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Fungal flora in groundwater-derived public drinking water

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Abstract

In order to assess the dissemination of hygienically relevant fungi via the public drinking water distribution system, a 12-month survey was performed on groundwater-derived drinking water from 29 water supplies in North Rhine-Westphalia, Germany. Frequencies of contaminated water samples, and the prevalent species and patterns of occurrence in raw water, waterworks, the network and house installations were studied on the basis of 2657 water samples. Results were obtained by long-term incubation of 1 ml aliquots of water samples on agar-based culture media, following bacteriological procedures documented in the German drinking water regulations (Anon, 1990). No correlation with standard hygiene indicators, such as *E. coli* or other coliform bacteria was observed. Common opportunistic and allergenic *Aspergillus* species were encountered only rarely. The fungal flora was dominated by a limited number of species of *Acremonium*, *Exophiala*, *Penicillium* and particularly *Phialophora*; some of them occurred throughout the entire drinking water system and are thought to constitute a resident fungal flora. *Phialophora* sp. nov., to be described as a new species elsewhere, was ubiquitous; it was found in 26.6% of the samples positive for fungi (7.5 % of 2657). Fungal diversity in the network itself was significantly lower than in raw water and house installations, indicating that not all fungi gaining access to the system are capable of surviving for longer periods. For species such as *Verticillium lecanii*, found exclusively after the introduction of newly buried pipes and remaining localized at those sites, introduction via arthropod vectors is likely. The resident species of *Phialophora*, *Exophiala* and *Acremonium* are particularly significant as they are shown to be disseminated efficiently by public drinking water.

Key words: Hygiene – fungi – *Phialophora* – *Acremonium* – *Exophiala*

Introduction

Fungi are receiving growing attention as agents of human infections and allergies (Guarro and Gené, 1992, Beck-Sagué et al., 1993). Factors involved in airborne fungal transmission, such as dampness and circulation promoted by air-conditioning systems, are well documented. In contrast, data on the role of drinking water in the dissemination of fungi are scarce and contradictory. Studies of potable water in hospitals and private dwellings indicate that the public drinking water system contributes to transmission of allergenic, toxigenic and opportunistic

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fungi. Dissemination of water-borne fungal propagules to hospitals and houses has been supposed, and a possible health impact was surmised (Annaissie et al., 1997; Arvanitidou et al., 1999; Warris, 2000). A wide diversity of fungal species has been isolated from water, from deposits and corrosion products of pipe surfaces, and from coatings in reservoirs, gaskets and sealings (Burman, 1965; Barth, 1969; Rosenzweig and Pipes, 1986; Roesch and Leong, 1983; Franke, 1993, Paterson et al., 1997; Kinsey et al., 1999; Doggett, 2000). Direct observation of isolation filters has shown that fungi are transported in water in the form of conidia but also as hyphal fragments (Kinsey et al., 1999). Some fungi are capable of sporulating when submersed, and can grow at low oxygen tension (de Hoog et al., 1994). It is largely unknown which fungi are resident and capable of surviving or even colonizing and contaminating the drinking water system. Therefore, data on the frequency of individual species throughout the water system is needed. In the present investigation, data is presented on the occurrence of fungi in mostly unchlorinated drinking water from 29 localities in the Lower Rhine region of North Rhine-Westphalia (NRW), Germany. Frequencies of the most common fungal species and the species diversity in small samples from wells, waterworks, distribution networks, from locations with newly buried pipes and house installations were determined on the basis of prolonged incubation of 1 ml samples on agar-based culture media. The results were correlated with bacteriological data assessed by the same method. Since several of the fungi encountered were difficult to identify phenotypically, additional RFLP and sequencing of the ITS domain of the rDNA operon was performed for the most prevalent species.

