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Research Article

Fungal contamination in residential water systems: A comparative study between hot and cold water samples

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Abstract: Some fungal species are known to have adverse health effects for humans and their presence in water systems may lead to alterations in the taste and odour of the water they occupy. Although a few country-based regulations are known, no universal legal restriction on the presence of fungi in drinking or utility water is present currently. Waterborne fungi have been a neglected part of microbial studies worldwide, and more sudies are needed in the current era of global warming. This study was performed to evaluate (i) the fungal load in randomly selected residential water systems connected to the municipal water supply in Istanbul, Türkiye, and (ii) the possible impact of water temperature on the number and biodiversity of fungi. Additionally, the relationship between bacterial loads, some water parameters and the determined fungi were investigated. Cold and hot water samples were taken from 20 randomly selected buildings in Istanbul and inoculated into SDA using the membrane filtration method for fungal isolation, and onto R2A and *Candida* Agar using the spread plate method for bacterial and *Candida* isolation, respectively. More microorganisms were detected in cold water samples than in hot water. The mean fungal and bacterial numbers in cold and hot water samples were 2.4, 1.47, 702.3 and 79.5 cfu/100 mL, respectively. No *Candida* was found. It was determined that temperature affected the biodiversity and frequency of fungi. *Penicillium* (41%) and *Aspergillus* (43.75%) were the dominant fungal genera in cold and hot water, respectively. *Aspergillus versicolor* was the most common fungal species found in both water samples. 9 of fungi were identified that are known to have the potential to cause allergies and/or opportunistic infections. No relationship was detected between fungal growth and pH and chlorine.

Özet: Bazı mantar türlerinin insanlar için olumsuz sağlık etkileri olduğu bilinmektedir ve su sistemlerinde bulunmaları, bulundukları suyun tadında ve kokusunda değişikliklere yol açabilir. Birkaç ülke bazlı yasal düzenleme bilinmesine rağmen, şu anda içme veya kullanma suyunda mantar varlığına ilişkin evrensel bir yasal kısıtlama bulunmamaktadır. Su kaynaklı mantarlar, dünya çapında mikrobiyal çalışmaların ihmal edilmiş bir parçasıdır ve küresel ısınmanın yaşandığı günümüzde daha fazla çalışmaya ihtiyaç duyulmaktadır. Bu çalışma, (i) İstanbul, Türkiye'deki belediye su şebekesine bağlı rastgele seçilmiş konut su sistemlerindeki mantar yükünü ve (ii) su sıcaklığının mantar sayısı ve biyolojik çeşitliliği üzerindeki olası etkisini değerlendirmek için gerçekleştirilmiştir. Ek olarak, bakteri yükleri, bazı su parametreleri ve belirlenen mantarlar arasındaki ilişki araştırılmıştır. İstanbul'da rastgele seçilmiş 20 binadan soğuk ve sıcak su örnekleri alınmış ve mantar izolasyonu için membran filtrasyon yöntemi kullanılarak SDA'ya, bakteri ve *Candida* izolasyonu için sırasıyla yayılmış plaka yöntemi kullanılarak R2A ve *Candida* Agar'a ekimleri yapılmıştır. Soğuk su örneklerinde sıcak sudan daha fazla mikroorganizma tespit edilmiştir. Soğuk ve sıcak su örneklerindeki ortalama mantar ve bakteri sayıları sırasıyla 2,4, 1,47, 702,3 ve 79,5 cfu/100 mL olarak bulundu. *Candida* bulunamadı. Sıcaklığın mantarların biyoçeşitliliğini ve sıklığını etkilediği belirlendi. Soğuk ve sıcak suda sırasıyla baskın mantar cinsleri *Penicillium* (%41) ve *Aspergillus* (%43,75) idi. Her iki su örneğinde de en sık bulunan mantar türü *Aspergillus versicolor* idi. Alerji ve/veya fırsatçı enfeksiyonlara neden olma potansiyeli olduğu bilinen 9 mantar türü tanımlanmıştır. Mantar üremesi ile pH ve klor arasında bir ilişki saptanmamıştır.

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Introduction

Mains water, also known as tap or municipal water, is supplied to homes, hospitals, and schools, through a network of pipes and treatment plants maintained by city managements. It is a prerequisite to treat and process this type of water supply to make it biologically safe for various usage purposes including drinking, cooking, bathing and

cleaning (Provincial Health Directorate of Istanbul, 2022). Studies addressing the microbial load of man-made water systems showed the presence of various bacteria and fungi of health concern in high numbers in these systems connected to the mains water supply (Hageskal *et al.* 2006, Kadaifçiler & Çotuk 2014). It is therefore crucial to monitor the mains water to prevent microbial contamination, particularly for presence of agents with the potential to have adverse effects on human health. The direct or indirect use of contaminated water in various ways, and inhalation of aerosols causes serious health problems and may even result in death over time (Babič *et al.* 2017). For this reason, it is essential to determine presence of a contamination in the mains water and microorganism responsible for the contamination. Although studies conducted on water systems have mainly focused on bacterial contamination (Zacheus & Martikainen 1995), the importance of fungal biocontamination has also been reported (Anaissie *et al.* 2002).

Fungi naturally live in soil and air environments, but they can also grow in aquatic systems. Some fungal species have developed an adaptation to grow in man-made water systems. In these systems, fungi can either be found in planktonic status in the water body, or they can be sessile in the biofilm layer. After increasing their colony number in the biofilm, they can break off from the biofilm layer and mix with the water (Kadaifçiler & Çotuk 2014, Babič *et al.* 2017). The initial contamination problems caused by fungi in mains water were identified in the 1960s and 1970s, and many fungal genera, mainly *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium*, *Arthroderma*, *Candida*, *Cladosporium*, *Chaetomium* and *Phialophora* have been detected in water systems (Hapçıoğlu *et al.* 2005, Hageskal *et al.* 2006, Kanzler *et al.* 2007, Kadaifçiler & Çotuk 2014, Babič *et al.* 2017, Kadaifçiler & Demirel 2018). The water contaminated with fungi can lead to adverse human health and environmental effects (Burman 1965, Bays *et al.* 1970). Fungi in water systems can cause various diseases, including invasive infections (i.e. aspergillosis and endocarditis) as well as superficial diseases (i.e. keratitis and otomycosis). Besides their potential health risks, some microfungi such as *Chaetomium globosum* can cause taste and odor problems in water (Alangaden 2011).

