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*Original Research*

# Occurrence of Fungi in Water Distribution System

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**Abstract**

It is demonstrated that contamination of water distribution systems with fungi is determined by the number and species composition of the mycoflora of waters supplying a given Water Treatment Plant (WTP) and the effectiveness of their removal in the unit processes of water treatment used. In the water distribution system examined, a significant number of microorganisms occurring in waters supplying the WTP was reduced in the water supplied to the system to 200 cfu $l^{-1}$ following sorption, coagulation, filtration and disinfection processes. Their number did not exceed 267 cfu $l^{-1}$ in the water phase in the distribution system while it was as many as 1000-5000-times greater in the biomass "suspended" in it. These organisms occurred sporadically in pipe sediments. Moulds, including species pathogenic and potentially pathogenic to humans and warm-blooded animals, constituted the mycoflora.

**Keywords:** fungi, pathogenic fungi, potentially pathogenic fungi, water distribution system

## Introduction

Secondary contamination[1] of water distribution systems has been investigated for many years by engineers and epidemiologists. The consequence of this phenomenon is an increase in the number of bacteria, actinomycetes, cyanobacteria, fungi and protozoa in the distribution system adversely affecting both the taste and odour of the water [1-10]. Moreover, it may also constitute a sanitary and epidemiological hazard to drinking water consumers as a result of the accumulation of pathogenic or potentially pathogenic species of these microorganisms, in the biomass "suspended" in the water phase, biological deposits and pipe sediments. Transmission of these microorganisms by water distribution systems was discussed in greater depth in review papers by Grabińska-Łoniewska [11, 12].

Growth of fungi in water distribution systems changes not only the water taste and odour for the worse but also may cause technological and operational difficulties. According to Lahti [13], they occur at fungal concentrations equal to 100 cfu 100 $ml^{-1}$. As a result of the production of organic acids in metabolic processes, microbiological corrosion of water pipes is accelerated and water disinfection is impeded because of the difficulties in maintaining

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[1] Increase of the quantity of mineral and organic compounds in treated water distributed by water supply system caused by their release from dead cells of organisms and by undergoing corrosion of water pipes inner surface as well as by outside contamination of the distribution system. This phenomenon may result in the increase of the number of microorganisms in the water exceeding the values permitted by legal regulations.

the appropriate concentration of chlorine remaining in the system. Water distribution systems may also be a source of fungal infections resulting in mycotoxicoses, mycoses and allergies. These organisms are inhaled (i.e. while showering or in the sauna), ingested, transmitted through abraded mucous membrane in the oral cavity, skin and cornea, or directly into the blood and internal organs in special circumstances such as catheter insertion, peritoneal dialysis or surgical procedures. Thus, monitoring of the contamination degree of water distribution systems with these microorganisms is recommended.

Studies on the mycoflora of water distribution systems have focused on the determination of the density and the type of microscopic fungi in the water phase and biofilm in water distribution systems, while their occurrence in other possible habitats of these organisms, i.e. the biomass "suspended" in the water phase and sediments on the inner surface of water distribution pipes, has not been examined. Rosenzweig and Pipes [14] showed that microscopic fungi colonized both distribution systems of groundwaters disinfected with chlorine at concentration 1.0-2.0 mg $Cl_2$ $l^{-1}$, and surface waters treated with coagulation methods, filtration through sand filters and chlorination (chlorine concentration 0.8-1.5 mg $Cl_2$ $l^{-1}$). Lahti [13] demonstrated that the number of microscopic fungi in water distribution systems may reach 100 cfu 10 $ml^{-1}$. As the results of these studies show, water treatment processes do not inhibit fungal presence in water systems. According to this author, 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 Rozenzweig et al. [15] showed that chlorine concentration 1.0-3.0 mg $Cl_2$ $l^{-1}$ did not have fungicidal effects on yeast cells and conidia.

