Rosenzweig, H. Minnigh, W.O. Pipes, Fungi in potable water distribution systems, *J. AWWA (Am. Water Works Assoc.)* 78 (1986) 53.
- [47] Department for Environment Food and Rural Affairs (DEFRA), in: *Review of Fungi in Drinking Water and the Implications for Human Health*. U.K, 2011, p. 107. <http://dwi.defra.gov.uk/research/completed-research/reports/dwi70-2-255.pdf>.
- [48] H.X. Zhao, T.Y. Zhang, H. Wang, C.Y. Hu, Y.L. Tang, B. Xu, Occurrence of fungal spores in drinking water: A review of pathogenicity, odor, chlorine resistance and control strategies, *Sci. Total Environ.* 853 (2022) 158626.
- [49] B. Yuksel, F. Ustaoglu, E. Arica, Impacts of a garbage disposal facility on the water quality of Çavuşlu stream in Giresun, Turkey: A health risk assessment study by a validated ICP-MS assay, *Aquat. Sci. Eng* 36 (2021) 181–192.
- [50] R. Amin, M.B. Zaidi, S. Bashir, R. Khanani, R. Nawaz, S. Ali, S. Khan, Microbial contamination levels in the drinking water and associated health risks in Karachi, Pakistan, *J. Water, Sanit. Hyg. Dev.* 9 (2019) 319–328, <https://doi.org/10.2166/washdev.2019.147>.
- [51] N. Afshan, M. Kazmi, S.A. Khan, N. Jafri, Bacteriological survey of drinking water in Karachi, in: *Proceedings of 2014 11th International Bhurban Conference on Applied Sciences & Technology (IBCAST) Islamabad, Pakistan, 14th - 18th January 2014, Islamabad, Pakistan, 2014*, pp. 92–94, <https://doi.org/10.1109/IBCAST.2014.6778129>.
- [52] F.A. Yousuf, R. Siddiqui, F. Subhani, N.A. Khan, Status of free-living amoebae (*Acanthamoeba* spp., *Naegleria fowleri*, *Balamuthia mandrillaris*) in drinking water supplies in Karachi, Pakistan, *J. Water Health* 11 (2013) 371–375, <https://doi.org/10.2166/wh.2013.112>.