Materials and methods

Study design

Between October 1998 and late September 1999 2657 water samples were collected from 700 locations in 29 water localities in North Rhine-Westphalia (NRW, Germany) (Table 1). Samples were taken routinely in the framework of bacteriological monitoring according to the German Drinking Water Regulations (Anon, 1990) and the Raw Water Guideline (Anon, 1991). These included ground water wells, waterworks and storage tanks, hydrants in the distribution network, and water taps immediately after the watermeter or elsewhere in house installations. Moreover, analyses were performed subsequent to the burial of new pipes. Water samples were described according to sampling date, disinfection regime and characteristics of the sampling location. The names of water suppliers and locations were kept anonymous. Since the major part of the drinking water supply in the selected area is derived from groundwater, most samples were taken from unchlorinated water.

Bacteriological analyses

Isolation of bacteria was performed according to German drinking water regulations (Anon, 1990). Colonies were enumerated by the pour-plate method (Anon, 1988) using blood agar base (Oxoid, Wesel). Aliquots of 1.0 ml and 0.1 ml of water were pipetted into culture plates and mixed with 10 ml of medium (48 °C). Plates were incubated at 36 °C ± 1 °C and 20 °C ± 2 °C for 44 ± 4 h. *E. coli* and other coliform bacteria were detected according to the German Standard DIN 38 411, Part 6 (Anon, 1991). For better interpretation of results, samples were classified according to categories of colony counts as shown in Table 2.

Mycological analyses

Isolation of fungi was performed using 1.0 ml water samples as described for the bacteriological analyses. Culture plates with blood agar base (Oxoid) were

Table 1. Type of water and number of sampling locations and water samples analysed.

Type	Location	Sampling locations n	Water samples total n	Samples unchlorinated n	Samples chlorinated n
I	Raw water	183	511	511	–
II	Waterworks	73	417	375	42
III	Distribution network	57	373	320	53
IV	House installations	144	686	617	69
V	Newly buried pipes	243	670	660	10

I: ground water ponds, II: during steps of water treatment in waterworks, at the outlet of waterworks and in storage tanks next to waterworks, III: at the outlet of drinking water tanks in the distribution network, at fire hydrants in the distribution net, after the watermeter; IV: in house installations; V: at newly buried drinking water pipes placed into operation.

Table 2. Categories of bacterial colony counts as used for evaluation of the bacteriological quality of drinking water (Anon, 1990)

Categories	(0–20]	(20–50]	(50–100]	>100
Bacterial colony counts 20 °C Bacterial colony counts 36 °C	Negligible	Slightly elevated	Distinctly elevated but within the legal limits	Above the notifiable level

incubated at 20 °C ± 2 °C for up to 4 weeks and examined weekly. Fungal colonies were enumerated and subsequently transferred to fresh potato dextrose agar (PDA, Merck, Darmstadt) and malt extract agar (MEA, Merck) and maintained at 20 °C ± 2 °C. Representative isolates were stored on PDA slants at 10 °C. Numbers of colony forming units on agar plates were expressed as numbers of colonies per ml water sample. Frequencies of fungi in water samples were given in total, as percentages of the total number of positive samples, and as percentages of fungus-positive samples in each of the following categories: raw water (I), waterworks (II), distribution network (III), and house installations (IV). Data obtained subsequent to the sampling of newly-laid water pipes (V) was given separately. Furthermore, in some cases samples taken directly after the watermeter (III) and elsewhere in the house installation (IV) were compared.

Identification of fungi

Whenever possible, filamentous fungi were phenotypically identified down to species level by morphology and microscopic characters (Booth, 1966; Domsch et al., 1980, Gams, 1971; Nelson et al., 1981; de Hoog et al., 2000). Slide preparations were stained with lactic acid / Cotton Blue either with or without ethanol, in Melzer's reagent, or in water. The morphological identity of a limited number of representative strains was confirmed at the Centraalbureau voor Schimmelcultures. Melanized fungi which were detected regularly but proved difficult to identify by morphology alone were characterized genotypically according to methods outlined by Gerrits van den Ende and de Hoog (1999); data will be presented elsewhere (de Hoog et al., in prep.). Yeasts which were cultivable under the conditions described were identified by their physiological and morphological characteristics (Barnett et al., 1990).