Studies on water supply systems have mainly focused on cold water systems (Hapçıoğlu *et al.* 2005, Hageskal *et al.* 2006, Kanzler *et al.* 2007, Kadaifçiler & Çotuk 2014, Kadaifçiler & Demirel 2018). There are only a limited number of studies that have investigated fungal loads in both cold and hot water systems simultaneously (Anaissie *et al.* 2002, Figueiredo Fonseca *et al.* 2010, Hayette *et al.* 2010). It is worth to mention that the water safety is traditionally monitored mainly by bacterial parameters that indicate faecal contamination. Therefore, the number of studies investigating fungal contamination is comparatively lower than that of bacterial contamination (Ma *et al.* 2015).

Temperature is an important parameter that affects the presence, diversity and distribution of microorganisms in water environments. With the climate changes affecting all ecosystems due to global warming, studies on fungi in freshwater have also accelerated. Global warming is expected to significantly affect nutrient and carbon cycles in ecosystems by changing the diversity, structure and activities of fungal communities (Hyde *et al.* 2016). Studies on fungal metabolism to temperature change often reach contradictory and location-specific results (Canhoto *et al.* 2016). Preciado *et al.* (2021) observed that temperature caused more dramatic changes in the fungal community in biofilm compared to tap water, such as loss of fungal diversity. However, certain fungal genera have been reported to become dominant and the number of opportunistic pathogenic bacteria increased with high temperatures. It has been concluded that the increased abundance of these microorganisms in water due to high temperatures is an undesirable situation as it may lead to health problems (Zacheus & Martikainen 1995).

The aim of this study is to investigate (i) the presence and concentration of fungi in residential water systems linked to a municipal water supply and (ii) the effect of water temperature variation on the quantity and biodiversity of fungi. In addition, the relationship between the presence of aerobic bacteria, water parameters, buildings' properties and the presence of fungi were also investigated. The findings of this research could be contribute to our understanding of the microbial composition and potential risks associated with water quality in residential water systems.

Materials and Methods

Water Sampling

A total of 40 water samples, 20 hot water and 20 cold water, were collected from 20 randomly selected residences in Istanbul, Türkiye. Samples were collected in 1 L sterile polypropylene bottles and were transferred to the laboratory in an insulated bag. Microbiological analyses were performed on the same day. The temperature, pH and free chlorine values of the samples were measured during samplings. The residences were multi-storey apartment buildings that are independent of each other, and only one flat in each apartment was sampled. 7 residences were on the Anatolian side and 13 residences are on the European side of the city. Building information including the age of the residences, districts, pipe material, presence of water tank were also noted.

The samples were taken from 15 different districts with the highest population density in Istanbul. There was no green area around the residences, and all of these residences were multi-story apartment buildings. The mean age of 20 randomly selected houses in Istanbul is 24.6 years. In 85% of the residences, polyvinyl chloride (PVC) was used as the pipe (surface) material, while in the remaining residences, galvanized steel (GS) was used. Only 15% of the buildings had water tanks and it was reported that there was no regular disinfection for them (Table 1).

Table 1. The general information of 20 residences.

Building Code	District	Building Age (Year)	Pipe Material	Presence of Water Tank
A	Kartal	15	GS	Yes
B	Esenler	22	PVC	No
C	Beylikdüzü	9	PVC	Yes
D	Zeytinburnu	22	PVC	No
E	Eyüpsultan	10	PVC	No
F	Bahçelievler	22	PVC	No
G	Eyüpsultan	7	PVC	No
H	Şişli	55	GS	No
I	Bahçelievler	20	PVC	No
J	Küçükçekmece	14	PVC	No
K	Avcılar	30	PVC	No
L	Ümraniye	20	PVC	No
M	Kartal	20	PVC	Yes
N	Maltepe	33	PVC	No
O	Şişli	20	PVC	No
P	Pendik	29	PVC	No
R	Kadıköy	45	GS	No
S	Fatih	50	PVC	No
T	Tuzla	27	PVC	No
U	Zeytinburnu	22	PVC	No

Isolation and Counting of Microorganisms

The membrane filtration method was performed for isolation and enumeration of fungi in the samples. Dilution series up to 10^{-3} were prepared for each sample. 100 mL of water samples were taken directly from the sampling bottles and dilution series were filtered through nitrocellulose membrane filters (47 mm diameter, 0.45 µm pore size, Millipore, UK) using membrane filtration device (Millipore, UK) in triplicates. Membrane filters were placed onto petri dishes containing Sabouraud Dextrose Agar (SDA) medium (Merck, Germany) supplemented with 500 mg streptomycine (Sammon *et al.* 2011). The petri dishes of cold and hot water were incubated at 25 ± 2°C for 14 days and at 37°C for 30 days, respectively (Hapçıoğlu *et al.* 2005, Figueiredo Fonseca *et al.* 2010). At the end of the incubation period, the fungal counts in the water samples were determined and expressed as colony-forming unit per 100 ml of the water (cfu/100 mL). Fungal isolates with different macromorphological characteristics were determined to be pure and transferred to SDA media and after 7 days of incubation cultures were stored at +4°C. In addition, cryotubes containing pure fungal spores were frozen at -86°C for the long-term storage of fungal isolates.

