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A REVIEW OF FUNGI IN DRINKING WATER AND THE IMPLICATIONS FOR HUMAN HEALTH

Final Report

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EXECUTIVE SUMMARY

Fungi are eukaryotic, heterotrophic organisms, including both single-celled yeasts and multi-cellular filamentous fungi. Many fungal species can survive in oligotrophic environments, through scavenging nutrients from the substrate which they colonise, or the air or water in which they live. Fungi also produce secondary metabolites, some of which are toxins. Some of the fungal species and the metabolites they produce are human pathogens or allergens.

Fungi can enter drinking water distribution systems through several contamination pathways, including treatment breakthrough, deficiencies in stored water facilities cross-connections, mains breaks and intrusions, and during mains installation and maintenance. Once introduced, fungal species can become established on the inner surfaces of pipes, induding interaction and reaction with sealings and coatings, and biofilms within distribution systems, or can be suspended in the water. Water companies in England and Wales have in place procedures to minimise the risk of microbial contamination.

The results of sample analysis from customer taps and other points within distribution systems often reveal higher numbers of fungi than the analysis of samples following treatment, prior to entry into the distribution system. Such increases through the distribution system could be due to two reasons: i) the fungi that remain present after treatment multiply within the system or that fungi that were only partially inactivated later recover, and ii) fungi enter the system via pathways of secondary contamination. Accumulation of fungi in stored water at the consumer end, such as in water tanks, has also been observed. For example, higher numbers of colony forming units of *Aspergillus* have been found in hospital water storage tanks than in the municipal water supply.

A number of different methods of analysing drinking water samples are used, including culture, measurement of ergosterol, quantitative PCR, gene markers and probes, protein probes, direct observation and mass spectrometry. There is currently no international standard specifically for the measurement of fungi in drinking water, and there is no widespread adoption of other relevant standards. Therefore, differences in analysis methods limit the extent to which results can be compared between studies. Furthermore, the most commonly used unit of quantification is numbers of Colony Forming Units (CFUs). However, this measure does not necessarily give an accurate representation of the number of fungi present in a sample, as not all species can be detected using culturing methods. It is also likely that one colony is formed of many different fungal structures, such as hyphae, conidia, conidiophores, from different “individuals” clumped together into one CFU.

Relatively few studies have investigated the fungi found in treated drinking water. The numbers of fungi found in the existing studies range from 1 CFU per litre to 5000 CFU per litre. Of the sixty-five genera that have been isolated in the studies analysed during this review, the majority were filamentous fungi. The most commonly isolated genera were *Penicillium*, *Cladosporium*, *Aspergillus*, *Phialophora* and *Acremonium*.

A number of factors influence the ecology of fungal taxa in drinking water distribution systems. Fungi are more likely to be isolated from surface-water derived drinking water than from that derived from groundwater. This may be related to the larger amounts of organic matter in surface water. Differences in acidity and calcium content may also account for some of the variation. Fungi were also more likely to be isolated from cold water than hot water, although this depends on the species considered and their optimum temperature range. Associations between fungi and bacteria are also relevant, in order to determine if fungal numbers correlate with commonly measured bacterial parameters of drinking water quality. However, there is no consensus in the literature of whether such a correlation exists.

Biofilms are an important habitat for fungi in drinking water. Their development is influenced by many factors including temperature, nutrient concentration, pipe material and water flow rate. However, how exactly such factors affect biofilm development and specifically the role of fungi in biofilms is not well known.

Water treatment appears to reduce the number of fungi in water, without removing all of them. Melanised species are particularly able to resist water treatment. Different treatment processes have different removal efficiencies, although it is not agreed which process is the most efficient method.

Many of the fungi that have been isolated from treated drinking water are known to be pathogenic, particularly *Aspergillus* and *Candida*. Although healthy individuals may suffer from superficial or localised fungal infections caused by these taxa, there is little evidence that their pathogenicity arises from their presence in drinking water. More severe invasive infections are limited to those with immune deficiency, due to for example HIV/AIDS, chemotherapy, immunosuppressive therapy following transplants, or other underlying health conditions, such as cystic fibrosis or diabetes mellitus. Such invasive infections carry a high mortality rate, estimated at between 50 and 100%, depending on the species involved. The extent to which infections arise from at-risk individuals is not well known. The continuing rise of *Aspergillus* infections in at-risk individuals despite hospital-based measures to control airborne fungal spores suggests that another environmental source exists. A small number of studies have linked the genotype of fungi recovered from patients to that of fungi from hospital water supplies. The significance of exposure via drinking the water, as opposed to washing with it, has not been specifically studied. Aerosolisation of fungi during showering or from running taps has received more attention; numbers of airborne fungi have been found to increase after running taps or showers. Infections caused by *Candida* species

are also significant, and while this genus has been isolated from drinking water the significance of exposure via drinking water is not known.

Fungi have also been linked to allergic disease, including worsening of asthma symptoms, hypersensitivity pneumonitis and skin irritation. Fungi known to provoke allergic responses in susceptible individuals, such as *Alternaria* spp., *Aspergillus*, spp., *Cladosporium* spp. and *Penicillium* spp., have been isolated from drinking water. Symptoms have arisen due to exposure when showering, bathing or using saunas, or from exposure to water-damaged buildings.

Some fungi, including *Penicillium* spp., *Aspergillus* spp., *Fusariam* spp. and *Claviceps* spp. are known to produce mycotoxins such as patulin, aflatoxins and zearalenone. It is thought that concentrations of mycotoxins in drinking water are low due to being diluted. No reports of disease caused by mycotoxins in drinking water have been identified.

Indirect health impacts may arise from association with other pathogens. For example, colonisation of the respiratory tract with *Candida* spp. increases the risk of ventilator-associated pneumonia from *Pseudomonas aeruginosa.* Biocorrosion of pipes by fungal species may represent a second indirect health impact. This process can lead to increased metal concentrations in drinking water and corrosion tubercles also provide habitat for fungi.

Secondary metabolites produced by fungi, particularly those growing in localised pockets near the consumer end may be responsible for altering the taste and odour of drinking water. It is thought that the threshold level for numbers of fungi that can cause such issues may be around 102-103 CFU l^{-1}. While problems with taste and odour do not necessarily imply a health risk they are often perceived as such by the consumer.

Due to the relative lack of literature on the topic of fungi in drinking water, there are a number of aspects that remain poorly understood. Research needs include a need to determine the importance of drinking water as the environmental source of fungal infection in vulnerable or at-risk population groups. Greater knowledge on the importance of ingestion as opposed to inhalation or skin contact as exposure pathways for fungi in drinking water will ensure that mitigation measures for at-risk patients are appropriate. Finally, greater understanding of the effect of the analytical method on the results obtained and development of a standard method would facilitate further research into fungi in drinking water.

- Fungi present in drinking water may cause severe fungal infections in immunosuppressed patients. In a small number of studies, drinking water supplies have been found to be the source of infection, although the pathway of infection (drinking vs. inhalation of aerosolised spores while showering) is uncertain
- Additional research would be required to further investigate the link between fungi in drinking water and infections in immunosuppressed patients, address its frequency from an epidemiological viewpoint and determine the fungal species and quantity in water that may cause such infections.
- The present risk of health impact for the general population is thought to be low based on current knowledge. Therefore current procedures for water system maintenance or water monitoring and treatment might be sufficient.
- The literature should be reviewed periodically in order to take account of potential environmental or procedural changes, such as climate change or altered water treatment processes.
- If future scientific results suggest an increase in risk, pilot epidemiological studies and surveillance may be justified.
- Further research and monitoring (if needed) would be facilitated by the use of a simpler and quicker method of fungal quantification and identification than culture.
- Greater knowledge of the associations between fungi and bacteria would help to ascertain whether commonly measured bacterial parameters of water quality correlate with fungi presence.

1. INTRODUCTION

Fungi are eukaryotic, heterotrophic organisms, including both single-celled yeasts and multi-cellular filamentous fungi. They primarily function as recyclers of organic material. Many fungal species can survive in oligotrophic environments, through scavenging nutrients from the substrate which they colonise, or the air or water in which they live. To maximise nutrient uptake, filamentous fungi form mats of fine hyphae. Dispersion is via spores. Fungi also produce secondary metabolites, some of which are toxins. Some of the fungal species and the metabolites they produce are human pathogens or allergens (Paterson and Lima, 2005).

Due to their tolerance of oligotrophic environments, some species of fungi are able to colonise drinking water distribution systems, which are typically low in nutrients. The significance of drinking water as an exposure pathway to pathogenic, allergenic or toxic fungal species or their metabolites is not well known.

Fungal infections are becoming of increasing concern due to the increasing numbers of immunocompromised patients and those with other risk-factors (Annaisie et al., 2002). Therefore, there is a need to ascertain what the exposure pathways are and whether treated drinking water has a role as a source of exposure to pathogenic fungi.

The presence of fungi in water distribution systems may cause other indirect challenges for water companies. For instance, the secondary metabolites produced by some species can alter the taste and smell of water, generating complaints from end-users. Organic acids produced by fungal metabolic processes can increase the rate of corrosion of water pipes, especially when it is difficult to maintain sufficient concentrations of water disinfectants, such as chlorine, throughout the distribution system (Grabinska-Loniewska et al., 2007).

There is a need to determine the extent of current knowledge regarding which fungal species have been reliably identified as present in treated drinking water and its distribution systems, their ecology and the extent to which they are a hazard to human health. **This report aims to synthesise and analyse the most significant recent literature regarding the occurrence and implications of fungi in treated drinking water and distribution systems.**

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2. METHODOLOGY

Literature was collected using keyword searches in Science Direct[1] and PubMed[2], focusing on publications from 2000 onwards, supplemented with older papers to provide theoretical knowledge where necessary. Several keyword combinations were used to search the title, abstract and keywords, including:

- 'fungi' AND 'drinking water'
- 'mycotoxin' AND 'drinking water'
- 'filamentous' AND 'drinking water'
- 'yeast' AND 'drinking water'
- 'biofilm' 'fungi' AND 'drinking water'
- 'fungi' AND 'water supply'
- 'fungi' AND 'water infrastructure'
- 'fungi' AND 'water network'
- 'fungal infection' AND 'water'
- 'allergy' 'fungi' AND 'drinking water'
- 'allergy' 'fungi' AND 'water'
- 'toxicity' 'fungi' AND 'water' (AND 'drinking water')
- 'taste' 'fungi' AND 'drinking water'
- 'odour' 'fungi' AND 'drinking water'
- 'drinking water treatment' AND 'fungi'
- 'drinking water purification' AND 'fungi'

The results that were obtained from each search were exported to EndNote.

The results obtained through the systematic literature search were supplemented by literature identified using broad searches using Google Scholar (for example for 'fungi' and 'protozoa') in order to include books and grey literature (i.e. unpublished reports and documents) and from the references of key papers, such as recent literature reviews. This was done in order to fill in gaps in coverage identified during the initial review of the literature collected.

[1] Available from: www.sciencedirect.com [Accessed 30/11/2010]

[2] Available from: www.ncbi.nlm.nih.gov/pubmed [Accessed 30/11/2010]

The complete list of references was then reviewed to identify any references that were not relevant to the topic of fungi in drinking water. These references were marked as such, but were retained in order to have a complete record of the search results.

Following the search on Science Direct and PubMed, 164 unique references were identified, of which 48 were found to be not relevant to this study following the initial review. Examples of those that were not relevant include papers where yeast was mentioned only as a culture medium and papers that were only focused on bacteria in drinking water. In these cases fungi may have been mentioned but not analysed sufficiently to be of use. This left 116 papers that could be of use in the literature review. The papers were prioritised according to those that provided the most useful and directly relevant information. This was determined by reading the abstract, on the basis of the following criteria:

- the study was conducted in the UK;
- the paper was focused on the ecology of fungi in treated drinking water;
- the paper was published recently (i.e. since 2000 in most cases); and
- the paper included inventories of species isolated from treated drinking water, or was a review of existing knowledge.