Physicochemical analyses

Free chlorine and chlorine dioxide was measured according to DIN 38 408, Part 4 (Anon, 1984). Briefly, at pH 6.2 – 6.5 chlorine or chlorine dioxide react with N,N-Diethyl-1,4-phenylendiamine under the formation of a red compound. The intensity of the red colour is measured with a photometer. Because of the higher molecular weight of chlorine dioxide the results of the measurements had to be multiplied by a factor of 1.9.

Statistics

All statistical tests were performed with SPSS for Windows, version 9, and the 5% level was used as a cut-off value of significance. The Chi-square test was used to test for significant differences in fungal and bacterial frequency distribution among the 5 groups considered (different sampling locations). The Kruskal-Wallis test was used to analyze differences in bacterial colony counts between sampling locations. Spearman's rank correlation coefficient was used to measure the monotone relationship between bacteriological and fungal occurrences. Difference in species diversity between the groups of sampling locations was assessed by the Shannon Index and the Phi scattering index (Vogel, 1994). Both indices are based on the numbers of taxa identified in the groups not considering sterile mycelia and unidentified species (Nübel et al., 1999). The Chi-square test was used to examine whether repeatedly isolated species were equally distributed between the different groups of water samples. This test was also used to determine possible correlations between fungal frequencies and chlorine dioxide on the one hand, and between *E. coli* or coliform bacteria and the particular sampling locations on the other. To analyse whether the distribution of the different groups is the same for the genera *Fusarium*, *Penicillium* and *Verticillium*, the method called "test for differences between two shares" was used, i. e. the two groups with the largest and the smallest relative frequencies were used for comparison.

Results

Frequencies of fungus-positive samples

Of the 2657 water samples examined 199 (7.5%) were positive for fungi (Table 3). Frequencies of distribution varied little for different locations (I – IV), with differences being insignificant. Numbers of positive samples were relatively low (5.1%) in raw water (I). Somewhat higher numbers were noted in samples taken after brief operation of newly laid pipes (V) and from waterworks (II) (9.1% and 8.4%, respectively). Downstream from the watermeter, counts were slightly lower (4.3%, data not shown) compared to other locations in the distribution network (III) (7.5%) and in household installations (IV) (7.1%), but differences proved to be insignificant.

Fungal recovery from water samples

Colony counts varied little between samples, with 1 cfu per ml measured in 68% of the positive samples, 2 cfu per ml in 13% of samples and higher numbers encountered only rarely. An exceptional recovery of 41 cfu per ml was found in a single case of *Paecilomyces farinosus*, but this high number is likely to be an artefact due to the masses of dry-conidia produced by this filamentous fungus. In most cases, colony forming units on culture plates were not visible within 2 days of incubation, while a maximum recovery rate was obtained after prolonged incubation (2 weeks), indicating the slow radial growth of many members of the water-inhabiting fungal flora.

Table 3. Numbers of samples positive for fungi and numbers of bacteria from 2657 samples from raw drinking water, waterworks, distribution networks, house installations and newly buried pipes. Results are expressed as categories of bacterial cfu on pour plates per ml.

	No. of samples	I Raw water	II Waterworks	III Net- work	IV House installation	V Newly laid pipes	Total
Positive samples							
Fungi 20 °C	n	26	35	28	49	61	199
	%	5.1	8.4	7.5	7.1	9.1	7.5
Coliform bacteria	n	10	13	7	15	n.e. [1]	45
	%	2,0	3,4	1,9	2,2		2,2
E. coli	n	4	1	1	1	n.e. [1]	7
	%	2,5	0,3	0,3	0,1		0.4
Bacterial colony counts 20 °C, [cfu / ml]							
0–20	n	476	375	345	658	n.e. [1]	1854
	%	93.5	93.5	92.5	96.1		94.2
20–50	n	15	13	17	15		60
	%	2.9	3.2	4.6	2.2		3.0
50–100	n	7	3	4	4		18
	%	1.4	0.7	1.1	0.6		0.9
>100	n	11	10	7	8		36
	%	2.2	2.5	1.9	1.2		1.8
Bacterial colony counts 36 °C, [cfu / ml]							
0–20	n	180	392	360	625	n.e.[1]	1557
	%	91.4	95.4	96.5	91.1		93.4
20–100	n	8	9	6	29		52
	%	5	2.2	1.6	4.2		3.1
50–100	n	2	2	5	13		22
	%	1.0	0.5	1.3	1.9		1.3
>100	n	7	8	2	19		36
	%	3.6	1.9	0.5	2.8		2.2

[1] n.e.: not evaluated.