Taxonomic studies showed the occurrence in the water distribution systems filamentous fungi belonging to the genera: *Phialophora* (*P. sessilis, P. cinerescens, P. malorum, P. verrucosa, P. hofmannii*), *Acremonium* (*A. berkeleyanum, A. arxii, A. strictum, A. psammosporum*), *Exophiala* (*E. angulospora, E. castellanii, E. cf. pisciphila*), *Penicillium* (*P. chrysogenum, P. brevicompactum, P. glabrum*), *Verticillium* (*V. lecanii, V. tenereum*), *Fusarium* (*F. merismoides, F. solani*), *Phoma* (*P. herbarum, P. homa leveillei*), *Aspergillus* (*A. fumigatus, A. flavus, A. niger, A. versicolor*), *Cladosporium* (*C. cladosporioides, C. herbarum*), *Paecilomyces* (*P. farinosus*), *Chara, Mucor, Geomyces, Ochroconis, Conidiobolus, Humicola, Myrothecium, Tilletiopsis, Plectosporium, Volutella, Sporocybe, Alternaria* and *Phaeococcus* [14, 16-18]. Yeast and yeast-like fungi are represented by the genera *Candida* (*C. albicans, C. tropicalis, C. naris, C. ciferrii, C. valida, C. quillermondii, C. parapsilosis*), *Rhodotorula* (*R. glutinis, R. mucilaginosa*), *Cryptococcus* (*C. laurentii*), *Kloeckera* and *Aureobasidium pullulans* [14, 19-21].

The majority of fungi in these genera are non-pathogenic saprophytes *Deuteromycotina*, widespread in lake waters, surface waters contaminated with wastewaters, seas and soils [22-26]. Thus, they enter the water treatment stations as contaminants from surface waters that are water supply sources and to the water distribution systems, from the air and soil if leaks or breaks occur in it.

According Göttlich et al. [17] melanization and production of slimy conidia were recurrent features of the fungi colonizing water distribution systems. West (cit. after above authors) found a predominance of the melanized genera *Cladosporium*, *Phoma*, *Alternaria* and *Exphiala* in drinking water derived from lake water. Species belonging to the genera *Fusarium*, *Acremonium*, *Exophiala* and *Phialophora* (this species has a broad capacity to survive desinfecion regimes) have been reported regulary among fungi isolated from municipal water systems in the USA, Germany and in the UK.

The aim of the present study was to determine the density and the species composition of fungi occurring in the water phase, the biomass "suspended" in it and sediments on inner pipe surfaces in samples collected from the water distribution system at various distances from the Water Treatment Plant (WTP). The results obtained were interpreted in relation to the density and the species composition of fungi in waters supplied for the purposes of water distribution systems and after individual treatment stages in the WTP.

## Materials and Methods

### Collection of Samples

Water samples were collected between December (2000) and November (2002) from different sites of municipal water supply system of the city of Warsaw. These included: intake waters supplying Water Treatment Plant (WTP), waters treated in technological lines I and II of WTP, and a mixture of the above waters disinfected with a mixture of $ClO_2$ and $Cl_2$ – transferred into distribution system and waters from 7 sites within the distribution network located on 4.2; 4.3; 5.4; 5.7; 8.7; 10.0 and 10.3 km from WTP.

The source of water supply for the first technological line is water taken from the bottom of the Vistula River and treated by filtration (so-called infiltration water) and aeration processes. Then it is treated on several slow sand filters and slow sand filters with inserts of activated carbon and finally disinfected ($Cl_2$, $ClO_2$).

The second technological line is supplied with the water from the open sedimentation basin. It is a mixture of water from the Vistula River and infiltration water. The treatment method of this water includes: chemical coagulation ($Al_2(SO_4)_3$, silica, pulverized carbon), lime alkalization, fast sand filtration and disinfection ($Cl_2$, $ClO_2$). Water delivered to the system is a mixture of the treated water from Ist and IInd technological lines prepared in different percentage ratio depending on treatment efficiency. The free chlorine concentration in the water supply net was in the range 0.15-0.20 mg $Cl_2l^{-1}$, at the terminal sections of the net occasionally drooped even to 0.01 mg $Cl_2l^{-1}$. All of the collection sites in the distribution network were located at hydrants outside buildings. All water samples were collected in sterile plastic bottles containing 10% (v/v) solu-

tion of thiosulfite to neutralize chlorine at the ambient temperature. Sediments were taken during water distribution net repairs from the water pipes' inner surface. Samples were always processed on the day of collection.