For *Candida* yeast isolation and enumeration steps, concentrated samples were prepared. For this purpose, water samples (500 mL) were filtered separately through nylon membrane filters (47 mm diameter, 0.22 µm pore size) using the membrane filtration device (Millipore, UK). The concentrated samples were obtained after shaking the filters with 10 mL of sterile tap water for 1 min in the stomacher device (IUL Instruments S.A.). Dilution series up to 10^{-3} were prepared for each sample. 1 mL of original and diluted water samples were spread plated in triplicate onto *Candida* Agar plates. Petri dishes used for cold and hot water samples were incubated at 30°C and 37°C, respectively, for 2 days. The growth in petri dishes was controled at the end of the incubation period. Incubation was extended to 30 days in case of no growth was observed (Hi-Media 2003, Kadaifçiler & Çotuk 2014).

Bacterial plating was performed using R2A agar medium to determine the number of aerobic bacterial counts. Petri dishes of cold and hot water samples were incubated at 28°C and 37°C, respectively, for 7-14 days and then the counts were obtained (Leginowicz *et al.* 2018).

Fungal Identification

Pure fungal isolates were first identified at genus level. Fresh fungal cultures were inoculated into Malt Extract Agar (MEA) medium and incubated at 25 ± 2°C for 14 days. Macroscopic properties of the colonies were examined under a stereo microscope and microscopic properties were examined using a light microscope (Barnett & Hunter 1999).

For species-level identification of isolates of the genus *Aspergillus*, fresh cultures were inoculated into petri dishes containing Czapek Dox Agar (CDA), Czapek Yeast Extract Agar with 20% Sucrose (CY20S), Czapek Yeast Extract Agar (CYA) and MEA media. All petri dishes were incubated at 25°C for 7 days. Additional petri dishes containing CYA medium were incubated at 37°C for 7 days (Klich 2002, Sammon *et al.* 2010). At the end of the incubation period, colony properties (colony diameter, colony texture, colony/ colony reverse color, pigmentation, exudates, sclerotia) of the isolates were first determined. Then, preparates were prepared with lactophenol cotton blue dye from fungal cultures. Micromorphological properties (spore size, spore color, vesicle shape, stipe size, Hülle cell, ascospore, cleistothecia) were examined under light microscope. Identification of the isolates was carried out by comparing the defined characteristics of the fungi with the accepted identification key (Klich 2002).

In order to identify isolates of the genus *Penicillium* at species level, fresh cultures were inoculated into petri dishes containing CYA, MEA, 25% Glycerol Nitrate Agar (G25N), Yeast Extract Sucrose Agar (YES) and Creatine Sucrose Agar (CREA) media. Petri dishes were incubated at 25°C 7 days. Additional petri dishes containing CYA medium were incubated at 5°C and 37°C for 7 days (Pitt 1979, Sammon *et al.* 2010). At the end of the incubation period, macromorphological characteristics of the isolates were observed. Then, fungal preparates stained with lactophenol cotton blue dye were examined under light microscope and

micromorphological characteristics (conidiophore branching patterns, metulae length, phialide shape, cleistothecium, spore size, spore color) were determined. Identification of the isolates was carried out by comparing the defined characteristics of the fungi with the universally accepted standards (Pitt 1979).

For species-level identification of fungal isolates including Dematiaceous Hyphomycetes, fresh cultures were inoculted into Potato Dextrose Agar (PDA) plates. Petri dishes were incubated at 25°C for 14 days. The macro- and micromorphological characteristics were examined, and identifcations were performed according to Ellis (1971).

Current names of fungi identified at genus or/and species level, as well as fungal authors, were standardized according to the "Index Fungorum Partnership" website (Hawksworth *et al.* 2011). The phyla of the identified fungi were updated according to the Mycobank website (http://www.mycobank.org/quicksearch.aspx).

Statistical Analysis

Statistical data were obtained using IBM SPSS Statistics software (version 29.0.0.0). The normal distribution of the arithmetic means was determined by the normality test and the confidence interval was accepted as $p < 0.05$. Since the number of subjects in both hot and cold water samples was below 30, the normality test was evaluated according to the Shapiro-Wilk method. It was determined that it did not show a normal distribution. Therefore, Non-parametric Mann-Whitney U test (among independent groups) was used to evaluate the difference in the mean numbers of fungi, aerobic bacteria in cold and hot water sample. Spearman correlation coefficients test was used to evaluate the relationship between fungi numbers in water samples, bacterial counts, water temperature, pH, free chlorine, age of residences, type of water systems and water tank. Significance level was accepted as $p < 0.05$.

Results

Water Parameters

It has been determined that the temperatures of the cold water samples randomly collected from 20 residences varied between 20°C and 25°C (the mean value was 21°C). The temperatures of the hot water samples were in the range of 40°C and 55°C (the mean value was 46.7°C). The mean pH values of the cold and hot water samples were 6 and 7, respectively. Free chlorine values in cold water samples were determined to be in the range of 0.1-1 ppm (the mean value was 0.73)**,** and free chlorine values in the hot water samples were determined to be in the range of 0.3-1 ppm (the mean value was 0.74).

Microbial Enumeration

Fungal growth was observed in 85% of cold and 45% of hot water samples (Table 2). The mean fungal counts in cold and hot water samples were 2.4 cfu/100 mL and 1.47 cfu/100 mL, respectively. The highest fungal counts in cold and hot water samples were detected in residences A and B, respectively.

Table 2. The mean microbiological counts in water samples of 20 residences.