Fungal diversity and species spectrum

The lowest fungal species diversity was found in samples from the distribution network (III), followed by the waterworks (II), whereas diversity was significantly higher in raw water (I), house installations (IV) and water obtained from newly buried pipes (V). Most species were either restricted to particular locations or were rarely encountered in the drinking water systems (Tables 4 and 5). In contrast, there was a restricted number of species within a relatively few, ubiquitous taxa. From a total of 21 genera represented by 66 species, only four genera, viz. *Phialophora, Acremonium, Exophiala* and *Penicillium* (listed in decreasing order of abundance) were determined in 4 or all sampling sites (Table 4). Characteristically, the first three genera were represented by small numbers of species in all types of water, sometimes isolated repeatedly from the same locality. *Penicillium* showed a slightly different distribution pattern, with isolates cultivated mostly from locations I, II and IV but not from the network (III) (Table 4). *Phialophora* sp. nov. was found in 26.6% of all fungus-positive samples and was equally distributed over all types of water (I–V). Sequence analysis of the ITS domain of the rDNA operon confirmed the presence of a previously undescribed species, differing significantly from its closest relative, the plant-inhabiting *Phialophora cinerescens*, by a large Indel in ITS1 and by more restricted colony sizes (de Hoog et al., in prep.). The second most widespread group was *Acremonium*, with *A. arxii, A. strictum* and *A. berkeleyanum* as prevalent species. *Aspergillus* species such as *A. fumigatus, A. niger, A. flavus* and *A. versicolor* were rarely encountered. The *Exophiala* species *E. angulospora* and *E. castellani* were less common in Category V than at other locations, while *Verticillium lecanii* and *Phoma leveillei* showed the opposite distribution pattern, being found exclusively at locations where newly buried pipes had been put into service (V). *Fusarium* species, especially *F. solani* and *F. merismoides* var. *merismoides*, were mainly found in Category V. The somewhat higher frequency of *Exophiala* in samples from waterworks was due to samples from a single waterworks.

Bacteria

Of the 1968 water samples from raw water (I), water works (II), network (III) and house installations (IV) evaluated, 2.2% did not conform with German drinking water regulations (Anon, 1990) (Table 3). Coliform bacteria were found in 1.8%, and *E. coli* in

Table 4. Fungal genera and numbers of fungus-positive samples from raw water, waterworks, distribution networks, house installations and newly laid pipes.

Fungal taxa	Number of positive samples in types of locations				
	I Raw water (total n 511)	II Waterworks (total n 417)	III Networks (total n 373)	IV House installation (total n 686)	V Newly laid pipes (total n 670)
Zygomycetes					
Conidiobolus	–	–	–	–	1
Mucor	1	–	1	–	–
Hyphomycetes					
Acremonium	1	1	4	12	6
Aspergillus	2	1	–	–	1
Chalara	–	2	–	–	1
Cladosporium	2	–	–	1	1
Exophiala	2	9	2	6	1
Fusarium	–	–	–	1	6
Geomyces	–	–	–	1	1
Humicola	–	–	–	–	1
Myrothecium	–	–	–	–	1
Ochroconis	–	–	1	1	–
Paecilomyces	1	–	–	–	3
Penicillium	5	2	–	4	3
Phialophora	8	10	11	19	16
Plectosporium	–	–	–	1	–
Tilletiopsis	1	–	–	–	–
Verticillium	1	–	–	–	7
Volutella	–	–	1	–	
Phoma	–	–	1	–	4
Yeasts					
Rhodotorula	–	–	–	1	1
Number of genera	10	6	7	10	16
Sterile mycelia	1	2	3	6	5
Unidentified	3	6	4	6	15

was found in 0.4% of the samples. Sampling locations with newly buried pipes were excluded from this analysis because they are not representative of long-term water quality.