No papers fit all the criteria; for example there was very little information from the UK. The one paper published before 2000 (Kelley et al., 1997) was included in the list due to it including information from the UK. It was this priority list (see Annex 3) on which the analysis was based, supplemented with references on specific points where appropriate.

3. FUNGAL TAXA IN TREATED DRINKING WATER

3.1. FUNGI ENTERING THE DRINKING WATER DISTRIBUTION SYSTEM

Fungi were isolated from treated drinking water in all the studies that were analysed in-depth (see Annex 3). A summary of the full results are presented in Annex 1. As can be seen, these studies were conducted in a limited number of countries, including UK, US, Germany, and Poland. While there are a number of species that are frequently isolated from drinking water systems, the precise species composition observed in different studies varies considerably. This indicates that the specific environmental characteristics of the individual distribution systems examined influence considerably the microbial communities found. However, the culturing method used may also affect the species isolated (see sub-section 3.2.2.). The current knowledge on how particular biotic and abiotic factors affect this variation is discussed further in chapter 4.

3.1.1. PATHWAYS OF CONTAMINATION OF DRINKING WATER DISTRIBUTION SYSTEMS

Contamination pathways are the entry points that allow microorganisms and pollutants to enter the water distribution system. Pathways can be either primary, i.e. where the source water contains microorganisms which survive treatment, or secondary, i.e. where contamination occurs after water treatment. There are a number of potential pathways, which are illustrated in Table 3-1.

Table 3-1: Contamination pathways for fungi and other microorganisms (US EPA, 2006)

	Pathway	Description	Level of importance
Primary contamination	Treatment breakthrough	Water treatment and disinfection processes may fail to remove/inactivate all microorganisms of concern from source water.	Many fungal species resistant to treatment and disinfection (Doggett, 2000). Higher risk following rainfall and flood events (US EPA, 2002).
Secondary contamination	Deficiencies in treated water storage facilities	Physical openings in storage facilities, and lack of cover allow microorganisms to be introduced from the air, animals, introduction of untreated surface or groundwater, etc (US EPA, 2002).	All service reservoirs in England and Wales are covered and vents protected by gauze to prevent animals gaining access. Contamination introduced earlier in the system may be amplified in stored water (e.g. through biofilm growth) and due to particle accumulation.
	Cross	Cross connections are where	Significance as a pathway for fungal

	Pathway	Description	Level of importance
	connections	the distribution system for treated water is conencted to any other system, including waste water, industrial process systems. If connections do not have devices to prevent backflow or back siphonage, other fluids can enter the treated water distribution system, particularly when pressure in the system drops (US EPA, 2002).	introduction unknown. In England and Wales the risk of this is minimised through water company enforcement of the Water Supply (Water Fittings) Regulations.
	Mains breaks and intrusions	Mains breaks include leaking joints and adapters, cracks in pipelines and deficient seals. Low and negative pressure events can allow intrusions of contaminants through such breaks). Changes in pressure can arise from pump startup and shutdown, flushing operations, sudden changes in demans, power failure, main breaks, large changes in demand etc. (US EPA, 2002).	Breaks are more common in ageing infrastructure, and can also result from thermal contraction and expansion arising from temperature changes. Frequency of breaks is variable by size of the system (US EPA, 2002). It is estimated that 3275 Ml/day were leaked in 2009/2010 (Ofwat, 2010).. The frequency and significance of low and negative pressure events is not well known. However, this is thought to be a key pathway for the introduction of soil-borne fungi (Doggett, 2000). Water companies have procedures in place to minimise the risk of ingress during bursts and repairs.
	Water main installation and maintenance	Insufficient treatment of materials, equipment or personnel can allow microbial entry to the distribution system.	Water companies have procedures in place to manage this. These procedure should in accordance with the "Principles of Water Supply Hygiene" and the associated technical guidance notes

Fungi may enter through any of these pathways, although the relative importance of each is not fully understood, controls are in place to minimise risks. In terms of allowing entry to microbes of concern, the following risk levels have been applied (US EPA, 2002):

- **high risk:** treatment breakthrough, intrusion, cross-connections, main repair/break (note that procedures are in place in England and Wales to minimise risk of microbial introduction during treatment and throughout the distribution system);
- **medium risk:** uncovered water storage facilities (note that there are no uncovered service reservoirs in England and Wales);

- **low risk:** new mains installation, covered water storage facilities, growth and resuspension, purposeful contamination.

For example, soil-borne fungi can enter distribution systems through leaks and mains joints if the main pressure is low, or during potentially during maintenance (University of Sheffield, 2009). Airborne species can be introduced from the air in contact with stored water (Göttlich et al., 2002 and Gonçalves et al., 2006). Physical entrapment of the spores may be responsible for the introduction of hydrophobic spores in water systems (Gonçalves et al., 2006).

Once introduced, fungal species can become established on the inner surfaces of pipes, including interaction and reaction with , sealings and coatings, and biofilms (see Box 1 for a full explanation of biofilms) within distribution systems, or can be suspended in the water (Göttlich et al., 2002, Grabinska-Loniewska et al., 2007 and Gonçalves et al., 2006). Some species are found throughout water distribution networks, while others may be restricted to localised sites (Kelley et al., 1997). For example, Göttlich et al. (2002) classified *Phialophora*, *Exophiala* and *Acremonium* as widespread and resident, and *Verticillium* and *Phoma* as transients with restricted distribution. The presence of transient species indicates that either such species grow at localised points within the system or that the system is regularly breached, allowing frequent local contamination (Kelley et al., 1997).

Water with long residence times in dead ends, tidal points and oversized pipes, and stored water on the consumer side[3], i.e. in tanks and other storage facilities, is particularly vulnerable to fungal colonisation (Paterson and Lima, 2005, Hageskal et al., 2007 and International Mycological Institute, 1996). Terminal pipe ends are favoured locations for fungal colonisation as they typically do not support sufficient concentrations of residual chlorine to kill fungi (Grabinska-Loniewska et al., 2007). At the consumer side, installations such as cisterns, heating tanks, taps, and shower heads can yield large numbers of fungi (in terms of Colony Forming Units (CFUs)) (Hageskal et al., 2007). For example, Anaissie et al. (2002) found that *Aspergillus* species were significantly more likely to be isolated in significantly greater concentrations (p=0.001) from cold water storage tanks than from municipal water or water from cold taps.

[3] The term "consumer side" refers to all water piping and installations in the consumer's premises.

Box 1: Fungi in biofilms

Biofilms are communities of micro-organisms, including bacteria, fungi and protozoa, that are attached to a surface, usually at phase boundaries such as the interface between a liquid and a solid (Paterson and Lima, 2005 and Doggett, 2000). They can include organic and inorganic material which, along with the microbes, is incorporated into an organic polymer matrix produced by microbes (US EPA, 2002). While bacteria are frequently the principal component of biofilms in water distribution systems, fungi and fungal spores can also become embedded on the biofilm surface or in encrustations. Fungi can also be primary colonisers of biofilms, if exposure time to pipe surfaces is long enough. Biofilms are a significant habitat for fungi in water distribution systems (Paterson and Lima, 2005 and Doggett, 2000). For example, Grabinska-Loniewska et al. (2007) found that the number of fungal CFUs held in biofilms was 1000-5000 times greater than that in water. The density of fungi in biofilms and the species involved vary between local sites (Doggett, 2000). For example, the number of yeasts in biofilms was found by Doggett (2000) to vary between 0 and 8.9 CFU cm^{-2} and for filamentous fungi between 4.0 and 25.2 CFU cm^{-2}. Inner surfaces of pipes in water distribution systems may have a continuous biofilm or, more commonly, patchy biofilms (US EPA, 2002).

The five stages of biofilm development are illustrated in Figure 3-1. Initial attachment to a solid surface occurs when bacteria penetrate a film of organic molecules on a surface by eddy diffusion (i.e. mixing of the liquid) and attach by weak electrostatic or Van Der Waals forces. Highly specific interactions between microorganisms and with the surface, such as dipole, ionic or hydrogen bonding, or hydrophobic interactions, create irreversible attachment. Pieces of biofilm periodically break off, due to shear forces (Wimpenny, 2000). This releases fungi and other microorganisms into the water transported through the network to end users (Hageskal et al., 2007).

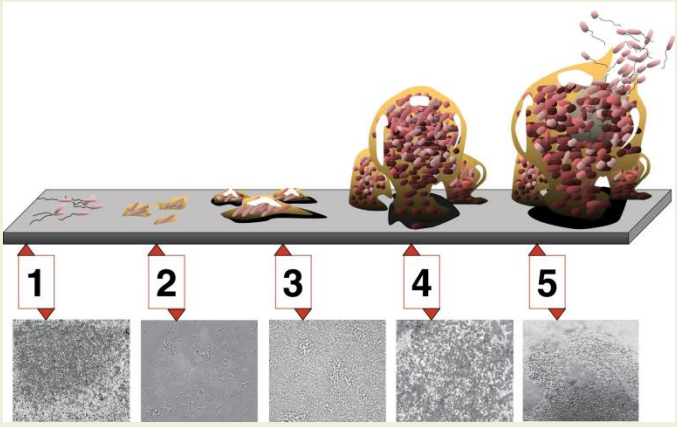

Figure 3-1: The five stages of biofilm development: 1. Initial attachment, 2. Irreversible attachment, 3. Maturation I, 4. Maturation II, 5. Dispersion (Monroe, 2007)

The organisms that make up biofilms may function as a community and thus have "emergent" properties, i.e. properties greater or different to those of the individual

components (Wimpenny, 2000). This is facilitated by the production of extracellular polymeric substances (EPS) which help to adhere the microorganisms to the surface, protect the community from environmental stresses and facilitate community interactions. Therefore, once fungi are established in biofilms they are less susceptible to water treatment or disinfection procedures (Hageskal et al., 2009 and Paterson and Lima, 2005). Fungal hyphae may also serve to strengthen the entire biofilm and make it more difficult to remove (Paramonova et al., 2009).

Interactions between fungi and bacteria, including in biofilms, are discussed in sub-section 4.2.1.

3.1.2. MULTIPLICATION AND SURVIVAL OF FUNGI WITHIN THE WATER DISTRIBUTION SYSTEM

The results of sample analysis from customer taps and other points within distribution systems often reveal higher numbers of fungi than the analysis of samples following treatment, prior to entry into the distribution system. For example, Grabinska-Loniewski et al. (2007) found a total of 200 CFU l^{-1} in newly treated water delivered to the distribution system. This increased to 5000 CFU l^{-1} in samples taken 10.3 km away from the treatment plant. Such increases through the distribution system could be due to two reasons: i) that the fungi that remain present after initial treatment/disinfection multiply within the system or are partially inactivated to later recover, and ii) that fungi enter the system via pathways of secondary contamination, or that fungi are not completely inactivated and later recover. Lack of sufficient concentrations of residual disinfectants throughout the system contributes to allowing the establishment of fungi entering the system. Accumulation in stored water at the consumer end has also been observed. For example, Anaissie et al. (2002) found higher numbers of colony forming units of *Aspergillus* in hospital water storage tanks than in the municipal water supply.