## Processing of Samples

The biomass of microorganisms constituting so-called secondary contamination was separated from 20 l water samples collected from the system using the filtration method through a membrane filter, pore size 5 μm, with a vacuum pump (Merck ME 2). The biomass retained on the filter was eluted for 30 min in a shaker (Elpan, type 357), with the amplitude 6 and 200 cpm/min., to 50 ml of 0.28% solution of sodium pyrophosphate. The prepared suspension was used for microscopic examination of the biocenosis constituting the biomass of secondary contamination. The suspension prepared as above, disrupted in an ultrasound disintegrator UD 20, vibration amplitude A=20 μm for 40 s, was used for microbiological examinations. Using this method of sample preparation, the obtained suspension contained both microorganisms occurring inside zoogleal aggregates of bacteria, as well as inside and on the surface of cells of algae and protozoa in the water phase in the water distribution system.

Samples from which the microorganism biomass was removed using the method described above were used to prepare the suspension of microorganisms collected from the water phase in the water distribution system. Microorganisms present in the water from the distribution system were thickened using the filtration method (as above) through a membrane filter, pore size 0.45 μm. Depending on the degree of water contamination, water filtration was performed from the volumes of 1.0; 0.5 and 0.1 litre. The filter was next placed in 10 ml of 0.28% solution of sodium pyrophosphate and shaken for 30 min. in a shaker (as above) to elute microorganisms from the filter.

Sediments were scraped from 62.8-80 $cm^2$ of the inner surface of sample sections, weighed, ground in a mortar, suspended in 0.28% solution of sodium pyrophosphate and shaken in a shaker (as above) to elute microorganisms from the mineral fraction of the sediments. Non-thickened samples were used for inoculation.

## Biological Examinations

The biomass isolated from the water was observed under a phase contract microscope (Opton). The total number of psychrophilic bacteria (PB) and pigmented bacteria was determined in culture on the broth agar medium MPA, and the number of fungi on Martin medium [27] containing in liter of distilled water: glucose – 10.0 g; peptone – 5.0 g; $KH_2PO_4$ – 1.0 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; agar – 20.0 g; rose bengal – 33.3 mg; streptomycin – 30.0 mg and chlorotetracyclin – 2.0 mg. Incubation was conducted at 26°C for 7 days. The results are reported as cfu $l^{-1}$. The taxonomic status of the fungi isolated from water samples and biomass samples was determined on the basis of macromorphological features. Studies were conducted on plates and micromorphological features conducted in microcultures using the following identification media:

- for the genera *Penicillium* and *Aspergillus* Czapek agar (28) containing in liter of distilled water: $NaNO_3$ – 3.0 g; $K_2HPO_4$ – 1.0 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; KCl – 0.5 g; $FeSO_4 \cdot 7H_2O$ – 0.01 g; sucrose – 0.5 g; agar – 15.0 g,
- for the genus *Fusarium* – MEA medium (28): malt extract – 20.0 g; peptone – 1.0 g; dextrose – 20.0 g; agar – 20.0 g; distilled water – 1 l. PDA medium: potato – 200.0 g; glucose – 20.0 g; agar – 20.0 g; distilled water – 1 l. SNA medium (29): glucose – 0.2 g; sucrose – 0.2 g; $KH_2PO_4$ – 1.0 g; $KNO_3$ – 1.0 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; KCl – 0.5 g; agar – 15.0 g; distilled water – 1 l.

The final classification was performed on the basis of the following taxonomic studies: Kwaśna et al. [30], Domsch et al. [31], Ellis and Ellis [32], Litwinow [33].

