

Fungi in water samples of a full-scale water work

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Abstract

This study aimed to assess the efficacy of the treatments used to eliminate fungal propagules in water plants and to investigate the biodiversity of fungal taxa in treated and drinking water. We investigated water from nine sites in two water plants and from one fountain. Up to 25 samples from each site were analysed. Identification of fungi was carried out mainly by matrix-assisted laser desorption ionisation–time of flight (MALDI-TOF) mass spectrometry (MS) or, if no MALDI-TOF MS reference spectra were available, by internal transcribed spacer (ITS), partial transcription elongation factor 1 (*tef1*) or partial beta-tubulin sequencing. A total of 92 taxa could be identified by either MALDI-TOF MS or sequencing. The taxonomic spectrum is in agreement with that reported in publications on fungal communities of drinking water. The treatment steps used reduced significantly fungal colonisation of the water: even if the final products (drinking water) from both plants differ statistically in their fungal colony-forming units (CFUs) content, the difference is not biologically meaningful. A multivariate analysis showed a separation of the sampling sites of the two plants, reflecting the different origin of the raw water and the different water treatment processes. Reduction of fungal CFU and number of taxa by the treatment steps was similar in the two plants and not influenced by the treatment methods. In both plants, the first step was responsible for at least 90% reduction of CFU. Low CFU levels were maintained over the whole process chain and the water transport to the drinking water fountain did not significantly modify the CFU number.

Keywords Ecology · Identification · ITS · MALDI-TOF · Taxonomy · Ascomycota · Drinking water

Introduction

Drinking water usually originates from aquifers, springs or surface water. Ground- or spring water is often suitable for human consumption without any treatment. In contrast, water from lakes, rivers and reservoirs often contains organisms (Rose and Botzenhart 1990; Schleupen 1996) that are

potentially harmful (WHO 2017) and need to be processed before human use.

In modern water plants processing surface water, several cleaning and disinfecting steps are used to produce drinking water. The purification process can be carried out by chemical methods such as oxidation with elemental chlorine, chlorine dioxide or ozone, and physical treatments, including filtration with a variety of materials such as sand, anthracite or activated carbon, and membrane filtration (Kelley 2001; Lemley et al. 1995; Mauclair et al. 2004; Niemi et al. 1982).

Chlorine and ozone are very potent disinfecting agents that inactivate microorganisms and viruses (Lezcano et al. 1999; Rennecker et al. 1999). In addition, ozone can induce flocculation and break down complex organic compounds into degradable molecules (Jekel 1994). Filters retain particles and microorganisms residing in the filters can eliminate biodegradable organic matter; modern membranes can also retain particles as small as viruses (Harman et al. 2010; Jermann et al. 2008; Teunis et al. 2009). UV irradiation can serve as a last disinfection step before the clean water is pumped into the reservoirs and the distribution network (Schwartz et al. 2003). Drinking water must meet high quality requirements and comply with local regulations. No legal

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limits have been set for fungi, although their presence in drinking water is unwelcome (Sonigo et al. 2011).

Fungi can be abundant in surface water sources (WHO 2017) but at least in Europe they are rarely present in water to be used for human consumption. Most fungi are not adapted to aquatic environments, but propagules of several yeasts and filamentous fungal taxa can survive in surface water, with some species having adjusted to living in aquatic systems (Jones et al. 2014) and playing a major role in the decomposition of organic matter (e.g. Cornut et al. 2012). Fungal spores can enter drinking water systems through several pathways (Sonigo et al. 2011), survive as planktonic forms in the water (Jones et al. 2014), or colonise the inner surface of pipes (and possibly filters) through biofilm formation (Douterelo et al. 2016).

Only scant information exists on the presence of fungi in treated drinking water (Doggett 2000; Göttlich and Flemming 2002; Göttlich et al. 2002; Grabińska-Loniewska et al. 2007; Hageskal et al. 2006; Hageskal et al. 2009; Kanzler et al. 2008; Pereira et al. 2010; Sonigo et al. 2011). The most recent studies still report colony-forming unit (CFU) frequencies from 1 to 5000 per litre, the most frequently isolated taxa being *Acremonium*, *Aspergillus*, *Cladosporium*, *Penicillium* and *Phialophora* species (Hageskal et al. 2006; Hageskal et al. 2009; Sonigo et al. 2011). While some of them may be opportunistic human pathogens or toxins and/or allergen producers, it is generally accepted that their presence at low frequencies in drinking water should not be of concern but for the development of foul taste and odour (Hageskal et al. 2006) and, most importantly, their potential for causing hospital-acquired infections (Mesquita-Rocha et al. 2013; Sonigo et al. 2011). Water treatment processes have been shown to be effective in reducing (but not eliminating) the number of fungal CFUs in water (Sonigo et al. 2011).

Limited data are available on the occurrence of fungal taxa in untreated vs. treated water and the efficiency of current disinfection and treatment practices on their frequency. This study aimed to assess the efficacy of the treatment schemes used to eliminate fungal propagules. Further objectives were to investigate the biodiversity of fungal taxa (most likely present as spores) in water undergoing treatment processes and in drinking water produced by two water plants obtaining their water from two ecosystems and using different treatment processes, as well as to obtain information on the potential occurrence of toxigenic or pathogenic species in the water systems tested.

Material and methods

Sites studied

We investigated water from different sites in two water plants (Hardhof and Lengg) in Zurich, Switzerland. Both water plants deliver drinking water to the town and its surroundings.

In Hardhof, groundwater, recharged with disinfected (chlorinated) river bank filtrate, is pumped from the aquifer; in Lengg, lake water passes several treatment steps before it is pumped into the drinking water reservoirs. The sites studied are shown in Fig. 1 and the water quality is described in Table 1.

At the Hardhof plant, sampling site H1 is untreated water from the River Limmat, an effluent of Lake Zurich. Its water quality varies according to the water level, the season and weather conditions. Site H2 is a natural river bank filtrate, and H3 is a chlorinated river bank filtrate (disinfection with chlorine and chlorine dioxide) after passage through a sand filter below the recharge basin. H2 and H3 contain varying amounts of natural groundwater. H4 is a mix of varying percentages of natural groundwater, recharged groundwater from injectors and recharge basins, and direct inflow of river bank filtrate.

Sampling sites at the Lengg plant included L1, with untreated water from the mesotrophic Lake Zurich. The water catchment is located 32 m below the surface: at this depth, the water has a constant temperature of 4–8 °C. The plankton and nutrients contents depend on the stratification of the lake: during summer the catchment is below the thermocline and the water is relatively poor in algae, while in winter the lake mixes and organisms from the epilimnion are frequent. L2 contains water after pre-oxidation with ozone; L3 water after rapid sand filtration; L4 water after intermediate ozonation (disinfection step); and L5 water after activated carbon filtration and slow sand filtration, to be then released as drinking water into the reservoirs.

Up to 25 samplings were carried out at each Hardhof and Lengg site from March 2014 to March 2015 and April 2015 to February 2016, respectively, at 2-week intervals. In addition, drinking water from a fountain in the town of Zurich, Switzerland, selected because of its remote location and the long retention time of the water in the pipes, was also sampled during the period March 2014 – July 2014 (Table 1).