3.2. IDENTIFICATION AND CHARACTERISATION OF FUNGI IN THE DRINKING WATER DISTRIBUTION SYSTEM

3.2.1. SAMPLING METHODS

Most studies take samples of water from the tap or from various places in the distribution system, often as part of routine bacteriological monitoring. It is difficult to obtain a representative sample; fungi are often unevenly distributed through water. Many are held in biofilms, fragments of which occasionally break off. Therefore, quantities of fungi are likely to be highly variable with time with occurrences in mobile phases often for short durations and small volumes (Hageskal et al., 2009 and Paterson and Lima, 2005). Other recent distribution system quality-related research is utilising 'large volume' sampling. However, this is relatively unproven at present. Biofilms have

been collected by taking pipe coupons (i.e. longitudinal sections of the inside of the pipe), from which biofilm fragments were removed (Doggett, 2000).

3.2.2. ISOLATION, IDENTIFICATION AND QUANTIFICATION

The main methods of isolating, identifying and quantifying the fungi in the samples taken are described in Table 3-2.

Results of the quantification methods described in Table 3-2 are usually given as the number of Colony Forming Units (CFUs) of fungi in a certain volume of water. However, this measure does not necessarily give an accurate representation of the number of fungi present in a sample; it is likely to be an underestimation. For example, it is likely that one colony is formed of many different fungal structures, such as hyphae, conidia, conidiophores, from different "individuals" clumped together into one CFU (Gonçalves et al., 2006 and Paterson and Lima, 2005).

The specific protocol chosen for culturing fungi in water samples can select for particular species and hinder the growth of others. For example, incubating samples at a temperature of 25°C allows growth of mesophiles[4], but for thermotolerant species such as *Aspergillus fumigatas* incubation 30°C is needed. This point is particularly important as species which are human pathogens can withstand human body temperatures and thus may also need higher incubation temperatures (Gonçalves et al., 2006). The medium used for isolation and culturing can also select for some species and exclude others, depending on its nutritional content (Hageskal et al., 2009). When resources allow, it is recommended that samples are cultured on both a low-nutrient and a high-nutrient medium (Kinsey el al., 1999).

International standards exist concerning specific aspects of the microbiological analysis of water and food stuffs, such as ISO 6222:1999 – Water quality (enumeration of culturable micro-organisms) and ISO 11133 (preparation, production, storage and performance testing of culture media) (joint water and food standard). At national level, the American Public Health Association, the American Water Works Association and the Water Environment Federation publish "Standard Methods for the Examination of Water and Wastewater". This includes a specific standard (no. 9610) on the detection of fungi[5]. In the UK, methods for the enumeration of micro-fungi and yeasts by membrane filtration or spread plate techniques are published in the "Microbiology of Drinking Water" (Environment Agency, 2004).

[4] Mesophiles are organisms that grow best at moderate temperatures of between 20 and 50°C (Brochier-Armanet et al., 2008).

[5] Available from: www.standardmethods.org/store/ProductView.cfm?ProductID=117 [Accessed 12/1/2010]

Table 3-2: Advantages and disadvantages of main methods of sample analysis

Method	Description	Advantages	Disadvantages
Culture of samples (detection and quantification)	Fungi are cultured either from filtered water samples or direct spread of the sample on to the plate. Samples may also be centrifuged prior to culture to collect the fungi. Ideally samples are cultured on both high and low nutrient media. Plates are kept at a constant temperature and examined at regular intervals. The number of CFUs present are then counted.	-Low cost and practical. Low level of expertise needed.	-The media, time and temperature of cultivation can all influence the taxa identified. -Not all fungi can be cultured successfully in laboratory environments (producing false negatives). -Slow-growing species are likely to be under-represented in counts if insufficient time for culture is given (International Mycological Institute, 1996). -Culturing water samples can give inaccurate results due to interactions between species. For example, competition for nutrients will reduce the counts of weaker competitors, and production of mycotoxins by filamentous fungi could inhibit the growth of other species (Gonçalves et al., 2006). A fungal toxin (rose bengal) is sometimes added to prevent overgrowth of dominant species, which then leads to them being under-represented (International Mycological Institute, 1996). -Fungi can be outcompeted on culture plates if overgrowth of bacteria occurs. To avoid this, antibacterial substances are sometimes used. However, such substances have the potential to also inhibit some fungal species (International Mycological Institute, 1996). - Different volumes of water used each have different detection limits (Hageskal et al., 2009).
HPLC of ergosterol (detection and quantification)	Provides estimation of total fungal biomass as this is directly correlated with ergosterol production. The concentration of ergosterol is measured using UV spectroscopy (Kelley et al., 2003 and Paterson and Lima, 2005).	-Is more sensitive than quantification by dry weight- quantities in water samples are likely to be too low to be detectable by measuring dry weight. -Good indication of fungal surface area.	-Does not discriminate between species. -Not a particularly accurate measurement of biomass.
Quantitative PCR (detection, identification and	DNA is extracted from water samples, and is mixed with species-specific DNA primer sequences and probes. The qPCR	-Sensitive and specific. - Rapid processing times, thus allowing real time analysis.	-Difficulties of determining which species are included in or excluded from the test. -Can create false positives, i.e. where cells are dead but

Method	Description	Advantages	Disadvantages
quantification)	instrument then detects the quantity of DNA for each species in relation to known quantities of species-specific reference stocks of DNA.		still detectable.
Gene markers and probes and protein markers (detection and identification)	Gene markers used to detect mycotoxin metabolic pathways. Protein markers can also be used to detect specific proteins using the Western Blot technique.	-Useful supplements to morphological identification (Hageskal et al, 2009 and Paterson and Lima, 2005).	
Direct observation (identification)	Light or scanning election microscopy used to identify taxa based on morphology.	-Low cost.	-Morphological identification is subjective (Paterson and Lima, 2005). -Impossible for non-sporing species, or those individuals that are not sporing at the time of the sample being taken (Hageskal et al, 2009 and Paterson and Lima, 2005). -Due to limitations in morphological identification, many studies identify fungi to genus rather than species level.
Mass spectrometry (identification)	Specimens are usually mixed with a matrix that absorbs a laser beam. Ions are produced from the resulting high-energy impact, which can be extracted and detected as a mass/charge spectrum/	-High precision, sensitivity and speed.	-Requires database to be completed (Marklein et al., 2008).
Standard methods for detection and quantification of fungi	No international standards currently exist.	-Will allow standardisation of methods and comparability between studies	-No widespread adoption – considerable variation exists between studies and many state the lack of international standardised methods as a hindrance.

However, such national standards are not widely adopted and there is currently no international standard method specifically for the analysis of fungi in drinking water (Hageskal, et al., 2009). This represents the main limitation in the detection, identification and quantification of fungi in drinking water samples and makes it difficult to compare results between studies (Paterson et al., 2009). Thus it is often not possible to determine the proportion of variation between studies that is attributable to differences in methodology and the proportion attributable to environmental variation.

The total number of fungal CFUs found in treated drinking water is highly variable between studies (see Table 3-3), ranging from 1 CFU per litre to 5000 CFU l^{-1}. Colony Forming Units are not an accurate measure of fungal numbers, as discussed above, which may explain a degree of the variation between studies. However, it is the most commonly used unit of quantification and is reported here for that reason.

Table 3-3: Fungal biomass in treated drinking water

Mean total number of CFUs	Location	Study
200 - 5000 CFU l^{-1}	Poland	Grabinska, 2007
90 CFU l^{-1}	Norway	Hageskal, 2007
2800 CFU l^{-1} (mean yeasts), 1000 CFU l^{-1} (mean filamentous fungi)	Brazil	Yamaguchi et al., 2007
180 CFU l^{-1}	US	Nagy and Olson, 1982
28 CFU l^{-1}	US	Kelley et al 2003
1-20 CFU l^{-1}	Portugal	Gonçalves et al., 2006
$3.7x10^{2}$ CFU l^{-1}	Greece	Arvanitidou et al., 1999
8.9-31.8 CFU cm^{-2}	US	Doggett, 2000
100-1500 CFU l^{-1}	US	West, 1986
91 CFU l^{-1}	Austria	Kanzler et al., 2008

Of the studies that were analysed in-depth in this review (see Annex 3), 65 genera were isolated from treated drinking water. Of these, the majority were filamentous fungi. More filamentous fungi than yeasts are also identified within individual studies of the same water distribution system (Göttlich et al., 2002, Doggett, 2000 and Grabinska-Loniewska et al., 2007). It should be noted when interpreting this finding that depauperate filamentous fungi can form yeast-like cells.

It should also be noted that findings from other countries may not be directly applicable to the UK. For example, chlorine concentrations in the US are commonly

higher than in the UK, and by contrast the water in the study by Göttlich et al. (2002) was not chlorinated. Climatic differences in mean temperatures and rainfall may also influence the taxa found. Furthermore, treatment and disinfection regimes vary locally, as will the source of the drinking water.

Figure 3-2 illustrates the most frequently isolated genera by the number of studies in which they were found. *Penicillium, Cladosporium* and *Aspergillus* were the most common genera.

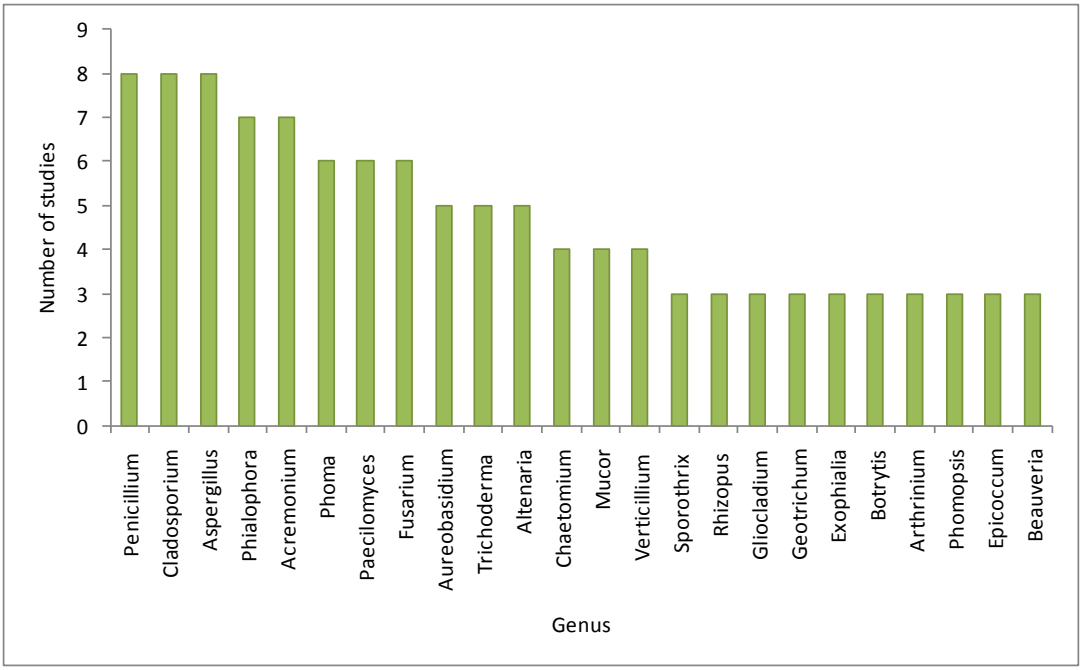

Figure 3-2: Number of studies in which most common genera were isolated from treated drinking water (those isolated by 1 or 2 studies excluded)

The temperature ranges that are tolerated by the taxa most frequently isolated from treated drinking water (see Table 3-4) affect the habitats within the water distribution system that they can inhabit. For example, some *Phialophora* species are thermotolerant (Göttlich et al., 2002), thus enabling them to colonise habitats such as hot water tanks. Differences in temperature tolerance between species may lead to seasonal variation in species composition. For example, numbers of *Acremonium* spp. isolated from drinking water samples taken in Braga, Portugal increased significantly between the months of November and February during the study period. During these months the abundance of other taxa declined to almost nothing, therefore suggesting that *Acremonium* spp. had a strong competitive advantage over winter. While this is likely to be due to the colder temperatures over winter other seasonal conditions such as rainfall may have had an effect (Gonçalves et al., 2006).