Building Code	Fungi		Bacteria	
	Cold Water (cfu/100 mL)	Hot Water (cfu/100 mL)	Cold Water (cfu/100 mL)	Hot Water (cfu/100 mL)
A	7.5 ± 6.36	2 ± 0	3340 ± 0.56	260 ± 1.13
B	5 ± 1.41	9 ± 2	0 ± 0	0 ± 0
C	2 ± 1.41	1.5 ± 0.71	420 ± 1.97	0 ± 0
D	2 ± 0	6 ± 0	0 ± 0	0 ± 0
E	6.5 ± 0.71	0 ± 0	106.6 ± 0.56	0 ± 0
F	2 ± 0	0 ± 0	0 ± 0	600 ± 0.56
G	1.5 ± 0.71	1 ± 0	40 ± 0	150 ± 0.42
H	1 ± 0	0 ± 0	330 ± 1.27	0 ± 0
I	0 ± 0	1 ± 0	0 ± 0	0 ± 0
J	1 ± 0	6 ± 1.41	60 ± 0.28	60 ± 0.28
K	2.66 ± 0.58	0 ± 0	70 ± 0.14	50 ± 0.4
L	6 ± 0	0 ± 0	150 ± 0.89	0 ± 0
M	3.5 ± 2.12	2 ± 0	7920 ± 1.13	0 ± 0
N	0 ± 0	0 ± 0	140 ± 0.28	0 ± 0
O	1 ± 0	0 ± 0	0 ± 0	0 ± 0
P	3.5 ± 0.71	1 ± 0	390 ± 0.98	0 ± 0
R	1 ± 0	0 ± 0	0 ± 0	0 ± 0
S	0 ±0	0 ± 0	0 ± 0	0 ± 0
T	1 ± 0	0 ± 0	640 ± 11.3	240 ± 0.7
U	1 ± 0	0 ± 0	440 ± 0.28	230 ± 0.7

Since the number of subjects in both cold and hot water samples was 20, the normality test was run according to the Shapiro-Wilk method ($W = 0.803$, $p = 0.001$ for cold water), ($W = 0.645$, $p = 0.001$ for hot water), and no normal distribution was determined. According to Mann-Whitney U test, a difference was found between the mean numbers of fungi in cold and hot water samples ($U = 120.000$, $p = 0.26$). Fungal counts were found to be higher in cold water samples than in hot water ones ($p < 0.05$). No *Candida* growth was observed in any of the water samples.

Bacterial growth was observed in 65% of cold and 35% of hot water samples (Table 2). The mean bacterial counts in cold and hot water samples were 702.33 cfu/100 mL and 79.5 cfu/100 mL, respectively. The highest bacterial counts in cold and hot water samples were detected in residence M and F, respectively. Since the number of subjects in both cold and hot water samples was 20, the normality test was run according to the Shapiro-Wilk method (W = 0.415, p = 0.001 for cold water), (W = 0.603, p = 0.001 for hot water), and no normal distribution was found. According to Mann-Whitney U test, a difference was found between the mean numbers of bacteria in cold and hot water (U = 130.500, p = 0.44). Bacterial counts were found to be higher in cold water samples than in hot water samples ($p < 0.05$).

According to the Spearman correlation coefficients test, no significant correlation was detected between the fungal and bacterial numbers detected in cold (r = 0.313, p = 0.180) and hot (r = -0.50, p = 0.833) water samples (Tables 3, 4).

In addition, a positive correlation was detected between the number of fungi in cold water samples and the presence of tanks (r = 0.482, p = 0.032). A negative correlation was determined between the number of fungi in hot water samples and the buildings' age (r = -0.460, p = 0.041). No correlation was found between fungi and other parameters (pH, chlorine, pipe material) in both cold and hot water samples.

Fungal Isolates

A total of 60 fungal isolates were obtained, 44 from cold water and 16 from hot water samples (Fig. 1). The highest total isolate number in cold and hot water was in building B with 8. The highest numbers of fungal isolates (with 5 each) were determined in cold water samples of residences A, B, and K. The highest number of fungal isolates (3 each) in hot water samples were detected in residences B, C, and J.

Table 3. Results of Spearman correlation analysis on the correlation between fungi in cold water and other parameters.

Fungi in cold water		Bacterial counts	pH	Chlorine	Temperature	Water Tank	Building age	Pipe material
Spearman's rho	Correlation Coefficient	0.313	0.243	-0.334	-0.014	0.482*	-0.441	0.012
	Sig. (2-tailed)	0.180	0.302	0.151	0.955	0.032	0.052	0.959
	N	20	20	20	20	20	20	20