## Results and Discussion

In total 55 isolates of fungi from 14 genera representing 20 species were found in mycoflora of water distribu-

Table 1. Fungal species isolated from water distribution system of Warsaw city.

| Taxa of the fungi |
|---|
| *Aspergillus niger* van Tieghem |
| *Aspergillus fumigatus* Fres. |
| *Aspergillus parasiticus* Speare |
| *Aspergillus ustus* (Bain.) Thom et Church |
| *Aspergillus versicolor* (Vuill.) Tiraboschi |
| *Cladosporium cladosporoides* (Fres.) de Viers |
| *Cladosporium sphaerospermum* Penz. |
| *Fusarium solani (Mart.) Sacc.* |
| *Fusidium terricola* Miller |
| *Geotrichum candidum* Link ex Leman |
| *Gonatobotrys simplex* Corda |
| *Paeciliomyces lilacinus* (Thom) Samson |
| *Penicillium frequentans* Westling |
| *Penicillium rubrum* Stoll |
| *Phialophora malorum* (Kidd et Beaum.) Mc Colloch |
| *Sclerotinia sclerotiorum* (Lib.) De Bary |
| *Sesquicillium candelabrum* (Bonord.) W. Gams |
| *Stachybotrys chartarum* (Ehrenb. Ex Link) Hughes |
| *Trichoderma viride* Pers ex Gray |
| *Verticillium lecani* (Zimm.) Vieges |

tion system investigated (Table 1). It was determined by the density and the species composition of fungi occurring in intake waters delivered to the WTP and treated in technological lines I and II.

Infiltration waters supplying technological line I were characterized by weak microbiological contamination (number of PB $13.3 \cdot 10^4$ cfu $l^{-1}$ and of fungi 130 cfu $l^{-1}$) (Table 2). *Aspergillus niger, Cladosporium cladosporioides, Phoma sp., Aspergillus fumigatus* and *Penicillum rubrum* dominated in the group of moulds, while *Stachybotrys chartarum* and *Verticillium lecanii* occurred in smaller numbers (Table 3).

A significantly greater contamination with psychrophilic bacteria and fungi ($99.8 \cdot 10^6$ and $6.6 \cdot 10^3$ cfu $l^{-1}$, respectively) was observed in the case of the open sedimentation basin from which water was supplied for technological line II. *Aspergillus fumigatus, Trichoderma viride, Fusarium solani, Phialophora malorum, Stachybotrys chartarum* and *Verticillium lecani* were dominant mould species in this basin; *Gonatobotrys simplex, Geotrichum candidum, Phoma sp.* and unidentified mitosporous fungi also occurred (Table 3). *Aspergillus fumigatus, A.niger, A.parasiticus, A,ustus, A.versicolor, Paeciliomyces lilacinus, Penicillium rubrum, Cladosporium cladosporoides* and *Geotrichum candidum* are species pathogenic or opportunistic pathogenic to humans in this group [31, 34-36]. *Aspergillus fumigatus, A.niger, A.parasiticus, A.ustus, A.versicolor, Penicillium frequentas, Paeciliomyces lilacinus* and *Geotrichum candidum* also belong to fungi occurring on plumage and in droppings of birds, including water-mud birds [34-36].

From Table 2 it should be noted that the number of PB slightly decreased during infiltration water treatment (aeration, filtration and disinfection) in technological line I while the number of fungi increased greatly. Although the quality of the water supplied was much worse, technological line II, which includes coagulation, alkalization, filtration and disinfection processes, was characterized by a high removal effectiveness of microorganisms. Consequently, the water pumped to the water distribution system that was a mixture of waters treated in technological lines I and II, contained PB to the amount of $11 \cdot 10^3$ cfu $l^{-1}$, and fungi – 200 cfu $l^{-1}$.

The water treatment process in the WTP resulted in the elimination of yeast-like fungi that constitute an important component of the mycofora, especially in the river water sedimentation basin. It is of great sanitary and epidemiological importance as the following potentially pathogenic species prevail in surface water reservoirs that supply the WTPs: *Candida albicans, C.glabrata, C.tropicalis, Rhodotorula glutinis* and *Trichosporon beigelli* [37-39]. Their density in water, depending on the location of the aquatic reservoir and the season, ranged between 80-52160 cfu $l^{-1}$, and correlated with the presence of *Escherichia coli*. Thus, Dynowska [37] as well as Wójcik and Tarczyńska [38, 39] believe these organisms should be regarded as indicators of sanitary contamination of water designed for consumption and recreation. The presence of *C. tropicalis*, *C. naris*, *C. ciferrii*, *C. valida*, *Kloeckera* spp. and *Rhodotorula* spp. in hospital and community potable waters in Greece was stated by Arvanitidou et al. [19].