Table 3-4: Optimum temperature range of most frequently isolated taxa

Taxon	Optimum temperature range
Penicillium	Some species psychrophilic or pyschrotolerant (4-12°C), such as *P. expansum* and *P. cyclopium* (Gesheva, 2009).
Aspergillus	A. fumigatus optimum = 37-42°C (Chang et al., 2004). Other species optimum=30°C. Others psychrophilic (4-12°C) (Gesheva, 2009).
Cladosporium	Most species approximately 20-25°C. Some species psychrophilic (Feller and Gerday, 2003)
Phialophora	Some species thermotolerant e.g. *P. verrucosa*
Acremonium	Some species thermophilic, e.g. *Acremonium alabamensis* (Johri et al., 1999), some psychrophilic, e.g. *Acremonium pyschrophilum*, some psychrotolerant e.g. *Acremonium cerealis* (Margesin et al., 2008), many others are mesophilic.

Many of the taxa most frequently isolated from treated drinking water, including *Peniciullium* spp., *Aspergillus* spp. and *Cladosporium* spp., are melanised, meaning they secrete pigment called melanin. This pigment provides protection (especially for spores) against a range of stresses. Such species have a competitive advantage and greater resistance to water treatment. Melanin increases virulence in pathogenic species due to the protection it gives against host species' defences (Langfelder et al., 2003). It is possible that fungal species develop further resistance following exposure to disinfectants found throughout the distribution system. However, there is little evidence that resistance by mutation to disinfectants is acquired, and little is known about potential mechanisms by which such resistance would be acquired (McDonnell and Russell, 1999). The factors that affect the ecology of fungi in the water system will be discussed in chapter 4.

The hydrophobic property of the spores of many of these frequently-isolated genera, including *Peniciullium* spp., *Aspergillus* spp. and *Acremonium* spp. provides further protection against water disinfection. Such spores tend to aggregate due to the hydrophobic molecules associating more with each other and other particles than with water. This aggregation appears to be associated with increased resistance to water disinfection using UV and chlorine (Marmane-Gravetz and Linden, 2005).

3.2.3. PATHOGENICITY OF ISOLATED SPECIES

Many of the species that have been observed in drinking water, including all of the five most commonly isolated genera, are either known pathogens or implicated in a number of diseases (see Annex 1). The implications of such pathogenicity will be discussed further in chapter 5.

3.2.4. CURRENT REGULATIONS

At present, regulations controlling levels of fungi in drinking water are rare. For example, in the UK fungi are not required to be monitored or controlled, according to

the Water Supply (Water Quality) Regulations 2000[6]. An exception is Sweden, which limits fungal numbers under the National Food Administration Regulation (SLVFS 2001: 30) regarding drinking water (amendments/new print 2005:10). The Regulation limits microfungi to 100 CFU per 100 ml. This limitation applies at the point of water use, and therefore takes into account fungi which enter the system through pathways of secondary contamination (National Food Administration, 2001).

[6] Available from: www.legislation.gov.uk/uksi/2000/3184/contents/made [Accessed 12/1/2010]

4. FUNGAL ECOLOGY IN WATER SYSTEMS

Numerous factors, both biotic and abiotic influence the ecology of fungi in drinking water, in terms of their prevalence, likelihood of colonisation, growth rate, establishment in biofilms, and the species composition of communities. However, it is difficult to generalise as to the precise effects of such factors, particularly in terms of biofilm development. This is because biofilm communities are also regulated by the interactions between components, and therefore may develop "emergent" properties (see section 3.1.) different to those of the individual components (Hamilton, 1987).

4.1. ABIOTIC AND ANTHROPOGENIC FACTORS INFLUENCING ECOLOGY OF FUNGAL TAXA IN WATER SYSTEMS

4.1.1. RAW WATER SOURCE

Studies that included analyses of both groundwater-derived and surface water-derived drinking water found that isolation of fungi was more likely from surface water-derived drinking water (Hageskal et al., 2006 and Hageskal et al., 2007). For example, Hageskal et al. (2007) found that a greater proportion of surface water- derived drinking water samples were positive for fungi than groundwater-derived samples. However, there was not a great difference in the total mean number of CFUs obtained from all samples of surface water-derived water taken by Hageskal et al. (2007), compared to all samples of groundwater-derived water (9.5 CFU 100 ml^{-1} and 8.4 CFU 100 ml^{-1} respectively). There was one anomalous data point in the groundwater sample – sampling of one shower head produced 100 CFU 100 ml^{-1}, which increased the total number of CFUs found in groundwater-derived water samples. In a study of untreated source water, Pereira et al. (2009) found significantly higher mean levels of fungi in surface and spring water (1750 CFU 100 ml^{-1} and 1025 CFU 100 ml^{-1} respectively) than in groundwater (66 CFU 100 ml^{-1}).

The source of the raw water affects the total number of CFUs found due to biotic and abiotic differences between surface and groundwater. Surface waters tend to contain larger amounts of organic matter, which both provide nutrients and a substrate for fungal growth. Differences in acidity and calcium content may also account for some of the variation – studies in Norway and Portugal found that surface water is slightly more acidic with a lower calcium content (Hageskal et al., 2007 and Pereira et al., 2009). Furthermore, groundwater has lower levels of turbidity and total organic carbon compared to spring and surface water (Pereira et al., 2009).

It could be expected that seasonal variation in the detection frequency of fungi is more prominent in surface-water derived water supplies, given the greater exposure that surface water has to climatic influences compared to groundwater. However, this hypothesis was not supported by the results of the study conducted by Hageskal et al. (2007) which looked at the frequency of positive samples by season.

4.1.2. WATER TEMPERATURE

Temperature is an important influence on fungal counts, as it affects survival, growth rate and ability to reproduce. Species differ in their particular temperature requirements (see Table 3-4 for examples). For example, filamentous fungi were found by Gonçalves et al. (2006) to be particularly prevalent during the winter when temperatures are colder. In Norway, fungi were 14 times more likely to be isolated from cold tap water than from hot tap water, although this depended on the precise temperatures considered (Hageskal et al., 2007). Göttlich et al. (2002) noted that many of the species that they identified were known as being psychrophilic[7], thus supporting these findings.

Studies of fungi in other environments such as soil and the laboratory have also observed that fungi can grow at low temperatures (Pietkainen et al., 2005 and Pasanen et al., 1991), even as low as -20°C. Furthermore, Pietkainen et al. (2005) noted that soil fungi are better adapted to cold environments than bacteria, in terms of having a higher growth rate at lower temperatures. This would therefore result in a change in the composition of microbial communities to favour fungi.

Biofilm formation, an important location of fungal colonisation, is affected by water temperature (Lund and Ormerod, 1995). The highest rates of biofilm formation in water distribution systems have been observed to be at water temperatures of 15-25°C (Donlan et al., 1994). Once established, the water temperature influences the microbial composition of the biofilm (Rogers et al., 1994) as different temperatures will favour different species. For example, the biofilms that formed at 20°C were dominated by bacteria with 96% of microbes being *Pseudomonas*, with several protozoa also being present. At 40°C, 50°C and 60°C, *Aspergillus* spp. were a key component of the climax community, along with several bacterial species but no protozoa (see section 4.2. for further discussion of the interactions of species in biofilms).

4.1.3. WATER FLOW RATE AND SYSTEM HYDRAULICS

Flow rate of water within distribution systems varies according to many factors, including the layout of pipes, system condition, system size, level of demand, elevation and pump operation (US EPA, 2002).

Numerous factors related to biofilm formation and development are influenced by water flow rate, including likelihood of initial attachment, nutrient availability, biofilm

[7] i.e. are organisms which thrive at cold temperatures.

structure, loss of extracellular polymeric substances (EPS), and biofilm removal. The effects of water velocity on such factors are summarised in Table 4-1.

Table 4-1: Effects of water flow rate on biofilms

Stage of biofilm formation/ development	Result	Mechanism	Reference
Likelihood of initial attachment and development	Biofilm formation increases and is more rapid at higher velocities	Higher flow rates reduce the thickness of the boundary layer between the substrate and the water, and increase mixing in the water. Thus, microbial cells come into contact with the substrate surface more frequently (Donlan, 2002). Biofilms appear to be able to compress under pressure and exhibit a high resistance to shear stress.	Manuel et al. (2007) Howsam, 1995 Percival et al., 1999 Lehtola et al., 2006
	Maximum biofilm accumulation at very low flow rates	Higher flow rates increased shear stress, which reduced biofilm accumulation. Low flows also result in longer residence times and thus a loss of disinfectant residual in stagnant water (US EPA, 2002).	Lau and Liu, 1993
Nutrient availability	Higher flow rates provide higher nutrient levels, and have thus been observed to lead to higher bacterial growth. This issue has not been specifically studied for fungi, and the role of competition for nutrients between fungi and bacteria should be considered.		Lehtola et al., 2006
Biofilm structure	Streamers of EPS which attach the community to the surface and bind cells together at high velocities	Streamers improve resistance of the biofilm to shear stress and increase its surface area.	Percival et al., 1999
	Patchy biofilms at low velocities		Percival et al., 1999
	Open and 'fluffy' structures formed at low velocities (0-5m s-1). Cells aligned in the direction of flow at high velocities (2-5 m s-1).		Santos, et al., 1991
	Biofilms developed at		Santos, et

Stage of biofilm formation/ development	Result	Mechanism	Reference
	lower velocities (0-5 m s-1) are less compact and thicker than those developed at 2-5 m s-1);		al., 1991
Loss of EPS	At a relatively high velocity (0.96m s-1), the EPS matrix developed faster than at 0.32 m s-1. However, at 1.75m s-1 the EPS matrix was not present, and bacteria were attached to the surface by fibrillar structures.		Percival et al., 1999
Biofilm removal	Fluctuating cell counts at higher velocities indicates sloughing of biofilm	Biofilms are viscous, giving fluid frictional resistance. Thus, at high velocities biofilms may become more compact and stabilised.	Christen and Characklis, 1989, cited in Percival et al., 1999
	Amount of pre-existing biofilms reduced when flow velocity increased.	Large shear stresses (greater than 10-12Nm^{-2}) resulted in significant cell detachment.	Duddridge et al., 1982
	Changes in flow rate remove biofilms and resuspend the microorganisms in water		Lehtola et al., 2006

In addition to the rate of flow, the type of flow can also influence biofilm formation. The biofilm formed in laminar flow had a greater total number of cells than that formed in turbulent flow. However, the biofilm in turbulent flow had a higher number of cells per unit volume and was more stable (Pereira et al., 2002). Reversal of the direction of flow caused by backflow can remove biofilms, resulting in release of biofilm microbes. Interrupted or pounding water flows may have the same effect (US EPA, 2002).

As can be seen in Table 4-1, the effects of water flow rate on biofilm development are complex, and sometimes contradictory. To some degree, the different findings in relation to water velocity and biofilm development may reflect different structures and composition of the biofilms, which gives them different emergent properties. Furthermore, the effects of water flow rate on such factors may interact with other biotic or abiotic factors, such as pipe material, the species composition, chlorine

concentration, etc. For example, it was found that in unchlorinated or low chlorine water, biofilm growth rate increases as shear stress increases. However, in water with higher chlorine concentrations, growth rate decreased as shear stress increased (Tsai, 2006).