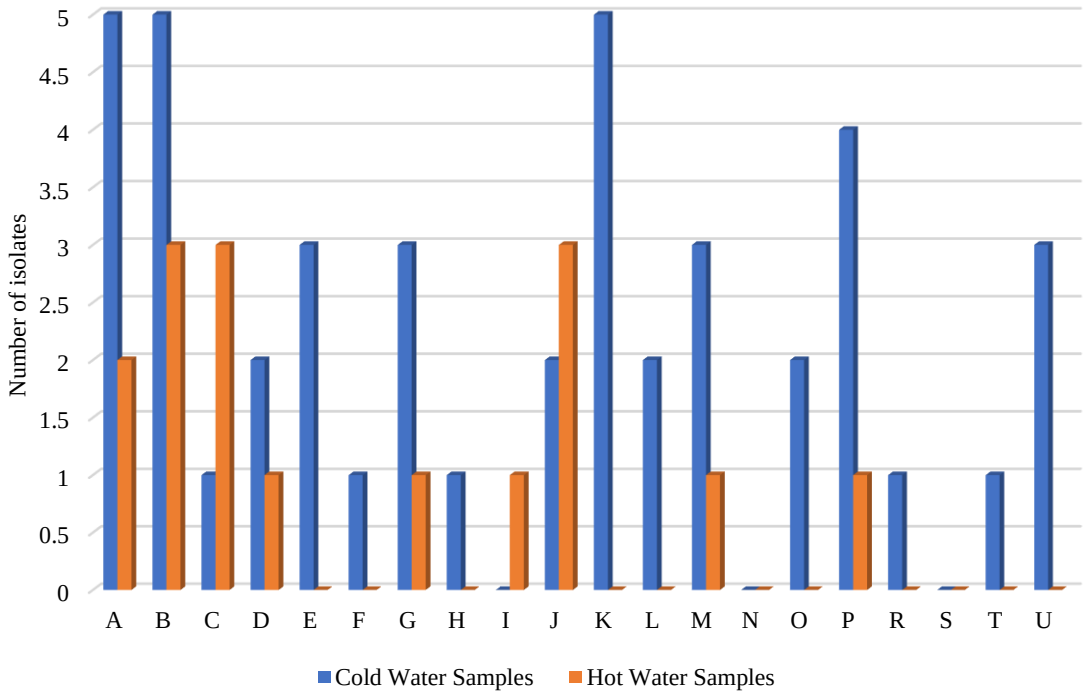

Fig. 1. Number of microfungal isolates in cold and hot waters sampled in the residences.

Table 4. Results of Spearman correlation analysis on the correlation between fungi in hot water and other parameters

Fungi in hot water		Bacterial counts	pH	Chlorine	Temperature	Water Tank	Building age	Pipe material
Spearman's rho	Correlation Coefficient	-0.50	0.114	-0.259	0.082	0.000	-0.460*	-0.080
	Sig. (2-tailed)	0.833	0.632	0.270	0.730	1.000	0.041	0.738
	N	20	20	20	20	20	20	20

Table 5. The identified microfungi in all water samples.

Building code	Isolate Code	Water	Genus/Species
A	F1	Cold	*Penicillium chrysogenum* Thom 1910
	F2	Cold	*Acremonium kiliense* Current: *Sarocladium kiliense* (Grütz) Summerb, 2011
	F3	Cold	*Scopulariopsis asperula* (Sacc.) S. Hughes 1958
	F4	Cold	*Penicillium aurantiogriseum* Dierckx 1901
	F5	Cold	Non-spore forming fungi
	F6	Hot	Non-spore forming fungi
	F7	Hot	Non-spore forming fungi
B	F8	Cold	*Penicillium chrysogenum* Thom 1910
	F9	Cold	*Chaetomium globosum* Kunze 1817
	F10	Cold	Yeast
	F11	Cold	*Penicillium citrinum* Thom 1910
	F12	Cold	*Eurotium amstelodami* Current: *Aspergillus amstelodami* (L. Mangin) Thom&Church 1926
	F13	Hot	*Penicillium citrinum* Thom 1910
	F14	Hot	*Penicillium raistrickii* G. Sm. 1933
	F15	Hot	*Aspergillus versicolor* (Vuill.) Tirab. 1908
C	F16	Cold	*Eurotium amstelodami* Current: *Aspergillus amstelodami* (L. Mangin) Thom&Church 1926
	F17	Hot	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F18	Hot	Non-spore forming fungi
	F19	Hot	Non-spore forming fungi
D	F20	Cold	*Penicillium expansum* Link 1809
	F21	Cold	Yeast
	F22	Hot	*Eurotium amstelodami* Current: *Aspergillus amstelodami* (L. Mangin) Thom&Church 1926
E	F23	Cold	*Penicillium brevicompactum* Dierckx 1901
	F24	Cold	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F25	Cold	*Arthroderma sp.* Curr. 1860
F	F26	Cold	*Penicillium brevicompactum* Dierckx 1901
G	F27	Cold	*Aspergillus flavus* Link 1809
	F28	Cold	*Chaetomium globosporum* Rikhy & Mukerji 1974
	F29	Cold	*Chrysosporium inops* J.W. Carmich. 1962
	F30	Hot	*Phialophora sp.* Medlar 1915
H	F31	Cold	*Penicillium olsonii* Bainier & Sartory 1912
I	F32	Hot	*Aspergillus niveus* Current: *Aspergillus neoniveus* Samson, S.W. Peterson, Frisvad & Varga 2011
J	F33	Cold	*Penicillium chrysogenum* Thom 1910
	F34	Cold	*Penicillium aurantiogriseum* Dierckx 1901
	F35	Hot	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F36	Hot	Non-spore forming fungi
	F37	Hot	Non-spore forming fungi
K	F38	Cold	*Penicillium expansum* Link 1809
	F39	Cold	*Chaetomium globosum* Kunze 1817
	F40	Cold	Non-spore forming fungi
	F41	Cold	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F42	Cold	Non-spore forming fungi
L	F43	Cold	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F44	Cold	*Penicillium expansum* Link 1809
M	F45	Cold	*Aspergillus versicolor* (Vuill.) Tirab. 1908
	F46	Cold	Non-spore forming fungi
	F47	Cold	*Penicillium chrysogenum* Thom 1910
	F48	Hot	*Aspergillus versicolor* (Vuill.) Tirab. 1908

Table 5. The identified microfungi in all water samples (Continued).