Sampling and isolation of fungi

One litre of each water sample was collected and one millilitre of a sterile 0.1 M sodium thiosulfate solution was immediately added to samples originating from ozonated water to stop ozone activity. Samples were brought back to the laboratory and dilution series (for samples of raw water) were prepared for further processing. The water was then filtered using sterile membrane filters (pore size 0.45 µm, Whatman cat. no. 10407314) and the filters were incubated for 7 days at 20 °C on Sabouraud glucose selective agar supplemented with chloramphenicol and gentamicin (Oxoid, article no. PO5096A) to suppress bacterial growth. Fungal growth was checked after 7 days and the CFUs were counted.

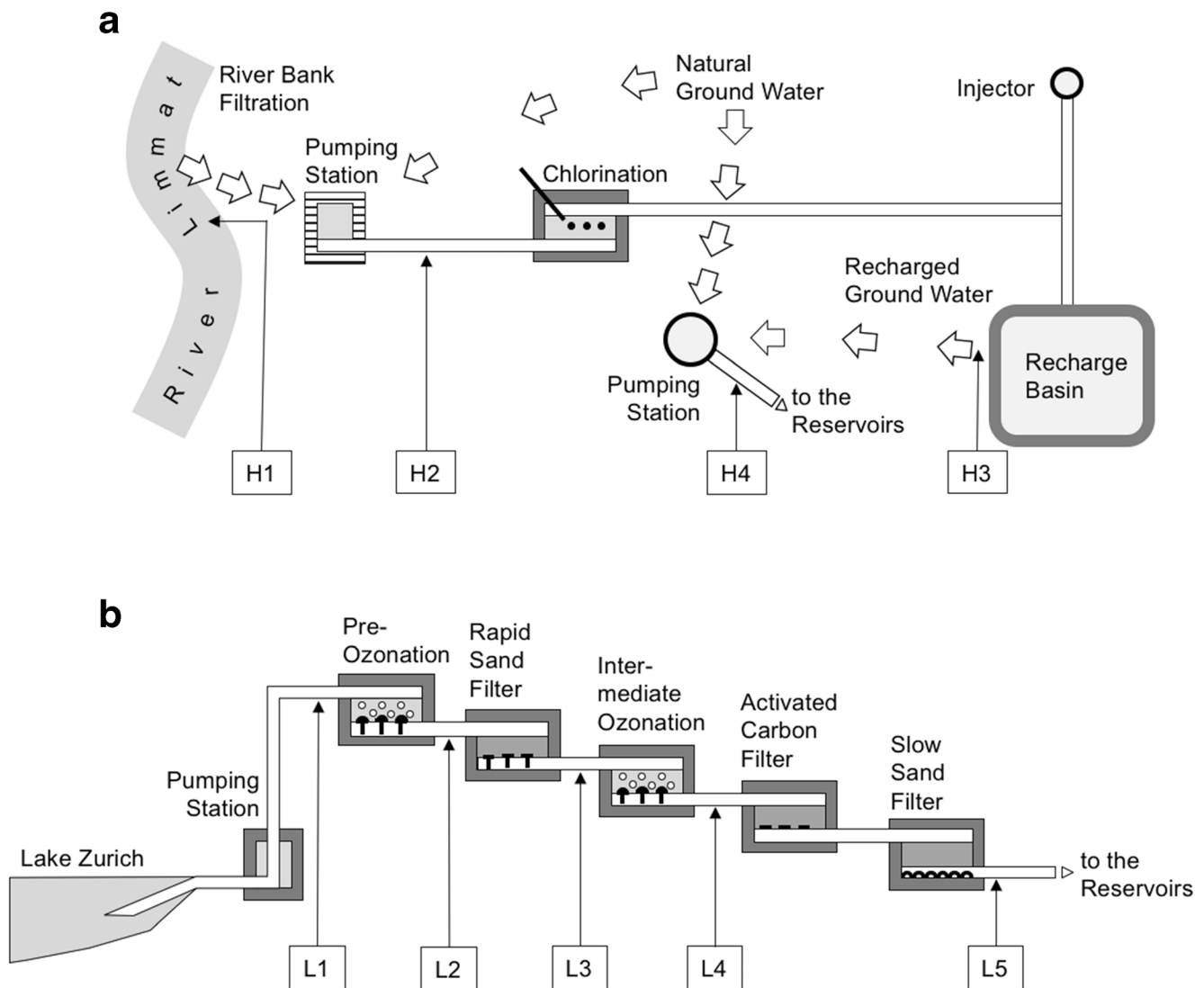


Fig. 1 Graphical display of the plants Hardhof (A) and Lengg (B). A: The drinking water obtained is a mix of natural groundwater, recharged groundwater and river bank filtrate. The water flow is represented by white arrows (in soil) and white bars (in pipes). The water is delivered

to the drinking water reservoirs. See text for additional details. B: The raw lake water passes several cleaning steps before it is pumped into the drinking water reservoirs. The water flow is represented by white bars

Fungal identification

Microscopy was used to identify sporulating isolates at the genus level. Identification at the species level was carried out mainly by matrix-assisted laser desorption ionisation–time of flight (MALDI-TOF) mass spectrometry (MS) or, if no MALDI-TOF MS reference spectra were available, internal transcribed spacer (ITS) region 1, ITS1 + 2 (ITS region 1, 5.8S rDNA, ITS region 2), partial transcription elongation factor 1 (*tef1*) or partial beta-tubulin sequencing.

For the unidentified sp. 47 and 62, MALDI-TOF MS could not resolve whether or not those were distinct taxa.

DNA sequencing

Strains were grown on Sabouraud gentamicin chloramphenicol 2 agar plates (SGC2, bioMérieux, France). Genomic DNA was extracted from individual isolates by phenol-chloroform (Ausubel et al. 1989) with a 10-mg/ml lysozyme pre-treatment of 2 h at 37 °C or using the QIAamp DNA blood mini kit (QIAGEN, Basel, Switzerland) according to manufacturer's instructions, after incubation in digestion buffer.

As a rule, only the ITS regions were sequenced, but depending on the outcome of the identification, different gene targets were subsequently selected for additional checks. *Paraphoma fimeti*, *Penicillium chermesinum*, *Phoma* cf.

Table 1 Features of the sites studied at the Hardhof and Lengg plants and of the drinking water fountain

Water plant	Site	Water type	No. of samples	No. of samples with fungal growth present – absent – n.e.	Water temperature (°C) Mean (min-max)
Hardhof	H1	Untreated river water	25	21 – 1 – 3	12.4 (4.6–20.8)
	H2	Natural river bank filtrate with a varying share of groundwater	25	22 – 3 – 0	15.4 (12.2–17.7)
	H3	Chlorinated river bank filtrate with a varying share of groundwater	10	5 – 5 – 0	n.a.
	H4	Groundwater (mix of different water types; already of drinking grade)	25	23 – 2 – 0	14.4 (10.1–17.1)
Lengg	L1	Untreated lake water	21	21 – 0 – 0	7.1 (6.5–8.2)
	L2	Pre-ozonated water	21	21 – 0 – 0	6.4 (5.8–7.3)
	L3	Rapid sand filtered water	21	19 – 1 – 1	6.5 (5.6–7.8)
	L4	Intermediate ozonated water	21	16 – 5 – 0	6.3 (5.6–7.0)
	L5	Activated carbon and slow sand-filtered water (drinking grade)	21	17 – 4 – 0	6.4 (5.8–7.1)
Fountain	F1	Drinking water	10	10 – 0 – 0	14.5 (9.3–19.1)

n.a.: not available; n.e.: not evaluable. Samples were collected at Hardhof and at the drinking fountain during the period 17 March 2014 to 3 March 2015 and at Lengg from 14 April 2015 to 23 February 2016 at 2-week intervals.