It should also be noted that the work examining the effects of water flow rate on biofilm formation and development is focused on bacterial biofilms. The interactions between fungi and bacteria are discussed in sub-section 4.2.1. How water flow rate affects fungal colonisation of biofilms specifically is not known, nor is whether the presence of fungi affects how biofilms respond to water velocity.

Fungi that have been observed to be able to grow in both stagnant and flowing water whether attached to surfaces or not. Indeed it has been hypothesised that the shape of spores may be an adaptation to allow anchorage to surfaces in flowing water (Kinsey et al., 2003).

4.1.4. NUTRIENT CONCENTRATION

Heterotrophic organisms such as fungi require nutrients for survival and growth, including assimilable organic carbon (AOC), phosphorus and ammonium. Such nutrients tend to concentrate at the solid-liquid interface, and can become trapped in biofilms at this interface. The level of nutrients often regulates the rate and extent of biofilm growth. Indeed, some countries such as the Netherlands prefer controlling AOC over disinfection for limiting biofilm growth. Phosphorus and ammonium concentrations may be limiting for microbial growth. Higher concentrations may facilitate the recovery of microbes that have been stressed by disinfectants (US EPA, 2002). Such studies have focused on bacteria when investigating the influence of nutrients on biofilm development, and further research is needed to determine the effect on fungi in biofilms. The overall influence of nutrient concentration on fungal establishment in water distribution systems is likely to be different from that for bacteria, given that fungi are able to grow in environments that appear to be nutrient free (Kinsey et al., 2003). Competition for nutrients between bacteria and fungi in culture is thought to occur (Gonçalves et al., 2006), but the extent to which such competition influences ecology of biofilms in water distribution systems is not known.

4.1.5. PIPE MATERIAL

The material from which the pipes in water distribution systems are made influences the deposition and presence of fungi. Grabinska-Loniewska et al. (2007) isolated fungi only in sections of the system made of iron and steel. Similarly, Doggett (2000) found fungi to be present in all samples of sections of iron piping, but not in the sample of PVC piping. However, it should be noted that this study only included one sample of PVC piping. Other studies have also found that bacterial biofilms develop more rapidly on iron pipe surfaces than PVC (Le Chevallier, 1999).

Differences between copper and polyethylene (PE) pipes in terms of biofilm formation have also been investigated. It was found that biofilm formation was more rapid in the PE pipes than the copper pipes, but after 200 days there was no difference in microbial numbers between the two materials (Lehtola et al., 2004).

Those piping materials with a high degree of surface roughness are more likely to be colonised, due to the greater surface area and the reduction in shear forces (Percival et al., 1999).

The hydrophobic/hydrophilic properties of the substrate will also influence biofilm formation (Momba et al., 2000).Theoretically, biofilms are more likely to attach to hydrophobic surfaces such as plastics, than hydrophilic ones such as metals (Donlan, 2002). However, the studies that have obtained this finding have not specifically assessed biofilm formation in drinking water distribution systems (Fletcher and Loeb, 1979, Pringle and Fletcher, 1983 and Bendinger et al., 1993). Therefore, it may be that other environmental factors in distribution systems are of greater influence than the hydrophobic/hydrophilic properties of the substrate.

The pipe material can also modify the effectiveness of water disinfectants. For example, the products of corrosion of iron pipes react with residual chlorine and prevent it from penetrating the biofilm (Le Chevallier, 1999). In a comparison of copper and PE pipes, it was found that chlorine was more effective in the PE pipes. Chlorine concentration declined more rapidly in the copper pipes, allowing microbial numbers to return to the pre-treatment level within a few days of chlorination (Lehtola et al., 2005).

Again, such studies focus on the effects of pipe material with respect to bacterial biofilms. The extent to which the pipe materials influence fungal establishment of biofilms or colonisation of existing biofilms requires further investigation.

4.1.6. PARTICLE ACCUMULATION

Organic and inorganic particles accumulate in areas of low flow within the distribution system. Water storage facilities are particularly vulnerable to particle accumulation due to the longer residence time of the water – it is usually only drawn on during periods of high demand. Such particles are important areas of microbial activity due to the nutrients and protection from disinfectants they provide, and many fungal species have been observed in particle accumulation. Furthermore, nutrients may be released from particles, leading to increased biofilm growth (US EPA, 2002). High biofilm growth may may lead to more particles being trapped, thus in turn leading to greater biofilm growth. As was discussed in sub-section, 4.1.4. further research is needed to determine the effect of nutrient concentration on fungi in biofilms specifically.

4.1.7. MAINTENANCE PROCEDURES

As introduction of fungi into water supplies during maintenance has been identified as a key secondary contamination pathway for soil species, maintenance procedures and

practices can have an influence on the species that enter the system. Personnel carrying out maintenance or repairs can be a pathway for introduction of contaminants. Any materials used, such as piping, filters, and seals, or equipment, such as tank cleaning equipment or video equipment used for inspection, can introduce contaminants if not disinfected before use. However, water companies have procedures in place to minimise the risk of introducing soil and microorganisms into the water distribution system during repairs.

4.1.8. WATER TREATMENT AND DISINFECTION

Under the Water Quality Regulations (2000 and as amended), water must not contain any microorganism, parasite, or other substance at a concentration or value which would constitute a potential danger to human health. This can be achieved through disinfection, which is defined in the Regulations as being 'a process of water treatment to remove or render harmless to human health every pathogenic micro-organism […] that would otherwise be present in the water'. This involves a number of processes carried out in a water treatment plant as well as maintaining a residual disinfection throughout the water distribution system to inactivate microorganisms introduced after the treatment plant.

- **Removal of fungi**

A number of different processes are used to remove microorganisms, including fungi. The main processes and the efficiency by which they remove fungi are provided in Table 4-2.

Table 4-2: Main removal processes and their efficiencies

Removal process	Removal efficiency	References
Filtration (sand or granular activated carbon)	90% of fungi removed.	Kelley et al., 2001
	13% of samples positive for thermophilic fungi and 100% positive for mesophilic fungi before treatment, compared to 14% and 92% positive respectively following sand filtration.	Niemi et al., 1982
Chemical coagulation – this involves adding a coagulant to remove contaminants from suspension.	56% of samples positive for thermophilic fungi and 100% positive for mesophilic fungi before treatment, compared to 0% and 46% positive respectively following treatment. The precise coagulation process used here is not known.	Niemi et al., 1982
Clarification – this involves allowing solids to separate out of the water and sink to the bottom of the tank. The term may also refer to the whole process of coagulation, flocculation and sedimentation.	70% of fungi removed. In the water treatment facility assessed in the study, the term clarification refers to 'floc blanket clarification'. In this case the blanket acts as both a coagulater and a filter.	Kelley et al., 2001

Overall, to varying degrees remove some but not all of the fungi found in the source water (Grabinska-Loniewska et al., 2007, Hageskal et al., 2007, Kelley et al., 1997, Paterson and Lima, 2005 and Kinsey et al., 2003).

However, fungal growth that is already well established within distribution systems is considerably more difficult to remove (Kinsey et al., 2003). The degree of treatment efficacy depends on a number of factors, including the particular processes used, and the species. For example, as has been discussed in section 3.1. , melanised, thick-walled species with hydrophobic spores are particularly resistant to treatment (Hageskal et al., 2009, Paterson and Lila, 2005 and Kinsey et al., 2003) .

Sand filtration has been suggested as an effective treatment method (Kinsey et al., 2003 and Paterson and Lima, 2005), and more so than clarification[8] (Kinsey et al., 2003). However, the filters can be colonised by fungi, thus increasing the biological load and reducing the effectiveness of the treatment processes (Hageskal et al., 2009 and Paterson and Lima, 2005). To remove already-established biofilms, flow jetting has been found to be the most effective method (Kinsey et al., 2003).

The efficiency of water treatment processes and the factors that influence it have not been widely studied (Hageskal et al., 2009). There is a need for greater research in this area, particularly in order to explain the discrepancies between existing studies, and to build consensus on the most effective techniques in particular sets of circumstances.

- **Inactivation of fungi**

Table 4-3: Main inactivation processes and their efficiencies

Removal process	Removal efficiency	References
UV radiation	Turbidity reduces effectiveness and no residual is provided. Pigmented spores better protected against radiation so less susceptible to UV treatment.	Betancourt and Rose, 2004. Hageskal et al., 2009
Copper and silver ionisation (not used in treatment of public drinking supplies)	29% of ionised water samples were positive for fungi compared to 77% of non-ionised water samples.	Pedro-Bodet, et al., 2007
Chlorine	99.36% inactivation of *Trichoderma harzianum* after 60 minutes, 98.11% inactivitation of *Epicoccum nigrum* after 40 minutes and 97.65% inactivation of *Aspergillus niger* after 10 minutes, all with an initial free chlorine concentration of 1.3 mg L^{-1}.	Kelley et al., 1997
Ozone	99% inactivation after 18 seconds at 0.02 mg L^{-1} ozone and after 5 seconds at 1 mg L^{-1} ozone.	Kawamura et al., 1986.
Chloramine	Not available	-

[8] Causing a precipitate to be formed in the water that can then be physically removed.

Ionisation of water with silver and copper, a well-recognised method of controlling *Legionella* in hospital water supplies, has resulted in a significantly lower prevalence of fungi compared to non-ionised water in hospital distribution systems. However, as the effectiveness of this method has only been investigated by one study, further research is needed to confirm the finding (Pedro-Bodet et al., 2007). Furthermore, it is not used as a method of treating public drinking supplies.

Chemical disinfectants are frequently also used as the last process in a water treatment plant and to maintain a residual concentration throughout the distribution system. Residual concentrations are needed to inactivate fungi that enter the system after the treatment plant and those which are initially only partially inactivated and thus can recover later in the system. The efficacy of chemical disinfectants against fungi is variable between species (Kinsey et al., 2003).

Efficacy of chlorine is the most dependent on temperature - inactivation of spores occurs less frequently at lower temperatures. The exposure time to free chlorine that is needed to inactivate fungi is longer than for other chemical disinfectants, particularly ozone and chlorine dioxide (Paterson and Lima, 2005). Spores are more resistant than hyphal cells, with some being extremely chlorine-resistant (Kelley et al., 1997). Such spores could thus allow the establishment of fungi in the water system even if treatment processes have removed the vegetative cells. Once fungi are established in the system, it can be difficult to maintain sufficient concentrations (i.e. of 0.4 to 0.5 mg l^{-1}) (Rosenzweig et al., 1983) of free chlorine to prevent colonisation and biofilm formation (Grabinska-Loniewska et al., 2007 and Lund and Ormerod, 1995). This is because the chlorine demand of fungi is high (Kelley et al., 1997 and Rosenzweig et al., 1983). Chlorine demand can also be affected by other microbes in the system and the material from which the pipes are made (Kelley et al., 1997). It has been suggested that initial free chlorine concentrations of approximately 1 mg l^{-1} are sufficient for spore inactivation and to provide sufficient residual chlorine in the system to assist in prevention of new growth (Kelley et al., 1997 and Kinsey et al., 2003) and development of biofilms (Lund and Ormerod, 1995 and Momba et al., 2000). However, concentrations of free chlorine are not always as high as 1 mg/l at UK treatment works and are likely to be much lower in distribution systems (0.3 mg l^{-1}),.Therefore, inactivation and prevention of regrowth within the UK's water distribution system is likely to be lower than suggested by these studies.

Chlorine dioxide and ozone have been found to be the most effective in studies by Kelley et al. (2001). However, chlorine dioxide is not widely used in the UK and ozone is not used in the UK to provide a residual disinfectant in the distribution system. Ozone has a lifetime of less than one hour in water due to its rapid decomposition. In most cases, i.e. apart from very short distribution systems, it does not remain long enough to provide a disinfectant residual throughout the distribution system. Therefore, it does not have an effect on biofilms and fungi present in the system after treatment.