Building code	Isolate Code	Water	Genus/Species
O	F49	Cold	*Botrytis sp.* Botrytis P. Micheli ex Pers. 1794
	F50	Cold	*Penicillium brevicompactum* Dierckx 1901
P	F51	Cold	Non-spore forming fungi
	F52	Cold	*Arthroderma flavescens* R. G. Rees 1967
	F53	Cold	*Penicillium sp.* Link 1809
	F54	Cold	Non-spore forming fungi
	F55	Hot	*Aspergillus versicolor* (Vuill.) Tirab. 1908
R	F56	Cold	*Monocillium humicola* Current: *Penicillium lagena* (Delitsch) Stolk & Samson 1983
T	F57	Cold	*Penicillium restrictum* J.C. Gilman & E.V. Abbott 1927
U	F58	Cold	*Penicillium brevicompactum* Dierckx 1901
	F59	Cold	*Penicillium griseofulvum* Dierckx 1901
	F60	Cold	Non-spore forming fungi

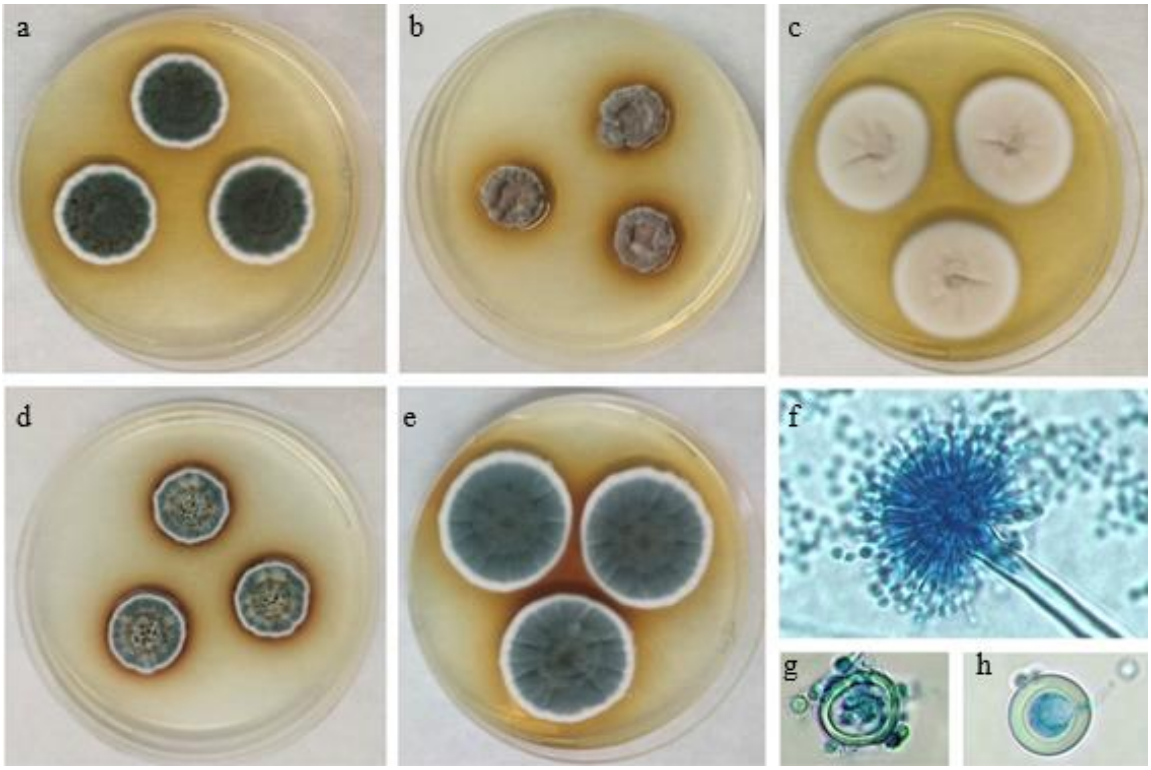

Fig. 2. *Aspergillus versicolor* **a-e.** Colony morphology after 7 days on CYA (25°C), CYA (37°C), MEA, CDA, and CY20S media, respectively, **f.** microscopic image of the conidial head at ×500 magnification, **g-h.** microscopic images of the hülle cells at ×1000 magnification.

Of the 60 fungal isolates obtained from the water samples, 96.6% were filamentous fungi and the others were yeasts. 21.6% of the fungal isolates were non-spore forming fungi. 45 filamentous fungal isolates belonged to 11 different genera (Table 5).

Penicillium was the most frequently isolated fungal genus in all water samples, constituting ~33.3%. *Penicillium* (41%) and *Aspergillus* (43.75%) were the most dominant fungal isolates in cold and hot water samples, respectively. *Aspergillus versicolor* was the most frequently isolated fungal species in all water samples (Fig. 2).

Among the cold water samples, the most frequently isolated fungal species were *Aspergillus versicolor* (9.1%), *Penicillium brevicompactum* (9.1%), and *Penicillium chrysogenum* (9.1%). However, in hot water samples, *Aspergillus versicolor* (31.25%) was the most frequently isolated fungal species.

Other fungi detected in water samples, according to their frequencies, were *Eurotium amstelodami* (current: *Aspergillus amstelodami*), *Chaetomium globosum*, *Penicillium aurantiogriseum*, *Penicillium citrinum*, *Acremonium kiliense* (current: *Sarocladium kiliense*), *Aspergillus flavus*, *Aspergillus niveus* (current: *Aspergillus neoniveus*), *Arthroderma* sp., *Arthroderma flavescens*, *Botrytis* sp., *Chaetomium globosporum*, *Chrysosporium inops*, *Monocillium humicola* (current: *Penicillium lagena*), *Penicillium* sp., *Phialophora* sp., *Scopulariopsis asperula*.

Discussion

Water is of paramount importance in human daily life. Tap water should be free from any microorganism or substance, both likely to constitute a potential danger to human health. There is no standardization regarding fungi in the mains water in Türkiye. The World Health Organisation (WHO) states that pathogenic or allergenic microorganisms should not be present in tap water, but does not specifically address microfungi in water standards (WHO 2022). However, Czech Republic and Sweden are the two countries that included microfungi in their drinking water quality standards. According to the water quality standards of the Czech Republic, the mains waters may contain 50 individuals/mL of microorganisms (bacteria and fungi) (Czech Republic Ministry of Health, 2004). Sweden has included fungi specifically in its water quality standards, allowing for 100 cfu/100 mL in mains waters (Swedish National Food Agency, 2001). However, the water quality standard is zero for fungi, 0 individuals/L in Hungary (Ministry of Health Hungary, 2001). Although fungi may not be specifically mentioned in water system standards of many countries, their presence should not be ignored due to their potential as opportunistic pathogens. in the present study, fungal numbers obtained in water samples of 20 residences included in the analysis were found to meet standards of both Czech Republic and Sweden. A general evaluation of data reported in former similar studies showed that our fungal counts are not high in comparison (Hapçıoğlu *et al.* 2005, Hayette *et al.* 2010, Kadaifçiler & Demirel 2018). This suggests that routine processes carried out by the Istanbul Water and Sewerage Administration (IWSA), such as ozonation and sand filtration in the municipal water supply systems before chlorine application, may contribute to the prevention of fungal growth along with disinfection.