Correlation of fungal and bacterial findings

No correlation ($P < 0.05\%$) in frequency distribution was observed between fungi and the standard hygienic indicators for faecal contamination, i. e. *E. coli* and other coliform bacteria. A negative correlation of fungal and bacterial counts was found in samples with bacterial colony counts above the acceptable level (Anon, 1990, Table 1). For samples in the categories of distinctly high bacterial counts (51 – 100) and slightly elevated bacterial colony counts (21 – 50), no indication for differences in frequencies of fungi was found. The presence of the most common dematiaceous fungal genera *Phialophora* and *Exophiala* was not correlated. Notable differences in the occurrence of fungi were observed between chlorinated and untreated water (3.4% and 7.8%, respectively), but these results are insufficiently supported due to the limited number (6.5%) of chlorinated water samples analyzed in this study.

Discussion

A limited number of investigations on fungi in drinking water has been performed to date (Rosenzweig et al., 1983; West, 1986; Rosenzweig and Pipes, 1986; Bagge and Mikkelsen, 1990; Nagy and Olson, 1982; Lahti, 1993; Frankova and Horecka, 1995; Arvanitidou et al., 1999; Kelley et al., 1997). Most authors agree with our observation that fungi are regularly present, but their significance for hygiene and public health seems to be less evident. Individual cases document the possible transmission of fungal spores in water-requiring devices attached to plumbing systems in hospitals (Anaissie et al., 1997; Warris, 2000), fungal contribution to odour production (Kikuchi et al., 1983; Rosenzweig et al. 1983; Wood et al., 1983; Sävenhed et al., 1991), damage of internal wall coatings in drinking water reservoirs (Barth, 1969; Franke, 1993), corrosion

Table 5. Predominant species and number of findings ranked according to the frequency of genera (%) in fungal positive water samples.

Fungal Genera	Species and number of findings in parenteses	Total occurrence (%)
Phialophora	*Phialophora* sp. nov. (55), *Phialophora* sp. 2 (1), *Ph. sessilis* (1), *Phialophora* sp. 3 (1), *Phialophora* sp. 4 (2), *Phialophora* sp. 5 (1), Not identified isolates (3)	32.7
Acremonium	*Acremonium* cf. *arxii* (8), *A. berkeleyanum* (4), *A.* cf. *berkeleyanum* (1), *A. strictum* (5), *A. psammosporum* (1), *Acremonium* spp. (5)	12.1
Exophiala	*Exophiala angulospora* (3), *E.* cf. *angulospora* (4), *E. castellanii* (6), *E.* cf. *pisciphila* (3), *E.* aff. *Pisciphila* (1), *Exophiala* spp. (3)	9.5
Penicillium	*Penicillium chrysogenum* (7), *P. brevicompactum* (1), *P. glabrum* (1), *Penicillium* spp. (10)	7.0
Verticillium	*Verticillium lecanii* (7), *V. tenereum* (1)	4.0
Fusarium	*Fusarium merismoides* (3), *F. solani* (2), *Fusarium* spp. (2)	3.5
Phoma	*Phoma herbarum* (2), *P. homa leveillei* (3)	2.5
Aspergillus	*Aspergillus flavus* (1), *A. fumigatus* (1), *A. niger* (1), *A. versicolor* (1)	2.0
Cladosporium	*Cladosporium herbarum* (2), *C. cladosporioides* (1), *Cladosporium*-like (1)	2.0
Paecilomyces	*Paecilomyces farinosus* (4)	2.0
Chalara	*Chalara sp.* (3)	1.5
Other filamentous fungi ($\geq$1.0%)	*Mucor*, *Geomyces*, *Ochroconis*, *Conidiobolus, Humicola, Myrothecium, Tilletiopsis, Plectosporium*, *Volutella*, Yeasts	4.5

(Emde et al., 1992), and interference with disinfection in drinking water supplies (Kelley et al., 1997); however, a general view is still lacking. Variation in the research methods, sample sizes and types of water analyzed differ widely. No standard methods for cultivation and enumeration of fungi in drinking water have been universally accepted yet.