herbarum, *Pochonia bulbillosa*, *Pseudocosmospora vilior*, *Saprolegnia* cf. *australis*, *Thysanophora penicillioides* and *Ulocladium* sp. were identified using ITS1 only. ITS1 + 2 were used for the genera *Acremonium*, *Bionectria*, *Cadophora*, *Epicoccum* (Aveskamp et al. 2010; Chen et al. 2015), *Exophiala*, *Geotrichum* (De Hoog and Smith 2004), *Mortierella* (Petkovits et al. 2011), *Ochroconis*, *Peyronellaea* (Aveskamp et al. 2010; Chen et al. 2015), *Phaeosphaeria*, *Pyrenochaetopsis* and some *Trichoderma* isolates (Druzhinina et al. 2005; Nagy et al. 2007). The beta-tubulin (*btub*) partial gene was additionally used for *Cladosporium* (Bensch et al. 2012; Zalar et al. 2007), *Ophiostoma*, *Penicillium* (Visagie et al. 2014) and *Phoma* (Aveskamp et al. 2010; Chen et al. 2015), and the *tef1* partial gene for *Fusicolla* (Lombard et al. 2015) and some *Trichoderma* isolates (Druzhinina et al. 2005; Nagy et al. 2007).

The ITS1 region was amplified using the forward primer ITS5 and the reverse primer ITS2 (White et al. 1990) and the sequences were generated on both strands using the primers ITS5 and ITS2. The ITS1 + 2 region was amplified using ITS1 (forward) and ITS4 (reverse; White et al. 1990) and sequences were generated with the ITS4 primer. The *btub* partial gene was amplified using Bt2a (forward) and Bt2b (reverse; Glass and Donaldson 1995) and sequences were generated with Bt2a. Amplification of the partial *tef1* gene was performed with EF1-728F (forward) and TEF1-Rev (reverse; Druzhinina et al. 2004; Samuels et al. 2002) and sequences were generated with EF1-728F.

ITS1 sequences were aligned with the SeqMan package (DNASar Inc., Madison, WI, USA) and the obtained consensus sequences identified with BLAST (www.ncbi.nlm.nih.gov/BLAST).

ITS1 + 2, *btub* and *tef1* sequences were processed using the Mega 6.06 software (Tamura et al. 2013). Identifications were carried out by constructing phylogenetic trees with sequences of previously well-identified fungal isolates (neighbour-joining method with the Kimura two-parameter distances bootstrapped with 1000 replicates), pairwise sequence alignment with BLAST (<http://blast.ncbi.nlm.nih.gov>) or the tool available in the CBS-KNAW culture collection (<http://www.westerdijknstitute.nl>). ITS sequences of *Trichoderma* spp. were identified using the trichOKEY barcoding (<http://www.isth.info/tools/molkey> (Druzhinina et al. 2005)).

Sequences of fungi identified to the species level by molecular methods have been deposited in the GenBank database and the corresponding accession numbers are included in Table 2.

Preparation of samples for MALDI-TOF MS analyses

Fungal material taken from freshly grown samples on solid agar plates was transferred into a 1.5-ml microtube containing 20 µl of 25% formic acid; mycelium and spores were crushed using a pestle or a loop and stored for up to 30 min before further processing. The resulting cell suspension was spotted in quadruplicate on a steel target plate and dried at room temperature. The spots were overlaid with 1 µl of matrix solution consisting of alpha-cyano-4 hydroxycinnamic acid (CHCA; Sigma-Aldrich, Buchs, Switzerland) in 33% acetonitrile (Sigma-Aldrich), 33% ethanol and 3% trifluoroacetic acid (TFA) and kept at room temperature for a few minutes until co-crystallisation was completed.

Table 2 Fungal taxa (raw CFU frequencies and percentages of the total number of CFUs) isolated from the Hardhof and Lengg plants

Taxon	H2	H3	H4	Total (treated sites)H2–F1	% of all taxaH2–F1	H1	Grand total	FountainF1
A – Hardhof								
<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud	0	0	1	2	0.16	9200	9202	1
<i>Bionectria solani</i> (Reinke & Berthold) Schroers (MG660848)	14	0	0	14	1.12	0	14	0
<i>Cadophora malorum</i> (Kidd & Beaumont) W. Gams (MG660849 to MG660852)	136	0	9	146	11.64	0	146	1
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	0	0	0	0	0.00	1000	1000	0
<i>Cladosporium</i> sp.	0	0	0	0	0.00	4100	4100	0
<i>Cladosporium</i> sp. A	11	0	2	13	1.04	1000	1013	0
<i>Cladosporium</i> sp. B	0	0	2	2	0.16	2700	2702	0
<i>Epicoccum nigrum</i> Link (MG660846, MG660847)	0	0	0	0	0.00	200	200	0
<i>Exophiala salmonis</i> Carmich. (MG660843)	101	0	2	106	8.45	0	106	3
<i>Fusicolla aquaeductum</i> (Radelk. & Rabenh.) Gräfenhan, Seifert & Schroers (MG660840)	0	0	9	17	1.36	0	17	8
<i>Penicillium bialowiezense</i> K.M. Zalessky (MG660833 to MG660837)	0	0	4	4	0.32	600	604	0
<i>Penicillium expansum</i> Link	0	0	0	0	0.00	100	100	0
<i>Pochonia bulbillosa</i> (W. Gams & Malla) Zare & W. Gams	1	3	0	6	0.48	0	6	2
<i>Pseudocosmospora vilior</i> (Starbäck) C. Herrera & P. Chaverri	95	43	208	354	28.23	0	354	8
<i>Purpureocillium lilacinum</i> (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	1	2	22	25	1.99	0	25	0
<i>Saprolegnia</i> cf. <i>australis</i> R.F. Elliott	0	0	0	0	0.00	200	200	0
<i>Thysanophora penicillioides</i> (Roum.) W.B. Kendr.	0	0	0	0	0.00	0	0	0
<i>Ulocladium</i> sp.	0	0	0	0	0.00	0	0	0
Unidentified sp. 11	0	0	0	5	0.40	200	205	5
Unidentified sp. 47**	0	0	0	0	0.00	0	0	0
Unidentified sp. 62**	0	0	0	0	0.00	0	0	0
Others*	386	25	75	493	39.31	63,700	64,193	7
Total (taxa >1%)	745	73	334	1187	94.66	83,000	84,187	35
Taxa representing <1% of total (70 taxa)	9	6	39	67	5.34	16,800	16,867	13
Grand total	754	79	373	1254	100.00	99,800	101,054	48
B – Lengg								
Taxon	L2	L3	L4	L5	Total(treated sites)L2–L5	% of all taxaL2–L5	L1	Grand total
<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud	4	0	0	0	4	1.50	80	84
<i>Bionectria solani</i> (Reinke & Berthold) Schroers	0	0	0	0	0	0.00	0	0
<i>Cadophora malorum</i> (Kidd & Beaumont) W. Gams	0	0	9	1	10	3.76	0	10
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	1	0	1	18	20	7.52	80	100
<i>Cladosporium</i> sp.	4	0	0	9	13	4.89	60	73
<i>Cladosporium</i> sp. A	4	0	0	1	5	1.88	130	135
<i>Cladosporium</i> sp. B	0	2	10	37	49	18.42	0	49
<i>Epicoccum nigrum</i> Link	21	0	0	0	21	7.89	0	21
<i>Exophiala salmonis</i> Carmich.	0	0	0	0	0	0.00	0	0
<i>Fusicolla aquaeductum</i> (Radelk. & Rabenh.) Gräfenhan, Seifert & Schroers	0	0	0	0	0	0.00	0	0
<i>Penicillium bialowiezense</i> K.M. Zalessky	0	0	0	5	5	1.88	160	165
<i>Penicillium expansum</i> Link	0	3	0	0	3	1.13	60	63
<i>Pochonia bulbillosa</i> (W. Gams & Malla) Zare & W. Gams	0	0	0	14	14	5.26	0	14
<i>Pseudocosmospora vilior</i> (Starbäck) C. Herrera & P. Chaverri	0	0	0	0	0	0.00	0	0