According to the monthly water quality reports published by IWSA during our study period, the presence of enterococcus, *Escherichia coli*, *Clostridium perfringens* and coliform bacteria, which are pollution indicators in tap waters, was investigated in determining water quality, and no bacterial contamination caused by these bacteria was detected in water samples (IWSA, 2022). Considering the bacteria counts in our study, it is thought that there may be a contamination in various ways in tap water systems known to have chlorine disinfection, as well as possible detachments from pipe surfaces, water tanks and also possible biofilm layers on shower heads. Bacteria and fungi passing into the water with these breaks may have caused the number and diversity of microorganisms in the water to be determined higher than they actually are during the collection of water samples. Chlorine is the most used disinfectant in water supply systems; it is effective against planktonic microbes but cannot penetrate biofilms (Kim *et al.* 2002). According to the monthly water quality reports of IWSA, there is quite high levels of chloride (28.76-170.18 ppm) in the mains water given from the facilities. Free chlorine levels in our water samples were low. Since chlorine is a volatile gas by its structure, the free chlorine in the mains water may decrease by being lost throughout the water system until it reaches the tap. Additionally, the presence of biofilms that may have formed along the pipeline of the water system can also trap chlorine in the water, leading to lower free chlorine levels being detected. The reduction in chlorine concentration caused by these factors might have been responsible for microbial growth in water samples. On the other hand, many microorganisms sensitive to chlorine can be easily eliminated from water even if the chlorine level is low. Although fungi were detected more than bacteria in both hot and cold water samples, fungal colony numbers were lower than bacterial colony numbers. This may suggest that bacteria are better adapted to oligotrophic water environments than fungi, and that the biofilm layer helps them in this adaptation by hosting them. As a matter of fact, it has been reported that fungi require longer time to take part in biofilms than bacteria and that they settle in the biofilm layer in a non-homogeneous and loose manner (Goksay Kadaifciler *et al.* 2024). The biofilm works as a reservoir, and fungus may be transported intermittently to the water. Although no correlation was detected between fungi and bacteria in our study, it is suggested that there may be synergistic or antagonistic effects, depending on the species, between fungi and bacteria in settling in biofilms in water systems (Goksay Kadaifciler *et al.* 2024). In a biofilm investigation using fungi and bacteria species, researchers reported that bacteria with high growth rates and metabolic activity reduced fungal spore germination, potentially due to nutrient competition (Barros Afonso *et al.* 2020, 2021). Different studies found relationships between fungi and bacteria with different results. It has been suggested that fungi usually colonize pre-established bacterial biofilms and their different ecological requirements may lead to a positive relationship between these microorganisms. In cultivation processes, negative relationships have been reported due to direct competition between both fungi and bacteria for resources. These findings may be a result of several factors such as differences in the composition of microorganisms isolated from water systems or differences in methodologies (Barros Afonso *et al.* 2020, 2021).

Cold water samples have a higher incidence of fungus than hot water samples, as shown in previous studies by Zacheus and Martikainen (1995) and Hayette *et al* (2010). This finding can be attributed to the decrease in dissolved oxygen levels in water as the temperature increases (Xing *et al.* 2014), creating an environment unfavorable for the existence of aerobic microorganisms. This situation can also be explained by the fact that the microorganisms in the water are sensitive to temperature and cannot increase their growth or survive. Microorganisms generally grow by feeding on organic matter that adheres to pipe surfaces in water systems in the presence of appropriate temperature and pH values. The type of the pipe materials can affect the ability of microorganisms to adhere depending on the roughness of their surfaces and the chemical components in their structure. Plastic pipes such

as PVC generally hold more water, and microorganism growth may be more common on such surfaces. The limited moisture retention capacity of metal surfaces and the fact that some metals may have antimicrobial properties may limit microorganism growth. However, it has been determined that microbial communities obtained from cast iron pipes are more stable than microbial communities obtained from non-ferrous pipe materials (Doggett 2000, Makris *et al.* 2014). Studies have stated that the type of pipe material may be associated with differences in the composition of microbial communities (Makris *et al.* 2014, Lee *et al.* 2021). It has been reported that fungi can also be present in water systems and can be included in the biofilm structure by interacting with the pipe surface material. These interactions may vary depending on the type of pipe surface material, environmental conditions (temperature, pH, etc.) and fungal species (Doggett 2000, Marangoni *et al.* 2013, Goksay Kadaifciler *et al.* 2024). For this reason, the relationship between these parameters mentioned above and fungi in water systems may differ for each study. Residence A, where the highest number of fungi were detected in cold water samples, has a water tank. The tank surface material is galvanized steel coated with zinc, and it is known that fungal biofilms form despite the toxic effect of zinc. It is known that sessile fungi can leave the biofilm over time and pass into water, causing the number of planctonic fungi to increase (Goksay Kadaifciler *et al.* 2024). In addition, the low amount of free chlorine in both the cold water sample of residence A and the hot water sample of residence B may have caused the fungal disinfection to be insufficient, causing the number of fungi to be detected high.