The examinations on the presence of fungi in the water distribution system conducted by us show that they occur in the water phase only in some sections of the system made of cast iron and steel, and their number does not exceed 267 cfu $l^{-1}$ (Table 4).

It is three times as small as the number at which, as Lahti [13] believes, operational difficulties of water distribution systems may occur. In the USA and Finland, the density of these organisms in the water phase in water distribution systems ranges between 20 and >160 – 1000 cfu $l^{-1}$ [13-15]. In the water distribution system studied by us, the mycoflora of the bulk water phase consisted mainly of species that are opportunistic pathogens to humans and warm-blooded animals, including *Cladosporium sphaerospermum, Aspergillus versicolor, A.parasiticus, Penicillium frequentans*. The species listed, as well as other species occurring in the biocenosis, i.e. *A.ustus* and

Table 2. Quantity of bacteria and fungi occurring in intake waters for WTP, after different stages of treatment and in the water pumped to water supply net.

| Sampling location | | Number of bacteria (PB) cfu $l^{-1}$ | | | Number of microscopic fungi cfu $l^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| | | non pigmented | pigmented | total | molds | yeast-like fungi | total |
| I technological line | infiltration intake of river water | $11.7 \cdot 10^4$ | $2.8 \cdot 10^4$ | $14.5 \cdot 10^4$ | 113 | 17 | 130 |
| | after different stages of treatment | $4.58 \cdot 10^3$ – $92.7 \cdot 10^3$ | $29.6 \cdot 10^3$ – $214 \cdot 10^3$ | $34.6 \cdot 10^3$ – $306 \cdot 10^3$ | 453 – 1373 | nf – 133 | 453 – 1506 |
| II technological line | river water sedimentation basin | $99 \cdot 10^6$ | $0.8 \cdot 10^6$ | $99.8 \cdot 10^6$ | $4.5 \cdot 10^3$ | $2.1 \cdot 10^3$ | $6.6 \cdot 10^3$ |
| | after different stages of treatment | $9.5 \cdot 10^3$ – $134 \cdot 10^3$ | $7 \cdot 10^3$ – $21 \cdot 10^3$ | $16.5 \cdot 10^3$ – $155 \cdot 10^3$ | 146 – 400 | 66 – 417 | 212 – 817 |
| Pure water pumped to water supply net | | $2.2 \cdot 10^3$ | $8.8 \cdot 10^3$ | $11 \cdot 10^3$ | 200 | nf | 200 |

Abbreviations: nf – not found

Table 3. Molds species occurring in intake river, water treated in WTP and pomped to water supply net.

| Sampling location | | Species | Percentage occurrence in mycoflora |
|---|---|---|---|
| I technological line | infiltration intake of river water | *Aspergillus niger* | 40 |
| | | *Cladosporium cladosporioides* | 15 |
| | | *Phoma* sp. | 15 |
| | | *Aspergillus fumigatus* | 10 |
| | | *Penicillium rubrum* | 10 |
| | | *Stachybotris chartarum* | 5 |
| | | *Verticillium lecani* | 5 |
| | after different stages of treatment | *Verticillium lecani* | 8-70 |
| | | *Aspergillus niger* | 50 |
| | | *Aspergillus fumigatus* | 7-46 |
| | | *Aspergillus versicolor* | 4-20 |
| | | *Cladosporium sphaerospermum* | 10-16 |
| | | *Penicillium rubrum* | 10 |
| | | *Fusarium solani* | 10 |
| | | *Phialophora malorum* | 10 |
| | | *Sesquicillium candelabrum* | 8 |
| | | *Phoma* sp. | 4-8 |
| | | *Paeciliomyces lilacinus* | 7 |
| | | *Cladosporium cladosporioides* | 6 |
| | | not identified mitosporous fungi | 2-6 |
| II technological line | river water sedimentation basin | *Trichoderma viride* | 20 |
| | | *Aspergillus fumigatus* | 12 |
| | | *Fusarium solani* | 12 |
| | | *Phialophora malorum* | 10 |
| | | *Stachybotrys chartarum* | 10 |
| | | *Verticillium lecani* | 10 |
| | | *Gonatobotrys simplex* | 5 |
| | | *Geotrichum candidum* | 2 |
| | | *Phoma* sp. | 2 |
| | | not identified mitosporous fungi | 2 |
| | after different stages of treatment | *Aspergillus fumigatus* | 30-35 |
| | | *Trichoderma viride* | 30 |
| | | *Geotrichum candidum* | 25 |
| | | *Phoma* sp. | 10 |
| Pure water pumped to water supply net | | *Aspergillus fumigatus* | 50 |
| | | *Aspergillus niger* | 50 |