Table 2 (continued)

Taxon	H2	H3	H4	Total (treated sites)H2–F1	% of all taxaH2–F1	H1	Grand total	FountainF1
<i>Purpureocillium lilacinum</i> (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	0	0	0	0	0	0	0.00	0
<i>Saprolegnia</i> cf. <i>australis</i> R.F. Elliott	0	9	0	0	9	3.38	0	9
<i>Thysanophora penicillioides</i> (Roum) W.B. Kendr.	0	3	0	0	3	1.13	0	3
<i>Ulocladium</i> sp.	2	3	0	0	5	1.88	0	5
Unidentified sp. 11	0	0	0	13	13	4.89	0	13
Unidentified sp. 47**	3	16	0	1	20	7.52	0	20
Unidentified sp. 62**	5	0	0	0	5	1.88	0	5
Others*	37	1	4	0	42	15.79	1290	1332
Total (taxa >1%)	81	37	24	99	241	90.60	1860	2101
Taxa representing <1% of total (70 taxa)	14	2	3	6	25	9.40	1070	1095
Grand total	95	39	27	105	266	100.00	2930	3196

Only those taxa that account for at least 1% of the total CFUs are reported in the table. The total for each site has been computed on different numbers of samplings and no direct comparisons can be made. For abbreviations for the sites, see Table 1. The drinking water site (F1) has been included in Table 2A, although it is not directly linked to the Hardhof plant. Unidentified sp.: no name match by MALDI-TOF or sequencing data. GenBank accession numbers of fungi identified to the species level by molecular techniques are provided in parentheses

Rare isolates (<1% of total in treated samples) include: *Acremonium kiliense* Grütz (MG660844, MG660845), *Aspergillus versicolor* (Vuill.) Tirab., *Aspergillus* cf. *thermolutatus* (Paden) S.W. Peterson, *Candida parapsilosis* (Ashford) Langeron & Talice, *Chaetomium globosum* Kunze ex Fr., *Cladosporium halotolerans* Zalar, de Hoog & Gunde-Cim. (MG660838), *Cladosporium* spp., *Debaryomyces hansenii* (Zopf) Lodder & Kreger, *Galactomyces pseudocandidum* de Hoog & M.T. Sm. (MG660853), *Mortierella* sp., *Ochroconis tshawytschae* (Doty & D.W. Slater) Kiril. & Al-Achmed (MG660854), *Ophiostoma piceae* (Münch) Syd. & P. Syd. (MG660839), *Paraphoma fimeti* (Brunaud) Gruyter, Aveskamp & Verkley, *Penicillium brevicompactum* Dierckx, *Penicillium chermesinum* Biourge, *Penicillium chrysogenum* Thom, *Penicillium* cf. *citrinum* Thom, *Penicillium commune* Thom, *Penicillium corylophilum* Dierckx, *Penicillium griseofulvum* Dierckx, *Penicillium* sp., *Peyronellaea* sp., *Phaeosphaeria avenaria* (G.F. Weber) O.E. Erikss., *Phaeosphaeria* sp., *Phoma* cf. *herbarum* Westend., *Phoma* spp., *Pichia guilliermondii* Wick., *Pochonia suchlasporia* (W. Gams & Dackman) Zare & W. Gams, *Pseudocosmospora* cf. *vilior* (Starbäck) C. Herrera & P. Chaverri, *Pyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Gruyter, Aveskamp & Verkley (MG660855), *Saccharomyces cerevisiae* Meyen, *Trametes versicolor* (L.) Lloyd, *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, *Trichoderma* cf. *asperellum* Samuels, Lieckf. & Nirenberg, *Trichoderma atroviride* P. Karst. (MG660841), *Trichoderma* cf. *harzianum* Rifai, as well as 29 unidentified taxa

*Several isolates (filamentous fungi and yeasts) could not be unambiguously identified and only the counted CFUs are reported

**MALDI-TOF MS could not resolve whether or not those were two distinct species

Acquisition of MALDI-TOF mass spectra and data processing

Spectra were obtained using a MALDI-TOF Axima™ Confidence mass spectrometer (Shimadzu-Biotech, Kyoto, Japan), with detection in linear positive mode at a laser frequency of 50 Hz and within a mass range of 3 to 20 kDa. The acceleration voltage was 20 kV and the extraction delay time 200 ns. A minimum of ten laser shots per sample was used to generate each spectrum. For each sample, a total of 50 mass fingerprints were averaged and processed using the Launchpad™ 2.8 software (Shimadzu-Biotech). For peak processing of raw spectra, the following settings were selected: (i) advanced scenario from the "Parent Peak Cleanup" menu; (ii) peak width set at 80 channels; (iii) smoothing filter width set at 50 channels; (iv) baseline filter width of 500 channels; and (v) dynamic threshold apex method. The threshold offset was set at 0.020 mV with a response factor of 1.2. Each target plate was calibrated using spectra of the reference strain *Escherichia coli* DH5α (Invitrogen, Carlsbad, CA, USA). Identification was carried out using available SuperSpectra™ or by comparison

with reference spectra present in the database used, which at the project start contained already more than 400 fungal and yeast species, in addition to 3500 bacterial, approximately 200 insect species and more than 100 reference spectra for cell lines. The cut-off level for a reliable species identification had to be above a confidence value of 90%. No identification, regardless of the frequency of occurrence of the isolate in the samples, was accepted if this threshold was not reached. All isolates that could not be identified by MALDI-TOF MS and accounted for more than five CFUs were sequenced. Overall, more than 20 new reference spectra were added to the database after identification of new isolates by DNA sequencing, but all spectra recorded have been included in the database.

Statistical analysis

All taxa for which an identification was available were treated as separate entities. When MALDI-TOF or sequencing data did not provide any useful identification but the taxonomic units were clearly distinguishable, taxa were labelled with

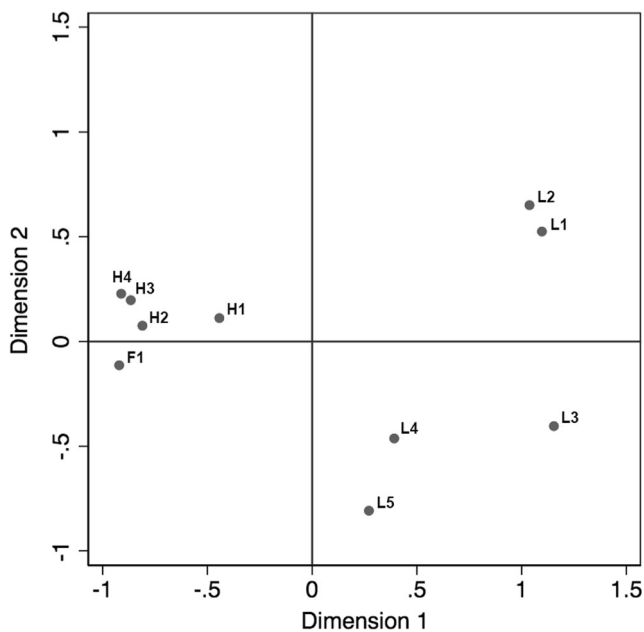


Fig. 2 Results of the NMDS analysis of all sites studied (normalisation: principal orientation). Loss criterion: 0.0931. Data are average values of all samples collected at the site

numbers (unidentified species). In a small number of cases, identification was hampered by technical problems and these were collectively included in the analysis as “other fungal organisms”.