In water systems, filamentous fungi are more commonly encountered than yeasts. Yeasts require an optimum pH value of 4.5-5 that supports their growth and a high nutrient content, and they are more sensitive to chlorine than filamentous fungi. Mains water do not provide suitable environmental conditions for the growth of yeasts, as they have low nutrient content and pH values that fluctuate between 6-7 (Rosenzweig *et al.* 1983, Barnett & Hunter 1999). For these reasons, yeasts may be less isolated than filamentous fungi in all water samples. The genus *Penicillium* was found to be dominant in cold water samples. It has been reported that fungal spores are more resistant to chlorination compared to bacteria and yeasts (Rosenzweig *et al.* 1983). Also, *Penicillium* spores are more resistant to chlorination than other fungal genera and are frequently detected in water environments (Pereira *et al.* 2013). On the other hand, the genus *Aspergillus* was found to be dominant in hot water samples. *Aspergillus* has the ability to survive under various challenging environmental conditions such as high temperature and low oxygen content (Babič *et al.* 2017), making *Aspergillus* spores to be commonly found in water systems (Richardson & Rautemaa-Richardson 2019). *Penicillium* and *Aspergillus* are known to be commonly found in hot water samples. These fungi are able to adapt to high temperatures by developing heat-resistant structures such as thick cell walls, which protect them from heat stress. Significantly these fungi have the ability to produce heat shock proteins, which help to protect them from the harmful effects of high temperatures (Tiwari *et al.* 2015). *Aspergillus*, *Penicillium*, *Candida*, and *Fusarium* that have been identified as pathogens, opportunistic pathogens, allergens, and toxigenic species have been found in man-made water system (Warris *et al.* 2001, Alangaden 2011). Opportunistic pathogenic fungi are known to thrive in warm and humid environments. Several studies showed that high temperatures can increase the growth rate and virulence of these fungi, making them more pathogenic to humans and animals (Klich 2002). The presence of opportunistic pathogens in hot water samples have been linked to an increased risk of infections, such as respiratory infections, skin infections, and systemic infections, particularly in immunocompromised individuals (Anaissie *et al*. 2001, 2002).

In the studies conducted on drinking and utility water connected to the mains water system in Türkiye, the focus has been on total and fecal coliform bacteria and pathogenic bacteria, which are important from a health standpoint. However, there are limited studies that investigate microbial load and diversity, including fungi. In one of these studies, which use the membrane filtration method, *Penicillium* spp., *Aspergillus* spp., and *Acremonium* spp. were detected in a hospital water system in Istanbul (Hapçıoğlu *et al*. 2005). *Penicillium*, *Aspergillus*, *Cladosporium*, and *Paecilomyces* were reported as dominant fungi in dental unit water systems connected to the mains water system in Istanbul (Kadaifçiler & Çotuk 2014). Similarly, in our study, it is noteworthy that *Aspergillus* and *Penicillium*, which are frequently detected in water samples, were also dominant in previous studies. *Penicillium* and *Aspergillus* spp. were identified as the predominant species among the 32 different species found in a study investigating the fungal diversity in water samples obtained from houses, hospitals, and shopping centers connected to the municipal water supply system in Istanbul. Furthermore, *Aspergillus* species with mycotoxigenic properties were also reported (Kadaifçiler & Demirel 2018). The comparison of findings of our present study with the fungal diversity of water based samples reported in relevant literature showed the results obtained were similar in general. Hageskal *et al.* (2006) investigated the presence of fungi in drinking and mains waters in Norway using the membrane filtration method. They detected 30 different fungal taxa, predominantly belonging to the *Penicillium* and *Aspergillus* genera. The fungi identified in our study, such as *Acremonium* sp., *Botrytis* sp., *Chaetomium* sp., *Chaetomium globosum*, *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium expansum*, *Penicillium olsonii*, *Penicillium raistrickii*, *Penicillium restrictum*, *Phialophora* sp., and *Scopulariopsis* sp. were also present in the study of Hagestal *et al.* (2006). Kanzler *et al.* (2007) investigated the presence of filamentous fungi and yeasts

in drinking and main waters and groundwater in Austria. They identified 32 different taxa of fungi, predominantly *Cladosporium* spp. and *Penicillium* spp. which were also the dominant fungi in our study. Additionally, the identified species, including *Aspergillus* sp., *Acremonium* sp., and a small number of yeasts, were also present in our study. In this study, the identified fungi such as *Acremonium kliense*, *Arthroderma* sp., *Aspergillus amstelodami*, *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus niveus*, *Botrytis* sp., *Chaetomium globosum*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Phialophora* sp., and *Scopulariopsis asperula* are known to have allergenic and opportunistic pathogenic properties. *Acremonium kliense*, in particular, has gained clinical significance as it can cause systemic fungal diseases in immunocompromised individuals. *Arthroderma* sp. can lead to fungal infections on the skin. *Aspergillus amstelodami*, *A. flavus*, *A. versicolor*, and *A. niveus* can cause invasive aspergillosis. *Botrytis* sp., also known as a plant pathogen, is an allergen. *Chaetomium globosum* can cause respiratory tract infections, rhinocerebral infections, and infections on the skin and nails. *Penicillium chrysogenum* and *P. citrinum* can cause invasive infections (Babič *et al.* 2017). *Phialophora* sp. can lead to systemic infections such as endocarditis and keratitis, particularly in immunocompromised patients (Migrino *et al.* 1995). *Scopulariopsis* sp. can cause fungal infections on the nails and keratitis.

The study shows that fungi, especially filamentous fungi, can be present in variable concentrations in building water systems and that temperature can change the number and diversity of fungi. In conclusion, analysis of water samples from building systems can reveal significant information about the microbial load, microbial community, and their effects on human health. Given the limited global research on waterborne fungi, especially in the context of climate change, further studies are necessary to understand the full scope of fungal contamination in water and its potential health impacts.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

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