Where water is treated with ozone it is usually replaced by chlorine or chlorine dioxide as a final step in order to maintain a disinfectant residual (Camel and Bermond, 1998).

Chloramines are another common choice of disinfectant. There are three types: monochloramine, dichloramine and nitrogen trichloride. Monochloramine is most commonly used as the other two negatively affect the taste and odour of the water (Chung et al., 2006). Monochloramine is more stable than chlorine, chlorine dioxide and ozone, and therefore may be more effective in the long-term, due to its greater persistence in distribution systems (Kelley et al., 2001). Monochloramine is a stronger fungicide than other chloramines (Arnitz et al., 2009).

Combinations of a number of removal and inactivation processes are likely to be the most effective. For example, in a Polish study, two different combinations of treatment processes were used successfully to remove all species but *A. fumigatus* and *A. niger*. The first treatment process involved filtration and aeration, including sand filters and sand filters with activated carbon, and disinfection with chlorine and chlorine dioxide. The second included chemical coagulation using aluminium sulphate, silica and pulverised carbon; alkalisation with lime; fast filtration with sand; and disinfection with chlorine and chlorine dioxide (Grabinska-Loniewska, 2007).

4.2. BIOTIC FACTORS INFLUENCING ECOLOGY OF FUNGAL TAXA IN WATER SYSTEMS

4.2.1. INTERACTIONS WITH BACTERIA

Understanding the interactions between bacteria and fungi is important in order to determine if bacterial content, a commonly measured parameter of drinking water, can be used as an indicator of fungal content (Gonçalves et al., 2006). If the absence of a correlation is common across distribution systems, it can mean that there is the potential for bacteriologically safe water to contain potentially pathogenic fungi.

As can be seen in Table 4-4, different studies have found different relationships between fungi and bacteria. These differences could arise from the different species compositions isolated from water systems, differences in methodologies, or different biological mechanisms affecting the relationship. For example, the interactions between fungi and biofilm-bacteria may explain the positive relationships (Jefferson, 2004). Fungi are often secondary colonisers of pre-established bacterial biofilms (Paterson and Lima, 2005 and Kinsey et al., 2003).

Table 4-4: Observed correlations between fungi and bacteria in drinking water

Positive correlations	Negative correlations	No correlation
A positive correlation was found between yeasts and total heterotrophic bacteria in tap water (Brazil) (Yamaguchi et	A negative correlation has been observed between fungi and bacteria in samples of high bacterial biomass (Germany)	No correlation was found between fungal and bacterial biomass in unchlorinated

Positive correlations	Negative correlations	No correlation
al., 2007)	(Göttlich et al., 2003)	groundwater-derived water in Germany (Göttlich et al., 2003) nor in treated water in Poland (Grabinska-Loniewska et al., 2007)
A significant positive correlation was observed between yeasts and total and faecal coliforms (Greece) (Aravanitidou et al., 1999)		No correlation was observed between filamentous fungi and total coliform (Brazil) (Yamaguchi et al., 2007)
A significant correlation was observed between filamentous fungi and total heterotrophic bacteria (Greece) (Aravanitidou et al., 1999)		No correlation found between levels of fungi and total coliform (untreated water) (Pereira et al., 2009).
Correlation between level of fungi and *Escherichia coli* and *Enterococcus* (untreated water) (Pereira et al., 2009).		

The different ecological requirements of the two organisms can theoretically lead to commensal relationships, in which one benefits while the other is unaffected (Jefferson, 2004). This theory suggests that negative correlations between fungi and bacteria in biofilms are unlikely. Furthermore, it has been demonstrated that fungi colonise pre-established bacterial biofilms, again indicating a positive relationship should be expected (Doggett, 2000). Negative relationships observed may be related to the culturing process, where bacteria and fungi are in direct competition for resources (Gonçalves et al., 2006).

These findings illustrate that correlations with bacteria depend on whether filamentous fungi or yeasts are being considered, and which bacteria are being assessed. Whether the remaining variation in findings between studies is due to differences in the specific composition of species, or to differences in methodology (such as the amount of time samples are cultured to allow for slow-growing fungi) is unclear. Therefore, there is a need for further research to investigate the different correlations between fungi and bacteria, and what factors influence such associations. This will allow it to be determined whether, and in which circumstances, bacterial contamination of drinking water indicates fungal contamination.

If bacteria and fungi inhabit the same location specific interactions have been observed. For example, culturing marine bacteria and fungi together has led to the production of novel compounds that are not produced by either species separately in laboratory conditions (Oh, et al., 2005 and Oh et al., 2007, in Shank and Kolter, 2009). Fungi-bacteria interactions can also inhibit secondary metabolite production. When a

bacteria (*Pseudomonas aeruginosa*) is cultured with a fungus (*Candida albicans*), farnesol, a metabolite produced by *C. albicans*, inhibits the production of secondary metabolites by *P. aeruginosa*, such as pyocyanin and Pseudomonas quinolone signal (Cugini et al., 2007, in Shank and Kolter, 2009). Farnesol also inhibits hyphal growth in *C. albicans* (Hogan, 2006). However, peptidoglycan, which forms bacterial cell walls has been shown to stimulate hyphal growth in *C. albicans* (Xu et al. 2008, Shank and Kolter, 2009).

Interactions and associations with other microorganisms are discussed in Box 2.

Box 2: Interactions and associations between fungi and other microorganisms

The importance of interactions and associations between other microorganisms and fungi in drinking water has not been well studied. Potentially important interactions that have been described in other circumstances are discussed below.

- **Interactions with protozoa**

Some species of amoebae, including *Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri* and *Sappinia diploidea* are known human pathogens (Visvesvara et al., 2007). In addition, free-living amoebae are known to be reservoirs for amoebae-resisting bacteria such as *Legionella,* which can survive and multiply within the amoeba host and exit it once environmental conditions become more favourable. The protection that the amoeba host provides the internalised bacteria allows them to avoid inactivation by water disinfection processes. It is this mechanism that is likely to explain the rapid recolonisation of some water systems immediately after a disinfection programme has stopped (Loret and Greub, 2010). An example of a fungus being phagocytosed by and replicating within an amoeba has been described in the literature. The melanisation of *Cryptococcus neoformans* is thought to be responsible for allowing it to survive within *Acanthamoeba castellanii* (Steenbergen et al., 2001).

However, associations between fungi and protozoa are also ecologically important. For example, in a study on microbial interactions in water-damaged buildings amoebae were observed to co-occur with several fungal species, including *Acremonium* spp., *Aspergillus versicolor, Chaetomium* spp. and *Trichoderma* spp. (Yli-Pirila et al., 2004). Given that amoebae have been found in treated drinking water (Singh and Coogan, 2005 and Berry and Raskin, 2006), such co-occurrences are potentially important and their significance in drinking water is not well known.

- **Interactions with viruses**

Many fungal species, including *Penicillium chrysogenum, Alternaria alternata* and *Aspergillus fumigatus*, are inhabited by viruses, forming fungi-virus complexes (Jamal et al., 2010). The effect on fungi of their infection by viruses varies depending on the species involved. Infection of *Aspergillus* species with mycoviruses has been observed to reduce mycelia growth rate, spore production and competitive ability (van Diepeningen et al., 2006).

- **Interactions with algae**

Some freshwater algae are infected with the chytrid fungus (Lopez-Llorca and Hernandez, 1996) and some other fungal genera, including *Penicillium* and *Aspergillus*, have been associated with green and red algae from marine environments Dewey et al., 1983). Marine algae also produce compounds which have been observed to have antifungal properties (de Félicio et al., 2010). Conversely, freshwater algal species have been observed to be destroyed by a fungus (*Trichaptum abietinum*) (Jia et al., 2010).

4.3. SUMMARY OF BIOTIC AND ABIOTIC FACTORS INFLUENCING FUNGAL ECOLOGY

The specific influences of the main biotic and abiotic factors on the most common taxa observed in drinking water systems are summarised in Table 4-5.

Table 4-5: Summary of biotic and abiotic factors influencing fungal ecology

Factor		Influence on fungal ecology on drinking water systems
Abiotic and anthropogenic factors	Raw water source	-Whether the raw water source is surface-water or groundwater influences the rate of fungal isolation. Surface water has more organic material and nutrients, thus leading to a greater likelihood of isolating fungi from surface-water derived systems.
	Water temperature	-Temperature affects fungal growth rate, reproduction, competition for nutrients with other elements of microbial community and survival. Studies of drinking water systems have found higher prevalence of fungi in cold water.
	Water flow rate and system hydraulics	-Flow rate affects biofilm formation, but no consensus as to the specific mechanisms by which this happens.
	Nutrient concentration	- Nutrients, particularly AOC, phosphorus and ammonium, are frequently a limiting factor for microbial growth, including in biofilms.
	Pipe material	-Pipe material influences fungal deposition and biofilm formation(e.g. iron and steel favour the colonisation).
	Particle accumulation	-Accumulated particles provide nutrients and protection from disinfectants and thus are a common habitat for fungi in distribution systems.
	Ingress and intrusion	-Introduction during maintenance procedures and intrusion during low and negative pressure events are a potentially important pathway for the introduction of soil and air-borne fungi .
	Water treatment	-Standard water treatment procedures are effective in removing most fungi from raw water. Melanised species have been found to be resistant to treatment however.

Factor		Influence on fungal ecology on drinking water systems
	Water disinfection	-Maintaining residual chlorine within the system can help to reduce biofilm formation and growth of fungi that enter the system after treatment.
Biotic	Interactions with bacteria	-Fungi colonise pre-established biofilms formed by bacteria, and may form commensal relationships with bacteria due to different ecological requirements, thus leading to positive correlations. -In culture fungi and bacteria are in competition for resources, thus leading to negative correlations being observed. This is probably true also in water distribution systems.
	Interactions with protozoa	-Some amoebae are known to attack and consume fungi. In addition, some species of amoebae can host bacteria and release them depending on the environmental conditions, thus having potential indirect impacts on fungal ecology. Although this interaction has not been sufficiently considered in drinking water systems
	Interactions with viruses	-Many fungi are inhabited by viruses.

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5. IMPACTS ON HUMAN HEALTH

A range of fungal taxa have been isolated from drinking water distribution systems, in a number of different countries (see Annex 1). Of greatest concern to consumers of drinking water is whether the presence of such fungi, some species of which are known to be pathogenic or allergenic, has negative impacts on health. The consumption of fungi-contaminated drinking water has, as far as is known, not caused acute disease, at least in immuno-competent individuals (Hageskal et al., 2009). However, there is a risk of superficial or localised infection in healthy individuals and more severe and invasive infection in immuno-compromised patients. Some species also have the potential to cause allergic reaction and disease. Furthermore, the health effects of fungal secondary metabolites should be the object of further research since some are toxic and others are thought to have caused taste and odour problems in tap water. Studies that directly assess whether fungi in drinking water are responsible for fungal infections and allergies are few. Therefore, while it is known that fungal species have been isolated from drinking water and that some fungal species cause the disease, the extent to which the two are linked is not well known.

This chapter assesses the various risks arising from fungi in drinking water for various population groups, and discusses how the risks are managed. A summary of health and taste/odour impacts is provided in Annex 2.

5.1. EXPOSURE PATHWAYS

- **Sources of pathogenic or allergenic fungi**

As has been discussed in section 5.2.1. there are a number of reasons to suggest that water should be considered as a potential transmission route for pathogenic or allergenic fungi. However, a number of other environmental sources exist, which are described in Box 3.