For several samples (see also Table 1), no evaluable data were available; these samples were thus not included in any analysis. Because of the small number of species and the usually low frequency of CFUs detected in each sample, data from all samples of each site were aggregated for multivariate statistical analyses: therefore, no analysis of the variation of fungal communities over time was carried out. Raw CFU frequencies observed at the different sites have been displayed in boxplots. When appropriate, a two-sided *t* test was carried out to confirm statistical differences between samples.

Confirmatory analyses using a Kruskal–Wallis test with Dunn’s test for multiple comparisons were carried out separately for Hardhof and Lengg, because the sites were sampled

Table 3 Results of the Kruskal–Wallis test comparing the sub-sites of each investigated site

Hardhof	Adjusted <i>p</i> value	Lengg	Adjusted <i>p</i> value
H1 vs. H2	<0.0001	L1 vs. L2	0.0017
H2 vs. H3	0.9511	L2 vs. L3	0.1739
H3 vs. H4	0.9999	L3 vs. L4	>0.9999
		L4 vs. L5	>0.9999
H1 vs. H4	0.0001	L1 vs. L5	<0.0001
F1 vs. H4	>0.9999	F1 vs. L5	>0.999

Statistically significant differences are highlighted in bold and italics. For abbreviations see Table 1 and text

over two different years and their ecological features were also quite different.

A nonmetric multidimensional scaling (NMDS) was carried out on the CFU data of all identified taxa (average number of CFUs over all samples studied) using the Pearson correlation coefficient as a similarity measure to compare the relationships of the fungal communities present in the sites sampled.

Statistical analyses were carried out with Stata SE ver. 14 (StataCorp LLC, College Station, TX, USA) and the graphical displays of frequencies prepared with Prism 7 (GraphPad Software, San Diego, CA, USA).

Results

A synopsis of all taxa isolated at the two water plants is presented in Table 2. A total of 92 fungal taxa could be identified to the species level or as distinct, unnamed entities by either MALDI-TOF MS or sequencing. *Aureobasidium pullulans* was very frequent in untreated water in both water plants and was present at a particularly high frequency in untreated water from Hardhof. *Cladosporium* and *Penicillium* species were also common at both plants in untreated, but rare in treated water. *Epicoccum nigrum* was present in untreated water only at Hardhof and, surprisingly, in treated water in Lengg. The protistan oomycetous genus *Saprolegnia* (Sandoval-Sierra et al. 2014) has been included because of its importance as a water inhabitant in the temperate zones: this taxon was comparatively frequent in untreated water samples of Hardhof but absent in all others, an exception being its (rare) occurrence in L3 (disinfected and filtered water) in Lengg. Overall, 21 taxa, accounting for 95% of all CFUs in Hardhof and 91% in Lengg, were present at frequencies of more than 1% of the total CFU numbers in each water plant and were common to both sites.

Figure 2 displays the results of the NMDS analysis. Dimension 1 separates the Hardhof from the Lengg sites. Within Lengg, dimension 2 separates clearly the untreated and pre-ozonated water (L1 and L2) from the samples that have undergone further treatments, whereas within Hardhof, the separation of untreated water samples (H1) from all others is less distinct. Based on the number of CFUs and fungal biodiversity, fountain water (F1) is separated from the other samples, being, however, closer to the Hardhof than to the Lengg samples.

Untreated water (L1 und H1) at both water plants contains significantly more fungal CFUs than any treated water (Table 3 and Fig. 3), with the untreated samples at Hardhof (median: 1700 CFUs) being statistically significantly more colonised than at Lengg (median: 80). The treatment steps are effective in reducing fungal CFUs in both plants (Fig. 3): the median CFU values (calculated over all sampling times) in

Fig. 3 Boxplots of CFU frequencies (number of CFU/L for each site) in water samples at both water plants. Line: median; hinges: 25th and 75th percentiles; whiskers: 10th and 90th percentiles; markers: outliers

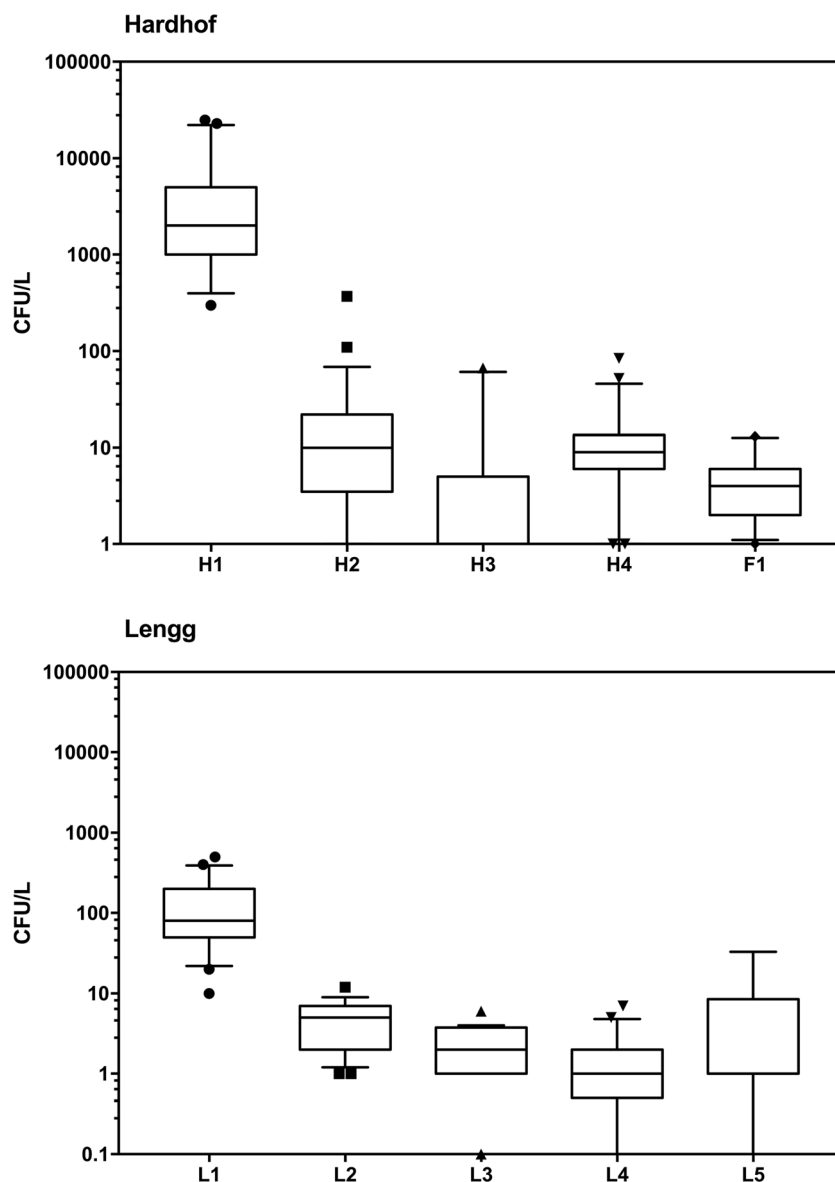


Table 4 Number of taxa isolated at the different sites (over all samples) of the two water plants

Hardhof	Site	H1	H2	H3	H4	
	H1	35				
	H2	2	12			
	H3	0	3	6		
	H4	5	5	3	18	
Lengg	Site	L1	L2	L3	L4	L5
	L1	21				
	L2	5	21			
	L3	1	1	10		
	L4	2	1	1	6	
	L5	4	4	3	4	14

Bold: total number of taxa; plain font: number of taxa common to the two sites

Hardhof range from 1 (H3) to 10 (H2) and in Lengg from 1 (L5, L4) to 5 (L2). Both treatment chains are effective in producing CFU-poor drinking water (Table 2 and Fig. 3): in Hardhof the cumulative number of CFUs recorded decreases by approx. 99% (from a total of 99,800 to 373, on average from 4536 to 15) and in Lengg by 96% (from 2930 to 105, on average from 140 to 5) over the whole treatment.