Geldreich (1986) reviewed older studies on filamentous fungi and yeasts in potable water. In Europe and the USA positive samples usually ranged from 17% and 60%, occasionally up to 90%. Similar frequencies were reported in similar studies (Siebert, 1976; Anaissie et al., 1997; Arvanitidou et al., 1999; Rechenburg et al., 2000). In Norway, the abundance of fungi in surface and treated water with little or no disinfectant varied between 1 and 10 cfu per ml (Ormerod, 1987), whereas cultivable fungi from surface water in Slovakia ranged from 1 to 45 cfu per ml (Tothova, 1999). The abundance of fungi in surface and ground water in selected localities in the USA were 18 cfu per 100 ml and 34 cfu per 100 m, respectively (Nagy and Olson, 1982) and thus well below numbers of culturable bacteria (Geldreich, 1986). For a water system in the USA drawing lake water, fungal numbers ranged from 1 to 15 cfu per 100 ml (West, 1986). Lathi (1993) stated that yeasts and filamentous fungi were frequent in water distributed from ground and surface supplies in Finland, but fungal densities rarely exceeded 100 cfu per 100 ml, a level above which taste and odour problems have been reported (Åkerstrand, 1987). In South Africa, fungal frequencies in drinking water from houses varied between 0 and 3×10^3 cfu per 100 ml (Augustionos et al., 1995). Average counts of 36.6 cfu per 100 ml were reported for filamentous fungi in tap water from hospitals and communities in Greece, with the finding that values in domestic water supplies were significantly higher (Arvanitidou et al., 1999).

In our one-year survey covering 29 drinking water supplies, we focused on the distribution pattern of fungal species as detectable in routinely analyzed drinking water samples. The average frequency of 7.5% of fungus-positive samples of 1 ml was considerably below values reported in the literature. This can partly be explained by differences in methodology. Most investigations have applied membrane filter techniques with volumes of 20–1000 ml, resulting in minimum detection limits down to 1 cfu per 1000 ml. The detection limit in the present study was 1 cfu per ml and thus approximates the upper range of values reported in most studies of fungi from drinking water. The critical value of >1 cfu per ml (Åkerstrand, 1987) was found in 2.4% of all samples. "High" levels ($\geq$10 cfu per ml, Holmberg, 1986) were observed in only three samples.

Species identification provided insight into the distribution of most of the abundant fungi throughout the municipal drinking water network. Diversity in fungal species detectable in 1 ml samples in the network (III) was significantly lower than in raw water (I) and in house installations (IV), indicating that not all species are able to survive following introduction into the system. Among the 66 species of 21 genera isolated here, only a few species were widespread. According to the model of Park (1972) concerning ecological classification of heterotrophic

microorganisms in fresh water, the fungi which occasionally enter drinking water and subsequently become inactive or lose viability may be regarded as transients with perhaps little ecological significance, whereas fungi of any ecological significance and activity in water (viability, decomposition, colonization) may be regarded as residents, irrespective of origin and whether they are temporary, periodic or permanent. The melanized genera *Phialophora* and *Exophiala*, as well as *Acremonium* were found to be particularly widespread. No localized peaks in frequencies were evident for these species, indicating a broad capacity to adapt to the drinking water environment. Therefore, they may be regarded as a main part of the resident mycoflora in unchlorinated water derived from ground water. Melanization and production of slimy conidia were recurrent features of resident fungi. West (1986) found a predominance of the melanized genera *Cladosporium*, *Phoma*, *Alternaria* and *Exophiala* in drinking water derived from lake water. Species belonging to the genera *Fusarium*, *Acremonium*, *Exophiala* and *Phialophora* have been reported regularly among fungi isolated from municipal water systems in the USA (Nagy and Olson, 1982; West, 1986), Germany (Siebert, 1976; Ossmann, 1979) and in the UK (Kinsey et al., 1999).