Determining the environmental source of a fungal infection or allergic disease requires genotyping and comparing fungal DNA taken from the affected patient and DNA taken from environmental sources. A number of different sources for a fungal infection are also possible (Menotti et al., 2005). For example, Warris et al. (2003) found that patients suffering from invasive aspergillosis were infected from either the air, water, or both. Furthermore, water was found to be the source of infections caused by *Fusarium* in a hospital in Houston, Texas, due to the molecular similarities between isolates from patients and isolates from water environments within the hospital (Anaissie et al., 2001).

Box 3: Sources of exposure to fungi

The air is thought to be a common source of pathogenic/allergenic fungi (Perlroth et al., 2007). Due to this, hospitals have implemented a number of measures to remove such fungi from the air to reduce exposure for patients at risk of fungal infection. Such measures include using high-efficiency particulate air filters and laminar airflow systems (Anaissie et al., 2002).

Fungal colonisation of food is also thought to be an important source from which patients' respiratory or digestive systems are colonised. Contaminated water used in food production processes may be a route by which fungi are introduced into food (Paterson et al., 2009 and Hageskal et al., 2006). Preventative measures include sterilising or disinfecting foods where possible, and banning some particularly contaminated foods such as soft cheeses for high-risk patients (Bouakline et al., 2000).

In some cases, such as the studies by Warris et al. (2003) and Anaissie et al. (2001), drinking water has been confirmed as at least one of the sources of fungal infections acquired in hospital. In other cases, fungal species that have been isolated from drinking water are involved, but drinking water as the infection source has not been confirmed.

Pathways of exposure

The four principal pathways by which people can be exposed to fungi in drinking water are:

- **ingestion**– drinking contaminated water directly;
- **inhalation** of aerosolised spores while showering or in the sauna;
- **skin contact** with contaminated water, such as while showering or bathing; and
- **introduction through mucous membranes**, such as the skin, eyes and oral cavity, while showering or bathing.

Aerosolisation of spores or fragments of hyphae from water has been particularly investigated as a pathway of exposure. For example, Anaissie et al. (2002) attempted to identify sources of *Aspergillus* infection in a hospital. They found that bathrooms had significantly higher numbers of airborne propagules than in patients' rooms (2.95 CFU m^{-3} and 0.78 CFU m^{-3} respectively, P=0.05). This was thought to arise from aerosolisation following running the tap or shower or flushing the toilet, allowing colonisation of damp microniches within the bathroom. Warris et al. (2001a) also found that airborne *A. fumigatus* levels increased after running the shower multiple times.

Skin contact with fungi in water while bathing can be a source of allergic skin irritation (see sub-section 5.2.2.).

5.2. DIRECT HEALTH IMPACTS OF FUNGAL INFECTIONS

5.2.1. SUPERFICIAL, SUBCUTANEOUS AND SYSTEMIC INFECTIONS

There a number of infections that are known to be caused by fungi, which can be classified according to the site of initial infection (Richardson and Warnock, 2003):

- **superficial mycoses**: infections of the skin, nails, hair and mucous membranes, such as topical candidiasis[9]. Such infections are relatively common and easily treated.
- **subcutaneous mycoses**: infections of the dermis, subcutaneous tissues and adjacent bone. These usually arise from implantation of fungi in soil or decomposing vegetation and are most common in tropical and sub-tropical regions when skin is exposed to soil (e.g. when barefoot). Disseminated infection is rare and usually only occurs in immunocompromised individuals.
- **systemic mycoses**: originate in an internal organ, often the lungs, and may spread to other organs (i.e. become invasive). These infections may be caused by true pathogens which can invade normal (i.e. immunocompetent) hosts, or by opportunistic pathogens which are less virulent and can only invade immunocompromised hosts.

A limited number of species are responsible for such diseases; it is thought that of the 50 000 to 250 000 known species of fungi, 500 have been linked to disease in humans and 100 can cause disease in otherwise healthy individuals (Richardson and Warnock, 2003). The most problematic species are *Candida* spp. (especially *C. albicans*), *Aspergillus* spp. (especially *A. fumigatus*) and *Cryptococcus neoformans* (Paterson et al., 2009 and Pfaller et al., 2006.).

The incidence rate of invasive fungal infections primarily in certain population groups, such as those that are immunosuppressed, continues to increase (Annaisie et al., 2002 and Arvantidou, et al., 1999). For example, the prevalence of invasive fungal infection at autopsy in a German hospital was found to increase 14-fold between 1978 and 1992 (Groll et al., 1996). The increase was found to continue during the follow-up study in the same hospital: 6.6% of patients autopsied in the period 1993-1996 had an invasive fungal infection, rising to 10.4% in the period 2001-2005 (Lehrnbecher et al., 2010). Several reasons for the observed increases have been suggested, including increases in incidence of HIV/AIDS; changes in medical procedures such as increased use of immunosuppressive medication, broad-spectrum antibiotics and prosthetic devices; and more invasive surgical procedures (Denning, 2006 and Enoch et al., 2006).

Immunocompetent individuals with no underlying health condition may experience superficial or localised infections but with fewer complications and a much smaller risk

[9] A general term for infections caused by Candida spp.

of disseminated or invasive disease and death (Anaissie et al., 1989, Chen et al., 2001, Walsh et al., 2004). For example, a study of both immunocompetent and immunocompromised patients with *Fusarium* infections found that skin infections in immunocompetent patients resulted from skin breakdown, were localised to this site, progressed slowly and responded well to treatment. By contrast, in immunocompromised individuals skin infections only occasionally resulted from skin breakdown. Infection progressed rapidly, was disseminated within the body including to the blood, and in some cases led to death (Nucci and Anaissie, 2002).

The following sections will discuss the taxa most frequently isolated from drinking water (Figure 3-2) and their direct health impacts. Subsequently, other taxa which are responsible for direct health impacts and which have been isolated from drinking water (although less frequently) will be discussed. It should be that noted not all species of the same genus have the same degree of toxicity, pathogenicity or allergenicity (Hageskal et al., 2009). However, the genetic boundaries between species are not well defined and can be misleading (Paterson and Lima, 2005).

In the discussion of fungal infections below, it is specified if infections are limited to a particular population sub-group or at-risk group. When such a group is not specified it indicates that the infection can occur in the general population, i.e. including healthy, immunocompetent individuals.

- ***Penicillium* spp.**

Penicillium marneffei has been identified as a pathogen endemic to south east Asia, India and China, that particularly affects HIV-infected individuals causing disseminated infection (Vanittanakom et al., 2006). However, there appear to be no reports of *P. marneffei* in drinking water or of infection being acquired via water. Invasive infection by other species is very rare, although superficial infection causing keratitis and otomycosis is more common (Lyratzopoulos, 2002). *Penicillium* is the genus that was most commonly identified in drinking water according to the studies examined (see Figure 3-2) and therefore drinking water is a source of exposure. However, whether *Penicillium* in drinking water is the source of *Penicillium* infections is not known.

- ***Cladosporium* spp.**

Fungi in the genus *Cladosporium* are not normally thought to be responsible for severe infections, although they have been isolated from skin and toenail infections (Tamiskar et al., 2006). They are allergenic, and can lead to hypersensitivity pneumonitis, sinusitis or asthma (Hayette et al., 2010) (see sub-section 5.2.2. for more details).

- ***Aspergillus* spp.**

Aspergillus spp. have been isolated from drinking water by many studies (see Figure 3-2).For example, Annaisie et al. (2002) isolated it from 33% of municipal water samples, 55% of samples from hospital water storage tanks and from 21% of samples

of water from hospital patient care areas. Infections caused by *Aspergillus* species are known as aspergillosis, a term which covers a range of invasive and non-invasive infections and allergic diseases.

Aspergillosis infections are primarily caused by inhalation of airborne spores (Annaisie et al., 2002). However, it is increasingly being recognised that water is an environmental source of *Aspergillus* spp. and has been identified as being the source of exposure. The genotype of *A. fumigatus* recovered from water was related to the genotype of isolates from three patients (Warris, 2003).There are a number of further arguments that suggest that water should be considered an important route of transmission of pathogenic *Aspergillus* spp. (Annaisie et al., 2002):

- incidence of aspergillosis continues to increase, despite measures to control fungi in air in hospital environments, such as the use of laminar air flow systems and high efficiency particulate air filters;
- there appears to be no correlation between airborne spore counts of *Aspergillus* spp. and rates of aspergillosis;
- the skin and the digestive system have been identified as points of entry for *Aspergillus* spp. (as opposed to lungs which are the point of entry for airborne fungi);
- *Aspergillus* species are similar to *Legionella* species, known water pathogens, in several aspects of their ecology, including amplification in water reservoirs, presence in biofilms in water distribution systems, and some requirements for growth; and
- invasive aspergillosis has been linked anecdotally with inhalation of contaminated surface water in patients who have suffered near drowning (Warris, 2001).

Aspergillus terreus is increasingly reported as a cause of pneumonia and disseminated infections in at-risk populations. This is an issue as *A. terreus* is relatively resistant to amphotericin B, the standard drug for treating fungal infections (Vesper et al., 2007). *Aspergillus ustus* has also been implicated as an emerging but rare opportunistic pathogen in immunocompromised individuals (Hageskal et al., 2006). An outbreak of *A. ustus* infections in a hospital in the US amongst patients that had undergone hematopoietic stem cell transplant stimulated a retrospective analysis of the likely cause. *Aspergillus ustus* infections result in onychomycosis, otitis media, primary cutaneous infection, endocarditis, pneumonia and disseminated infection. Eighty-three per cent of the patients in this outbreak had graft-versus-host disease following transplant that required immunosuppressive therapy, thus making them vulnerable to opportunistic infections (for more details see sub-section 5.2.4.). Water was not specifically tested, but a common environmental source (such as air, water, or surfaces) was thought likely. This was due to the genetic similarity of the fungal isolates

and the spatial proximity of the patients while in the hospital (Panackal et al., 2006). Hageskal et al. (2006) hypothesised that slight differences in time between infections may be a result of the biofilm theory, i.e. that sloughing of biofilm may periodically occur, leading to temporal differences in prevalence. They also suggested that the hot water tank in the hospital may have been a source as *A. ustus* is able to establish in such installations.

- ***Phialophora* spp.**

Infections caused by a number of *Phialophora* species have been observed, including rare superficial infections in healthy patients (Kimura et al., 2003). *Phialophora europaea*, a member of the *P. verrucosa* complex, has been isolated from cutaneous and nail infections in north-western Europe (de Hoog et al., 2000).

Phialophora is one of the genera that were most commonly identified in drinking water according to the studies examined (see Figure 3-2). However, whether *Phialophora* infections arise from exposure to *Phialophora* in drinking water or whether other sources such as spores or hyphae in air or food for example are the source of exposure is not known.

- ***Acremonium* spp.**

Acremonium infections have been observed in vulnerable individuals, for example pulmonary infection with *Acremonium strictum* was observed in a patient with chronic lymphocytic leukaemia (Herbrecht et al., 2002). However, *Acremonium* infections are rare, even in immunocompromised hosts (Mattei et al., 2003).

- **Other pathogenic taxa isolated from drinking water**

Candida spp. are a frequent cause of infections, which can range from superficial candidiasis infections that are common and easily treated, to systemic candidiasis. Superficial infections can occur in the skin and mucous membranes, and can arise from the overgrowth of normal yeast flora. Systemic or invasive candidiasis includes disseminated candidiasis, candidemia (i.e. the presence of *Candida* spp. in the blood), endocarditis and meningitis. It has a mortality rate of 40-50% (De Rosa et al., 2009).