Table 4. Quantity of psychrophilic bacteria (PB) and fungi in the bulk water phase of different sections of water supply net.

| Sampling location | | Number of bacteria (PB) cfu $l^{-1}$ | | | Number of fungi cfu $l^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| | | non pigmented | pigmented | total | molds | yeast-like fungi | total |
| Water distribution net – distance from WTP (km) | 4.2 | $60.4 \cdot 10^3$ | $14.1 \cdot 10^3$ | $74.5 \cdot 10^3$ | nf | nf | nf |
| | 4.3 | $28.9 \cdot 10^3$ | $30.7 \cdot 10^3$ | $59.6 \cdot 10^3$ | nf | nf | nf |
| | 5.4 | $6.3 \cdot 10^3$ | $5.3 \cdot 10^3$ | $11.6 \cdot 10^3$ | 267 | nf | 267 |
| | 5.7 | $5.2 \cdot 10^3$ | $35.3 \cdot 10^3$ | $40.5 \cdot 10^3$ | nf | nf | nf |
| | 8.7 | $22.5 \cdot 10^3$ | $15.7 \cdot 10^3$ | $38.2 \cdot 10^3$ | nf | nf | nf |
| | 10.0 | $129.7 \cdot 10^3$ | $76 \cdot 10^3$ | $205.7 \cdot 10^3$ | 97 | nf | 97 |
| | 10.3 | $109.1 \cdot 10^3$ | $10.9 \cdot 10^3$ | $120 \cdot 10^3$ | nf | nf | nf |

Table 5. Quantity of psychrophilic bacteria (PB) and fungi in biomass "suspended" in bulk water phase of different sections of water supply net.

| Sampling location | | Number of bacteria (PB) cfu $l^{-1}$ | | | Number of fungi cfu $l^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| | | non pigmented | pigmented | total | molds | yeast-like fungi | total |
| Water distribution net – distance from WTP (km) | 4.2 | $71.8 \cdot 10^3$ | $14.1 \cdot 10^3$ | $85.9 \cdot 10^3$ | nf | nf | nf |
| | 4.3 | $43.9 \cdot 10^3$ | $136.9 \cdot 10^3$ | $180.8 \cdot 10^3$ | nf | 83 | 83 |
| | 5.4 | $12.4 \cdot 10^3$ | $4.7 \cdot 10^3$ | $17.1 \cdot 10^3$ | 33 | nf | 33 |
| | 5.7 | $8.9 \cdot 10^3$ | $87.9 \cdot 10^3$ | $96.8 \cdot 10^3$ | 1000 | nf | 1000 |
| | 8.7 | $15.2 \cdot 10^3$ | $1.9 \cdot 10^3$ | $17.1 \cdot 10^3$ | nf | nf | nf |
| | 10.0 | $42.6 \cdot 10^3$ | $44.7 \cdot 10^3$ | $87.6 \cdot 10^3$ | 1117 | 160 | 1277 |
| | 10.3 | $7.6 \cdot 10^3$ | $5 \cdot 10^3$ | $12.6 \cdot 10^3$ | 5000 | nf | 5000 |

*Fusidium terricola* (Tables 3 and 6), colonize bird feathers, nests and droppings [34, 35].

The degree of microbiological contamination of water distribution systems is defined not only by the density of microorganisms in the water phase that is monitored in routine control examinations but also by their number in the biofilm and sediments on inner surfaces of water pipes, not monitored so far. According to Fleming et al. [40], the participation of these two fractions in the total number of microorganisms occurring in water distribution systems equals 5 and 95%, respectively.