The decrease in the number of taxa (Table 4) is less relevant; in Hardhof the river bank filtrate (H2) contains only 33% (12 vs. 35) of the number of taxa detected in untreated water (H1) whereas in Lengg no changes are seen [21 taxa before (L1) and after pre-ozonation (L2)].

Fountain water (F1) does not contain a large number of fungal propagules: the cumulative number of CFUs detected is 48 (average: 4.8 CFU/L), which is biologically not significantly different from the frequencies observed in the

disinfected samples; the number of taxa reported from F1 is 14 vs. 18 in the Hardhof site H4 and 14 in the Lengg site L5 (drinking water).

Discussion and conclusions

Our study has shown that the treatment steps used to obtain drinking water from river and groundwater or lake water reduce significantly and effectively fungal colonisation of the water, both in cell count and number of species (probably present in the water as living spores): even if the final products (drinking water) from both plants differ statistically in their fungal CFU content (Mann–Whitney: $p = 0.0012$), the difference is biologically not meaningful (median CFU/L values of W305 = 9, W207 = 1).

After the first treatment step, a drastic, statistically significant reduction of fungal CFUs in the samples is achieved (Table 3). The reduction at Lengg can be a consequence of the treatment, whereas at Hardhof this effect is probably at least partly due to a dilution of the river bank filtrate with natural groundwater. The fountain water contains only a very limited number of CFUs, thus raising no concerns as to its use for human consumption. The data also suggest that fungal propagules do not germinate again in the pipeline.

The NMDS analysis (Fig. 2) shows a clear separation of the sampling sites of the Hardhof and Lengg plants by dimension 1. This reflects the different origin of the raw water and the different water treatment processes in the two plants, as well as the 1-year shift of the sampling time. The further separation of the process waters in Lengg shown by dimension 2, as compared to the compacter cluster of the Hardhof samples, cannot be easily explained. Pre-ozonation (the step applied to the L1 site) probably exerts only a small effect on fungal propagules in comparison with the other processes, as shown for other micro-organisms (Gallard et al. 2003). This could explain why in Fig. 2 the two sites L1 and L2 are very close.

The three sampling sites with treated water at Hardhof are more closely related with regards to the number of CFUs detected and their fungal biodiversity. Natural soil, serving as a large filter and reservoir, provides most likely a much more homogenous cleaning than the separated steps at Lengg. In addition, H2, H3 and H4 are located in the same aquifer. The distance from the river, the injectors and the recharge basins at specific sampling sites lead to varying contribution of each water quality to the samples. This mix can change and, over time, originate similar samples in different sites.

Samples from the drinking fountain (F1) are more similar to the Hardhof than Lengg plant (Fig. 2). This could suggest that the water sampled originated chiefly from Hardhof; on the other hand, it also indicates that the pipelines do not substantially influence the water with regards to the presence of

fungal taxa or CFUs. This is also supported by the statistical analysis presented in Table 3, which shows that the CFU numbers of the water from the public fountain are statistically not different from those detected in drinking water from either plant (sites H4 and L5), with no statistical difference between Hardhof and Lengg.

Fungal CFU reduction by the different treatment steps was similar in the two plants and was apparently not influenced by the different treatment methods used at the plants. At both plants, the first process step (Fig. 1a and b) was responsible for the bulk reduction (90% and more) of fungal propagules. The following downstream processes are important to guarantee a constant drinking water quality, but with regard to the CFU frequencies, the decrease of fungal CFUs is negligible (Table 3 and Fig. 3). The last step (leading to water obtained from sampling sites H4 and L5) seems to have almost no effect, considering that an increased, albeit statistically non-significant, number of CFUs is observed in samples from these sites. This could be due to the growth of propagules still present after the last disinfection step or the colonisation by new fungi originating from a filter or an installation. In any case, the numbers are so small that such a colonisation can be considered negligible for drinking water quality assurance.

The very low CFU levels are maintained over the whole process chain (Table 3) and apparently even the transport of the water through the pipeline to the fountain (F1) does not significantly modify the number of CFUs (Fig. 3).

The largest reduction of taxa number is achieved in both plants after the first filtration step (Table 4). This is the river bank filtration for H2 at Hardhof and the rapid sand filtration for L3 at Lengg (Fig. 1). The first process step at Lengg (pre-ozonation) does not reduce the number of taxa (Table 4) but the fungal community experiences a shift, with 16 taxa disappearing and 16 new ones emerging. The origin of these newly appearing taxa is difficult to explain; on the other hand, most of them were found only once and in small numbers. We believe, however, that the shift in the fungal communities is most likely due to random rather than selection events during pre-ozonation. Random changes in the community composition can be expected also for the other process steps (Table 4). At Lengg, important emerging taxa were *Epicoccum nigrum* and the unidentified taxa 47 and 62 at site L2; at L3 *Saprolegnia* sp.; at L4 *Cadophora malorum* and at L5 *Pochonia bulbilosa* and the unidentified taxon 11. At Hardhof, the shift in taxa was prominent between H1 and H2. Important emerging taxa in H2 were *Cadophora malorum*, *Exophiala salmonis*, *Pseudocosmospora vilior*, *Bionectria solani* and the unidentified taxon 55. Differences among the Hardhof sites reflect most likely the differences of the water origin rather than the influence of the individual process steps.

The taxonomic spectrum of the fungi detected in this investigation coincides roughly with that reported in previous

publications on fungal communities in drinking water, regardless of the isolation methods and conditions used. Species of *Acremonium*, *Aureobasidium*, *Candida*, *Cladosporium*, *Fusarium s. l.*, *Exophiala*, *Paecilomyces*, *Penicillium*, *Phialophora s. l.* and *Phoma s. l.* were already reported by several authors (Doggett 2000; Göttlich and Flemming 2002; Göttlich et al. 2002; Grabińska-Łoniewska et al. 2007; Hageskal et al. 2006; Heinrichs and Haase 2013; Kanzler et al. 2008).

Seventeen fungal species were isolated only at the H1 site in Hardhof, and 10 at the L1 site in Lengg from untreated water. With regards to treated water, we were able to detect plant or site-specific as well as unspecific taxa, although the specificity would need to be verified in more appropriate studies. Five plant- or site-specific species were detected more than once and at frequencies of more than five CFUs. These include *Bionectria solani* (site H2, in 3 different samplings), undetermined species 55 (H2, in 2 samplings) and 24 (F1, in 2 samplings). Species 62 was isolated from three samplings at site L2 and *Saprolegnia cf. australis* at site L3 (seven samplings). The presence of *Bionectria* in river bank filtrate is not surprising because *Bionectria* spp. live saprobically (rarely parasitically) on *Myxomycota*, *Oomycota* and nematodes and have rarely been isolated from water, air or from the built environment (Schroers 2001).