The predominance of the genus *Phialophora* (32.7%) in our study is notable, indicating the capacity to survive and possibly colonize drinking water systems. This is particularly characteristic of *Phialophora* sp. nov. (26.6%), a species previously recorded as *P. cinerescens* in drinking water in Finland (de Hoog et al., 1999), but which to date has not been reported from Germany. Occasionally, other *Phialophora* species have been mentioned in the literature concerning drinking water and ponds; these include *P. malorum*, *P. verrucosa* and *P. (Lecythophora) hofmannii* (Ossmann, 1979) and *P. fastigiata* (Ossmann, 1979; Kinsey et al., 1999). However, some of these species are thermotolerant, e.g. *P. verrucosa* which tolerates 37 °C (de Hoog et al., 1999). Thus, perhaps misidentifications for *Phialophora* sp. nov. are concerned, a species unable to grow at temperatures above 32 °C (de Hoog et al., 2000). *Phialophora* sp. nov., an ubiquitous melanized resident, is likely to have a broad capacity to survive disinfection regimes (Philipps et al., 1999).

Less common members of the genus *Acremonium* are also relevant. *A. arxii* has been isolated from drinking water in Germany and Sweden, as has *A. berkeleyanum* from Turkey (Centraalbureau voor Schimmelcultures List of Cultures). Siebert (1976) found *Acremonium* spp. to be the most common isolate in drinking water from house installations in North Rhine-Westphalia (NRW), Germany. *Acremonium* and *Fusarium* contain species encountered in soil and especially in cold climates (R. C. Summerbell, pers. comm.). Also, *Fusarium* and *Acremonium* species have been reported from opportunistic infections in humans (de Hoog et al., 2000). Occurrence of *Fusarium* spp. in groundwater, surface water and in tap water has been documented (Gerlach and Nirenberg, 1982; Frankova, 1993; Anaissie et al., 1997). *Fusarium* sp. has been isolated from the polyurethane caulking material used in a municipal water system in the USA, together with *Petriellidium boydii* (*Pseudallescheria boydii*) (Roesch and Leong, 1983). In our study, *F. merismoides, F. solani* and two unidentified species were present in an average of 3.5% of all fungus-positive samples. *F. merismoides* var. *crassum* was shown to be able to survive in a building's plumbing system for several years (Gerlach, 1972). *F. solani* is the most common species in human *Fusarium* infections (Guarro and Gené, 1992).

The genus *Exophiala* contains a number of human pathogens (de Hoog et al., 2000). Other *Exophiala* spp. are known to occur in freshwater (Uijthof et al., 1997), shower recesses (Listemann and Freiesleben, 1996), swimming pools and public bathing facilities (Nishimura et al., 1987; Aho and Hirn, 1981, Nishimura and Miyaji, 1982, Haase, et al., 1994; Matos et al., 2001). We found some members of the *E. pisciphila* complex which have been reported from opportunistic infections in young cultivated fish, probably after the fungus passed through a sand filter (Uijthof et al., 1997). *Exophiala angulospora* was reported from a drinking water well in Japan (Iwatsu, 1991). Another taxon had the characteristic morphology of *E. angulospora,* but was clearly different in its ITS sequence and may be an undescribed species (G. S. de Hoog, unpublished data); it is here referred to as *E. cf. angulospora*. Another repeatedly encountered *Exophiala* species was *E. castellanii*. This species is otherwise known only from the type strain (CBS 158.58) which was reported to have originated from human skin (Castellani, 1905) but actually the origin of this strain is unclear; other clinical reports of *E. castellanii* concern misidentifications (G.S. de Hoog, unpublished data). The frequent occurrence of several *Exophiala* species in drinking water suggests a marked ecological preference of these species for this environment. Notably, these species were more common in the network with lower general biodiversity than at locations with newly buried pipes (V). In contrast, *Verticillium lecanii*, a fungus known from insects and mites in soil (Domsch et al., 1980) and *Phoma leveillei* were found exclusively at

locations of the latter. Thus, these species seem to belong to a transient fungal flora with localized distribution in the network. For *Verticillium lecanii*, introduction via soil and arthropod vectors is likely (W. Gams, pers. comm.). *Phoma leveillei* has been isolated in a case of heavy fungal colonization of a plumbing system and cistern in a private house, where contamination via repair works was suspected (E. Göttlich, unpublished data). In general, the prevalence of fungi from newly buried pipes (V) did not differ significantly from the other sampling sites (I–IV), but it must be kept in mind that sample volumes were small and therefore not suitable for detection of low quantities of fungi.