Our studies confirm the above claim. The results show that the number of living bacterial cells determined with the breeding method, "immobilized" in the biomass suspended in water, may be even 10-30-times greater than that in water. Zoogleal aggregates of bacteria whose density ranged between 13000-155333 $l^{-1}$ were the main component of the biomass. Furthermore, filamentous bacteria, fungal hyphae and spores, algae (*Chlorella sp., Melosira sp., Tabelaria sp., Nitschia sp.*), protozoa belonging to flagellates and amoebae, as well as rotifers occurred in the biomass "suspended" in the water phase. The number of fungi in this biomass in some system sections was even 1000-5000 times greater than that in the water phase (Table 5).

Mycoflora composition in the biomass suspended in water was also greatly similar to that identified in water without the biomass. The species dominant in water (*Cladosporium sphaerospermum, A.vesicolor* and *A.parasiticus*) were the main components of the biomass mycoflora differentiated from it, together with *Penicillium rubrum, A.ustus* and *Cladosporium cladosporioides,* as well as one phytopathogenic species – *Sclerotinia sclerotiorum* (Table 6).

The genera and species found in water distribution systems in the USA by Rosenzweig et al. [14, 15] and in Germany by Göttlich et al. [17], i.e. *A.niger, A.fumigatus, Penicillium sp., Cladosporium sp*., occurred both in water and in the biomass separated from it. The findings that moulds were the main components of the mycoflora of drinking-water distribution systems in terms of abundance as well as percent frequency are in agreement with obser-

Table 6. Species of fungi occurring in the water and biomass "suspended" in it, on the sampling location situated at 5.3 km from WTP.

| Date of samples intake | Water | | Biomass "suspended" in water | |
|---|---|---|---|---|
| | Species | Percentage occurrence in mycoflora | Species | Percentage occurrence in mycoflora |
| 28.01.2001 | *Cladosporium sphaerospermum* | 30 | *Cladosporium sphaerospermum* | 30 |
| | *Aspergillus parasiticus* | 10 | *Penicillium rubrum* | 10 |
| | *Aspergillus versicolor* | 10 | *Aspergillus parasiticus* | 10 |
| | *Fusidium terricola* | 5 | *Aspergillus versicolor* | 10 |
| | *Penicillium frequentans* | 5 | | |
| 22.10.2001 | *Cladosporium sphaerospermum* | 30 | *Cladosporium sphaerospermum* | 30 |
| | *Aspergillus versicolor* | 10 | *Cladosporium cladosporioides* | 15 |
| | *Aspergillus parasiticus* | 7 | *Aspergillus ustus* | 10 |
| | *Aspergillus ustus* | 5 | *Aspergillus parasiticus* | 8 |
| | not identified mitosporous fungi | 7 | *Aspergillus versicolor* | 2 |
| | | | *Sclerotinia sclerotiorum* | 1 |

Table 7. Quantity of bacterial biomass and different groups of microbes in the water distribution systems sediments depending on the kind of pipes and period of their exploitation.

| Determination | Pipe characteristics | | | | | |
|---|---|---|---|---|---|---|
| | Cast iron joints ϕ 50 mm | | | Spheroidal graphite iron pipe ϕ 250 mm | Steel joints ϕ 40 mm | |
| | Period of exploitation (years) | | | | | |
| | 52 | 42 | 31 | 2 | 46 | 15 |
| Quantity of sediment (mg $cm^{-2}$) | 458 | 1024 | 616 | 1291 | 427 | 261 |
| Quantity of biomass (ng 100 $g^{-1}$) | 454 | 147 | 147 | 7.2 | 2683 | nf |
| Total number of psychrophilic bacteria (cfu 100 $g^{-1}$) | $454 \cdot 10^3$ | $147 \cdot 10^3$ | $147 \cdot 10^3$ | 7189 | $2683 \cdot 10^3$ | nf |
| Total number of pigmented bacteria (cfu 100 $g^{-1}$) | nf | nf | 2066 | 7053 | nf | nf |
| Moulds (cfu 100 $g^{-1}$) | nf | 4273 | nf | nf | nf | nf |