Other plant-specific and comparatively frequent species include *Exophiala salmonis*, *Fusicolla aquaeductum* [syn.: *Fusarium aquaeductum* (Rabenh. & Radlk.) Lagerh. & Rabenh.], *Ochroconis tshawytschae*, *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus* Thom) and *Pseudocosmospora vilior* at the Hardhof plant; at Lengg, only undetermined species 47 was plant-specific and found at frequencies higher than 1%. Recent data have shown that *Exophiala* species may be present in drinking water (Göttlich et al. 2002) and *E. salmonis* is known to be a fish parasite (De Hoog et al. 2011; Langvad and Engjom 2016). *Fusarium* species usually live saprobically in the soil and in water (*Fusarium*-like clade: Triest et al. 2016), and several species are known plant pathogens (Summerell et al. 2010). *Fusicolla aquaeductum*, on the other hand, is frequently isolated from river water and water pipes (Gerlach and Nirenberg 1982). *Ochroconis* species are mostly cosmopolitan and have been isolated from different substrates, including immunocompromised human hosts and the built environment (Giraldo et al. 2014; Samerpitak et al. 2015), but have so far never been observed in water. *P. lilacinum* is a saprobe and is known as an opportunistic human pathogen in immunocompromised patients (Luangsa-ard et al. 2011) that has so far never been reported in processed water. *Penicillium brevicompactum* was isolated exclusively at the Lengg plant (L1 and L2) and *P. chrysogenum* at the Hardhof plant (H1 and H4). Both species are known to also inhabit the built environment, in particular where the relative humidity is high (Scott

et al. 2008). *Cadophora malorum*, a soil-borne or wood-inhabiting fungus (Harrington and McNew 2003), as well as the nematode parasite *Pochonia bulbillosa* (Zare et al. 2001) were isolated from processed water from both water plants.

Some fungal species known to produce mycotoxins (see e.g. Fog Nielsen 2003; Fogle et al. 2008; Frisvad and Samson 2004; Klich 2002) were detected during this investigation, albeit in very small numbers. These include *Aspergillus versicolor* (site H4), *Chaetomium globosum* (H1), *Penicillium brevicompactum* (L1 and L2), *P. chrysogenum* (H1 and H4) and *P. expansum* (H1, L1 and L3).

With regards to the taxa representing the comparatively high CFU number (493) recovered from the Hardhof plant (Table 2), only 75 CFUs were present in drinking water and 2 taxa accounted for 65 of them. Among the sequenced isolates that could not be unambiguously identified (Table 2), the presence of mycotoxin-producing taxa cannot be excluded, even if the obtained sequences did not suggest any close relationship of the isolates with known mycotoxin-producing species. Mycotoxin production, however, has not been studied specifically within this project. It is unlikely that the fungi isolated produce mycotoxins in water but this needs to be further investigated.

This study has some limitations. For technical and logistic reasons, sampling had to be carried out during two different study periods. In addition, the origin of the water collected at the fountain could not be unequivocally assigned to either plant. The data structure, with many taxa appearing only sporadically in the samples, did not allow carrying out a temporal analysis of the changes in the fungal communities occurring in the different sites. Finally, several isolates could not be identified by any of the methods used, which suggest a remarkable, hitherto not completely studied fungal biodiversity in water. Nevertheless, this investigation has shown that fungal propagules are present in raw water and their number is effectively reduced by the treatment steps applied to produce drinking water.

References

- Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1989) Current protocols in molecular biology. Greene Publishing Associated & Wiley Interscience, New York
- Aveskamp MM, de Gruyter J, Woudenberg JH, Verkley GJ, Crous PW (2010) Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Stud Mycol* 65:1–60
- Bensch K, Braun U, Groenewald JZ, Crous PW (2012) The genus *Cladosporium*. *Stud Mycol* 72(1):1–401
- Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW (2015) Resolving the *Phoma* enigma. *Stud Mycol* 82:137–217