Species of *Penicillium* found in 6.3% of fungus-positive samples at all locations except for the network (III) seem to have a different strategy to survive in drinking water. Conidiation in these species is adapted to spread via air (hydrophobic conidia), and thus a contamination via air or soil is probable. They may be found in water storage tanks above the air-water interface (Barth, 1969; Franke, 1993), and are likely to dominate in surface water rather than in groundwater (Frankova, 1993). Nevertheless, *Penicillium* species have been reported from contaminated groundwater, distribution systems, and household installations in Slovakia (Frankova, 1993), the U.K. (Kinsey et al., 1999), the USA (Nagy and Olson, 1982), and Greece (Arvanitidou et al., 1999), sometimes among the most numerous species.

Aspergillus fumigatus, *A. niger* and *A. flavus* are common allergens and may cause opportunistic invasive infections in hospitalised, immunocompromised patients (de Hoog et al., 2000). Allergenic, toxigenic and opportunistic fungi may be found in drinking water, and the potential health hazards they pose have been outlined by Niemi et al. (1982) and Geldreich (1986). Muittari et al. (1980) reported an epidemic of extrinsic allergic alveolitis caused by tap water in Finland, although the implication of the fungi was not convincing. Arvanitidou et al. (1999) found *Aspergillus* spp. to be one of the more common genera in drinking water. In Norway, where hospital water was discovered as a source of *A. fumigatus*, transmission via the public drinking water system was supposed (Warris, 2000). In our survey, fungi of the genus *Aspergillus* were rarely encountered in the drinking water system; *A. fumigatus* was found in a single case in a waterworks. This marked discrepancy is probably explained by the fact that in our case the isolation method was not suited for the detection of single active or inactive fungal propagules entering the water system. Moreover, the drinking water originated from groundwater, whilst most drinking water in Norway is derived from surface water (Ormerod, 1987) which is purified by sand filters, through which *Aspergillus* conidia can easily pass (A. Warris, pers. comm).

In the present investigation, the frequency of filamentous fungi in small water samples was much higher than that of yeasts, which is in agreement with the observations of Hinzelin and Block (1985), Alvarez (1993), and Arvanitidou et al. (1999). This can probably be explained by the fact that yeasts are mostly associated with nutrient-rich substrates whereas among filamentous fungi oligotrophs are more common. There was no correlation between total counts of bacteria below the acceptable value (100 cfu per ml) and filamentous fungi. This was also found to be the case for studies in the USA (Nagy and Olson, 1982) and in Denmark (Bagge and Mikkelsen, 1990). In case of higher bacterial counts, a negative correlation was found, probably reflecting antagonistic behaviour of bacteria and fungi in culture (Griffin, 1972; Hinzelin and Block, 1985). These data contradict an analysis of drinking water in Greece, where a positive correlation was found between filamentous fungi and heterotrophic bacteria (Arvanitidou et al., 1999). These authors however, did not find any correlation between numbers of fungi and *E. coli* and coliform bacteria, which corresponds to our results.

Conclusions

One of the central issues confronting water suppliers is whether harmful contaminants in hospitals and private houses may originate from the public drinking water system. Although this may yet hold true for drinking water derived from surface water, it seems unlikely for groundwater-derived potable water in NRW. Potentially harmful fungi were rarely noted in the course of our study. In contrast, we revealed the existence of a resident fungal flora containing a limited number of adapted, somewhat psychrophilic species not found outside oligotrophic waters. Their function in drinking water ecology is unknown, as is their significance for drinking water hygiene and routine maintenance of the distribution system. Since the majority of these organisms are heavily melanized, they are likely to have a broad capacity to survive disinfection. However, our results indicate that public drinking water seems to play a key role in dissemination of these species. The existence of preferred colonization sites, such as biofilms and

other deposits in the network, and sealings and coatings in drinking water reservoirs seems likely. To investigate this hypothesis, the colonization pattern of materials used in the drinking water is currently under investigation.

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