vations of Lahti [13], Göttlich et al. [17] and Arvanitidou et al. [19]. According to Doggett [20] filamentous fungi (mainly of the genera *Aspergillus* and *Penicillium*) are typically more prevalent than yeast and yeast-like fungi (*Aureobasidium pullulans*, *Candida quilliermondii*, *C. parapsilosis, Cryptococcus laurentii, Rhodotorula glutinis, R. mucilaginosa*) in the mycoflora of drinking water systems biofilm. This author stated that densities of filamentous fungi ranged from 4.0 to 25.2 cfu $cm^{-2}$, where as yeast densities ranged from 0 to 8.9 cfu $cm^{-2}$. This can probably be explained by the fact that yeasts and yeast-like fungi are mostly associated with nutrient-rich environments, whereas among filamentous fungi oligotrophs are more common. And fungal conidia show greater resistance to chlorine inactivation than yeasts cells.

Our examinations showed that pipe sediments are not, as suggested by Fleming et al. [40], the main place of the accumulation of biomass microorganisms in water distribution systems. In the system examined by us it consisted mostly of saprophytic bacteria. Assuming that the mass of one bacterial cell is $10^{-12}$ g, the quantity of the biomass accumulated in sediments of cast iron joints over 31-52 years ranged between 147-454 ng 100 $g^{-1}$. More biomass accumulated in steel joints that had been in operation for 46 years (2685 ng 100 $g^{-1}$). A smaller quantity of the biomass (7.8 ng 100 $g^{-1}$) was recorded in the sediment in a pipe

made of spheroidal graphite iron used for 2 years, characterized by the highest copper concentration (0.654% dw) in the mineral fraction of the sediment. The occurrence of fungi in sediments was not common in the studied water distribution system. Opportunistic pathogenic species, i.e. *Aspergillus niger, A.fumigatus* and *A.versicolor*, were found in them. However, as they occurred in the biocenosis occasionally and in small numbers (4273 cfu 100 $g^{-1}$), they do not seem to pose a health hazard to water consumers (Table 7).

Both in water as well as in biomass "suspended" in bulk water phase and in pipe sediments there was no correlation between total counts of PB and filamentous fungi. These results were similar to that obtained by Hapcioglu et al. [18] and Göttlich et al. [17]. They found no positive correlation between the filamentous fungi counted in public drinking water distribution system and standard hygiene indicators such as *E.coli* or other coliform bacteria. However, these findings conflict with conclusions of Arvanitidou et al. [19] who stated a positive correlation between the number of filamentous fungi and heterotrophic bacteria in hospital and community potable waters.

Literature data indicate that different relationships exist between the number of yeast-like fungi and bacterial hygiene indicators. Positive correlation between the cell quantities of *Candida* species in surface waters and colony counts of *Enterococcus* organisms was stated by Brinkman et al. [21]. This finding corresponds with the conclusion of Arvanitidou et al. [19], who found that the number of colony-forming units of yeasts in hospital and community potable waters positively correlated with those of total and faecal coliforms. These authors suggest that tap water is a potential transmission route for yeast-like fungi in hospital and communal distribution systems and may pose a health hazard mainly for the immunocompromised hosts.

## Conclusions

The examinations conducted show that the contamination degree of the water distribution system by fungi depends mostly on the effectiveness of their removal in technological processes of water treatment. Despite the high microbiological contamination of intake waters delivered to the WTP, unit processes (sorption, coagulation, filtration, disinfection) used in the water distribution system examined prevented fungi from getting into the water distribution system in the number that could adversely affect the taste and odour of the water as well as consumers' health. The biomass of microorganisms (zoogleal aggregates of bacteria, algae, protozoa) "suspended" in the water phase is the main site of the occurrence of fungi belonging to moulds, including species pathogenic and potentially pathogenic to humans, in the water distribution system. Therefore, apart from the bacteriological determinations referred to in legal regulations, the scope of control examinations monitoring the contamination degree of water distributed through water distribution systems, especially in sections designed for a small number of consumers or following system failures and renovations, should include the determination of the density and the species composition of fungi in samples of water and biomass isolated from it.

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