- Cornut J, Clivot H, Chauvet E, Elger A, Pagnout C, Guérol F (2012) Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams. *Water Res* 46:6430–6444
- De Hoog G, Vicente VA, Najafzadeh M, Harrak M, Badali H, Seyedmousavi S (2011) Waterborne *Exophiala* species causing disease in cold-blooded animals. *Persoonia* 27(1):46–72
- De Hoog GS, Smith MT (2004) Ribosomal gene phylogeny and species delimitation in *Geotrichum* and its teleomorphs. *Stud Mycol* 50: 489–515
- Doggett MS (2000) Characterization of fungal biofilms within a municipal water distribution system. *Appl Environ Microbiol* 66(3):1249–1251
- Douterelo I, Jackson M, Solomon C, Boxall J (2016) Microbial analysis of *in situ* biofilm formation in drinking water distribution systems: implications for monitoring and control of drinking water quality. *Appl Microbiol Biotechnol* 100:3301–3311
- Druzhinina I, Chaverri P, Fallah P, Kubicek C, Samuels G (2004) *Hypocrea flavoconidia*, a new species with yellow conidia from Costa Rica. *Stud Mycol* 50:400–407
- Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genet Biol* 42(10): 813–828
- Fog Nielsen K (2003) Mycotoxin production by indoor molds. *Fungal Genet Biol* 39(2):103–117
- Fogle MR, Douglas DR, Jumper CA, Straus DC (2008) Growth and mycotoxin production by *Chaetomium globosum* is favored in a neutral pH. *Int J Mol Sci* 9(12):2357–2365
- Frisvad JC, Samson RA (2004) Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and airborne terverticillate *Penicillia* and their mycotoxins. *Stud Mycol* 49(1):1–174
- Gallard H, Von Gunten U, Kaiser H (2003) Prediction of the disinfection and oxidation efficiency of full-scale ozone reactors. *J Water Supply Res Technol AQUA* 52(4):277–290
- Gerlach W, Nirenberg H (1982) The genus *Fusarium* – a pictorial atlas. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* (209)
- Giraldo A, Sutton DA, Samerpitak K, de Hoog GS, Wiederhold NP, Guarro J, Gené J (2014) Occurrence of *Ochroconis* and *Verruconis* species in clinical specimens from the United States. *J Clin Microbiol* 52(12):4189–4201
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 61(4):1323–1330
- Göttlich E, Flemming HC (2002) Auftreten von obligat und fakultativ pathogenen Organismen in Trinkwasser-Biofilme: Pilze. *Berichte aus dem IWW*, vol 36. Mülheim an der Ruhr
- Göttlich E, van der Lubbe W, Lange B, Fiedler S, Melchert I, Reifenrath M, Flemming HC, de Hoog S (2002) Fungal flora in groundwater-derived public drinking water. *Int J Hyg Environ Health* 205(4): 269–279
- Grabinska-Loniewska A, Koniłowicz-Kowalska T, Wardzyńska G, Boryn K (2007) Occurrence of fungi in water distribution system. *Polish J Environ Stud* 16(4):539–547
- Hageskal G, Knutsen AK, Gaustad P, de Hoog GS, Skaar I (2006) Diversity and significance of mold species in Norwegian drinking water. *Appl Environ Microbiol* 72(12):7586–7593
- Hageskal G, Lima N, Skaar I (2009) The study of fungi in drinking water. *Mycol Res* 113(Pt 2):165–172
- Harman BI, Koseoglu H, Yigit NO, Sayilgan E, Beyhan M, Kitis M (2010) The removal of disinfection by-product precursors from water with ceramic membranes. *Water Sci Technol* 62(3):547–555
- Harrington TC, McNew DL (2003) Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the *Helotiales*. *Mycotaxon* 87:141–152
- Heinrichs G, Haase G (2013) Dunkel pigmentierte Biofilme an Trinkwasserarmaturen – mykologische Analyse und hygienische Bewertung, Ursachen und Abhilfemaßnahmen. *Vom Wasser* 110(2):41–43
- Jekel MR (1994) Flocculation effects of ozone. *Ozone Science & Engineering* 16(1):55–56
- Jermann D, Pronk W, Kägi R, Halbeisen M, Boller M (2008) Influence of interactions between NOM and particles on UF fouling mechanisms. *Water Res* 42(14):3870–3878
- Jones EBG, Hyde KD, Pang K-L (2014) Freshwater fungi and fungal-like organisms. De Gruyter, Berlin/Boston
- Kanzler D, Buzina W, Paulitsch A, Haas D, Platzer S, Marth E, Mascher F (2008) Occurrence and hygienic relevance of fungi in drinking water. *Mycoses* 51(2):165–169
- Kelley J (2001) Identification and control of fungi in distribution systems. Amer Water Works Assn; illustrated edition (January 2003), Denver, USA
- Klich MA (2002) Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures, Utrecht
- Langvad F, Engjom (2016) Infection with *Exophiala salmonis*, vol 42. ICES Identification leaflets for diseases and parasites of fish and shellfish. International Council for the Exploration of the Sea
- Lemley A, Wagenet L, Kneen B (1995) Activated carbon treatment of drinking water. *Water Treatment Notes, Fact Sheet 3*. Cornell Cooperative Extension, New York State College of Human Ecology
- Lezcano I, Pérez Rey R, Baluja C, Sánchez E (1999) Ozone inactivation of *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella sonnei* and *Salmonella typhimurium* in water. *Ozone Sci Eng* 21(3):293–300
- Lombard L, Van der Merwe N, Groenewald J, Crous P (2015) Generic concepts in *Nectriaceae*. *Stud Mycol* 80:189–245
- Luangsa-ard J, Houbraken J, van Doorn T, Hong S-B, Borman AM, Hywel-Jones NL, Samson RA (2011) *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. *FEMS Microbiol Letters* 321(2):141–149
- Mauclaire L, Schürmann A, Thullner M, Zeyer J, Gammeter S (2004) Sand filtration in a water treatment plant: biological parameters responsible for clogging. *J Water Supply: Research and Technology – Aqua* 53(2):93–108
- Mesquita-Rocha S, Godoy-Martinez PC, Goncalves SS, Urrutia MD, Carlesse F, Seber A, Silva MA, Petrilli AS, Colombo AL (2013) The water supply system as a potential source of fungal infection in paediatric haematopoietic stem cell units. *BMC Infect Dis* 13:289
- Nagy V, Seidl V, Szakacs G, Komon-Zelazowska M, Kubicek CP, Druzhinina IS (2007) Application of DNA bar codes for screening of industrially important fungi: the haplotype of *Trichoderma harzianum sensu stricto* indicates superior chitinase formation. *Appl Environ Microbiol* 73(21):7048–7058
- Niemi RM, Knuth S, Lundstrom K (1982) Actinomycetes and fungi in surface waters and in potable water. *Appl Environ Microbiol* 43(2): 378–388
- Pereira VJ, Fernandes D, Carvalho G, Benoliel MJ, San Romao MV, Barreto Crespo MT (2010) Assessment of the presence and dynamics of fungi in drinking water sources using cultural and molecular methods. *Water Res* 44(17):4850–4859
- Petkovits T, Nagy LG, Hoffmann K, Wagner L, Nyilasi I, Griebel T, Schnabelrauch D, Vogel H, Voigt K, Vagvolgyi C, Papp T (2011) Data partitions, Bayesian analysis and phylogeny of the zygomycetous fungal family *Mortierellaceae*, inferred from nuclear ribosomal DNA sequences. *PLoS One* 6(11):e27507
- Rennecker JL, Mariñas BJ, Owens JH, Rice EW (1999) Inactivation of *Cryptosporidium parvum* oocysts with ozone. *Water Res* 33(11): 2481–2488
- Rose J, Botzenhart K (1990) *Cryptosporidium* und *Giardia* im Wasser: Nachweisverfahren, Häufigkeit und Bedeutung als Krankheitserreger. *Gas- und Wasserfach Wasser, Abwasser* 131(10):563–572

- Samerpitak K, Duarte A, Attili-Angelis D, Pagnocca FC, Heinrichs G, Rijs A, Alfjorden A, van den Ende AG, Menken S, de Hoog G (2015) A new species of the oligotrophic genus *Ochroconis* (*Sympoventuriaceae*). *Mycol Progr* 14(2):6
- Samuels GJ, Dodd SL, Gams W, Castlebury LA, Petrini O (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia* 94(1):146–170
- Sandoval-Sierra JV, Martín MP, Diéguez-Urbeondo J (2014) Species identification in the genus *Saprolegnia* (*Oomycetes*): defining DNA-based molecular operational taxonomic units. *Fungal Biol* 118(7):559–578
- Schleupen E (1996) *Cryptosporidium parvum* and *Giardia lamblia*: Literaturrecherche. Gas- und Wasserfach Wasser, Abwasser 137(2):83–93
- Schroers HJ (2001) A monograph of *Bionectria* (*Ascomycota*, *Hypocreales*, *Bionectriaceae*) and its *Clonostachys* anamorphs. *Stud Mycol* 46:1–211
- Schwartz T, Hoffmann S, Obst U (2003) Formation of natural biofilms during chlorine dioxide and u.V. Disinfection in a public drinking water distribution system. *J Appl Microbiol* 95(3):591–601
- Scott JA, Wong B, Summerbell RC, Untereiner WA (2008) A survey of *Penicillium brevicompactum* and *P. bialowiezense* from indoor environments, with commentary on the taxonomy of the *P. brevicompactum* group. *Botany* 86(7):732–741
- Sonigo P, Detoni A, Reilly K (2011) A review of fungi in drinking water and the implications for human health. WD 0906 DEFRA
- Summerell BA, Laurence MH, Liew ECY, Leslie JF (2010) Biogeography and phylogeography of *Fusarium*: a review. *Fungal Divers* 44(1):3–13
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729
- Teunis PF, Rutjes SA, Westrell T, de Roda Husman AM (2009) Characterization of drinking water treatment for virus risk assessment. *Water Res* 43(2):395–404
- Triest D, De Cremer K, Pierard D, Hendrickx M (2016) Unique phylogenetic lineage found in the *Fusarium*-like clade after re-examining BCCM/IHEM fungal culture collection material. *Mycobiology* 44(3):121–130
- Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CH, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA (2014) Identification and nomenclature of the genus *Penicillium*. *Stud Mycol* 78:343–371
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1):315–322
- WHO (2017) Guidelines for drinking-water quality: fourth edition incorporating the first addendum
- Zalar P, de Hoog GS, Schroers HJ, Crous PW, Groenewald JZ, Gunde-Cimerman N (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. *Stud Mycol* 58:157–183
- Zare R, Gams W, Evans HC (2001) A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. *Nova Hedwigia* 73(1–2):51–86