The incidence of candidaemia in UK hospitals has been assessed as part of the European Confederation of Medical Mycology epidemiological survey of candidaemia. It found that there were 18.7 episodes per 100 000 finished consultant encounters or 3.0 per 100 000 bed days, with a 30 day mortality rate of 26.4%. *Candida albicans* was isolated in the majority (64.7%) of cases. The patients demonstrated a number of predisposing factors, including use of antibiotics, intensive care treatment, surgery, cancer and intravascular catheters (Tortorano et al., 2004). The mortality rate is falling over time (Kibbler et al., 2003).

Candida spp. in biofilms have been observed a cause of hospital-acquired infections via implanted catheters and other devices (Douglas, 2003). While *Candida* spp. have been

observed in drinking water (see Annex 1), it is not known whether this is a significant pathway for infection.

Infections caused by *Fusarium* spp. are increasing in frequency in immunocompromised patients. They carry a high mortality rate; 79-87% of patients die within 90 days of being diagnosed. *Fusarium* species have been isolated from drinking water, and as was discussed in section 5.1. the drinking water in one hospital in Texas has been identified of the environmental source of *Fusarium* infections.

Discussion of pathogenicity for each taxon found in drinking water can be found in Annex 1.

- **Conclusions**

Superficial or localised, easily treated fungal infections occur in healthy people without risk factors for more serious disease, but there is little evidence that such infections are caused by exposure to fungi in dinking water. Invasive disease is much rarer and limited to immunocompromised patients or those with underlying conditions. Occurrence of invasive disease per year in the US is estimated at 72-228 infections per million population for *Candida* species, 30-66 infections per million population for *Cryptococcus neoformans* and 12-34 infections per million population for *Aspergillus* species (Pfaller et al., 2006). Such invasive infections can cause severe disease and tend to have high rates of mortality associated with them (see sub-section 5.2.5.). In a small number of studies, fungi in drinking water are thought to be the source of infection in vulnerable patients (Warris et al., 2003). However, in the majority of cases it is known that the taxa involved have been isolated from drinking water, but it is not known if this is the source of infection.

Hageskal et al. (2006) concluded that the concentrations of fungi that they isolated from drinking water in Norway were unlikely to cause severe infection in healthy individuals. The concentrations that they reported were in similar ranges to concentrations reported by other studies for the same species (see Annex 1). The study conducted in the UK (Institute of Mycology, 1996) did not quote CFU numbers per species, and therefore it is difficult to determine if concentrations of individual species are in the same range. However, the total CFU numbers for all fungi reported by the Institute of Mycology were broadly within the same range as other studies (see Table 4-3).

5.2.2. ALLERGIES

Many species of fungi, including some found in drinking water (see Annex 1), are known to be potential allergens (Paterson and Lima, 2005). These include *P. richardsiae*, *A. fumigatus*, *A. niger*, *A. flavus, Penicillium* spp. and *Cladosporium* spp. Allergies are the main negative health impact for healthy individuals. Allergic symptoms may also arise in response to dead spores and other fungal debris that would not be culturable (Kauffman and van der Heide, 2003). Therefore, water that is

found to be free of fungi from testing by culture may in fact still provoke allergic disease.

Allergic respiratory disease

There is strong evidence of a correlation between fungal exposure and severity of asthma (Hogaboam et al., 2005). For example, a study of children on the Isle of Wight found that 0.5% were sensitive to *Alternaria* species and 2.9% to *Cladosporium* species. A US study of asthmatic patients found that the percentage sensitive to fungal extracts was as high as 80% (Bush and Portnoy, 2001). A small study of young people suffering a severe asthma attack and respiratory arrest found that 10 of the 11 patients were sensitive to *Alternaria* species (O'Hollaren et al., 1991). Whether this is a causal relationship has not yet been fully confirmed. Much of the evidence is related to associations between frequency of asthma attacks and numbers of airborne spores. Such spores may have been aerosolised from a water source. For example, inhabiting damp and mouldy buildings has also been linked to a worsening of asthma symptoms (Denning et al., 2006).

Allergic fungal rhinitis has also been reported, causing nasal obstruction and congestion. Symptoms are similar to allergic fungal sinusitis, which is caused by a wide range of fungal species, including *Alternaria* spp., *Aspergillus*, spp., *Cladosporium* spp. and *Penicillium* spp., many of which have been isolated from drinking water (Ponikau et al., 1999).

Hypersensitivity pneumonitis or extrinsic allergic alveolitis, is a condition where the alveoli of the lung become inflamed due to oversensitivity to inhaled particles, including microorganisms. Fungi have been implicated in incidents in Finland in which exposure was attributed to taking baths, showers and saunas (Muittari et al., 1980, in Hageskal et al., 2009). In other cases, disease has arisen from exposure to fungal spores in water-damaged buildings (Seuri et al., 2000).

Skin irritation

Outbreaks of allergic disease have in some cases been linked to presence of particular fungal species in water supplies, and have also been associated with exposure when taking baths or showers and using hot tubs or saunas (Paterson et al., 2009, Jacobs et al., 1986 and Hageskal et al., 2009). For example, this association was observed during an outbreak of skin irritation in Sweden, where the water was found to contain 77-3100 CFU 100 ml^{-1} of *Phialophora richardsiae* (Hageskal et al., 2009).

Conclusions

There is clear evidence that fungi trigger a range of allergic responses, particularly within the respiratory system and on the skin. Allergic sensitivity to fungi occurs in the general population and is particularly common in asthmatic patients. However, determining the proportion of people who are sensitive to fungi as allergens is

complicated by the fact that sensitivity may be localised. This occurs when allergic reactions in specific locations such as the nasal cavity arise but the patient does not respond to skin-prick tests, the usual test for allergies (Ponikau et al., 1999). Prevalence of allergic fungal disease is not well known. It is thought that the majority (93% in one study) of patients suffering from chronic rhinosinusitis meet the diagnostic criteria for allergic fungal sinusitis (Ponikau et al., 1999 and Schubert, 2006).

The gravity of fungal allergic disease depends on the type of reaction. In cases of hypersensitivity pneumonitis, removal of the patient from the source of exposure may be sufficient to reduce symptoms (Jacobs et al., 1986, Apostolakos et al., 2001 and Churg et al., 2006). Chronic stages may be more difficult to treat.

A number of cases, such as the outbreak of skin irritation in Sweden, imply that fungi in drinking water may be the source of exposure, particularly via skin contact when bathing or through aerosolisation of spores when showering or using saunas.

The correlation between ingestion of fungi in drinking water and allergic reactions has not been studied, and would require larger-scale epidemiological studies to confirm or reject such correlations (Hageskal et al., 2009).

5.2.3. MYCOTOXIN-MEDIATED HEALTH IMPACTS

- **Mycotoxins and mycotoxin producers**

Some fungal taxa, including *Penicillium* spp., *Aspergillus* spp., *Fusariam* spp. and *Claviceps* spp., produce mycotoxins during their metabolic processes. Of these genera, the first three have been isolated from drinking water. *Alternaria* is another potential mycotoxin producer which has been observed in drinking water (see Annex 1). Of the thousands of mycotoxins that can be produced, only about ten cause problems in food, feed and beverages. Of these, aflatoxins and zearalenone are the most relevant and have been detected in drinking water. (Paterson and Lima, 2005 and Paterson et al., 2009).

Penicillium expansum produces patulin (Paterson et al., 2009), however large amounts of *P. expansum* does not imply that there will be large amounts of patulin. Patulin is sensitive to physicochemical parameters; for example, it is sensitive to pH and becomes increasingly stable as pH decreases. In culture studies, production of patulin has been observed to occur when fungal growth rate decreases, for example because of limiting nitrogen (Paterson et al., 2007).

The concentrations of mycotoxins in drinking water are likely to be very low as they will be diluted (Hageskal et al., 2009 and Gonçalves et al., 2006). For example, only trace amounts of aflatoxins were detected by Kinsey et al., 2003 and the levels of zearalenone produced in water inoculated with *F. graminearum* by Paterson (2007) were 10^5 lower than the dietary concentration at which it mimics oestrogen (<1 mg kg^{-1} feed). Stored water, such as bottled water, and processes in which water is evaporated, such as in some food production processes, may present more of a risk as

the mycotoxins become more concentrated (Gonçalves et al., 2006, Paterson et al., 2009 and Paterson and Lima, 2005).

- **Health effects of mycotoxins**

The effects of mycotoxins can be mutagenic (induces or increases mutations), teratogenic (disturbs embryo development), oestrogenic (mimics the action of oestrogen) or carcinogenic (produces a cancer). The frequency of such impacts and their severity depends on the mycotoxin in question, its concentration, the exposure pathway and duration of exposure. They can also damage major organs or systems such as the nervous, endocrine or immune system (Paterson et al., 2009). Schütze et al. (2010) found that in an animal model chronic exposure to mycotoxins (gliotoxin and patulin) increased allergic response in asthmatic individuals by worsening chronic airway inflammation. However, no reports have been identified of disease attributed to mycotoxins produced in the water distribution system (Kelley et al., 1997 and Paterson and Lima, 2005).

- **Conclusions**

Mycotoxin-producing taxa have been identified in the drinking water system. However, while consumption of mycotoxins is known to produce health impacts, drinking water has not been identified as the source of symptoms attributable to mycotoxins.

5.2.4. AT-RISK GROUPS FOR FUNGAL INFECTIONS

As has been discussed in section 5.2. , healthy individuals may suffer from superficial or localised fungal infections, for example of the skin, nails or hair, but are not at risk of invasive infections. Individuals at greater risk of invasive or disseminated infections include:

- immunocompromised patients, arising from HIV/AIDS, chronic granulomatous disease, chemotherapy, immunosuppressive therapy, graft-versus-host disease following allogeneic bone marrow transplant, allogeneic haematopoietic stem cell transplants (HSCT), etc.;
- those with underlying health conditions, e.g. diabetes mellitus and cystic fibrosis;
- those undergoing treatment for inflammatory conditions such as rheumatoid arthritis and Crohn's disease;
- recipients of haemodialysis;
- those with reduced integrity of the skin barrier, such as following surgery or burns, or through use of indwelling medical devices such as catheters; and
- very low birth weight babies.

The degree of risk may vary between these groups and depends on the species of fungus. For example, the incidence of invasive aspergillosis in a number of at-risk groups is given in Table 5-1.

Table 5-1: Incidence of invasive aspergillosis in at-risk groups

At-risk group	Incidence of invasive aspergillosis	Reference
Acute leukaemia	5-24%	Warris et al., 2001
Chronic granulomatous disease	25-40%	Warris et al., 2001
AIDS	0-12%	Warris et al., 2001
Allogeneic stem cell transplant	12.8%	Cornet et al., 2002
Autologous stem cell transplant	1.1%	Cornet et al., 2002
Bone marrow stem cell transplant	6%	Cornet et al., 2002
Peripheral stem cell transplant	1.6%	Cornet et al., 2002
Heart-lung transplant	11.1%	Cornet et al., 2002
Small bowel/liver-small bowel transplant	10.7%	Cornet et al., 2002
Lung transplant	2%	Cornet et al., 2002
Liver transplant	1.9%	Cornet et al., 2002
Heart transplant	1.3%	Cornet et al., 2002
Kidney transplant	0.4%	Cornet et al., 2002
Kidney-pancreas transplant	0%	Cornet et al., 2002

- **Immunocompromised patients**

The number of cases of invasive infections caused by filamentous fungi has increased significantly recently, which is thought to be due to increases in the number of immunocompromised patients (Paterson et al., 2009, Hageskal et al., 2006 and Denning, 2006). The increase is linked to growing elderly populations, increased incidence of cancer and increased numbers of transplantations (Perlroth et al., 2007). Furthermore, modern treatment regimes, for example for cancer or following organ transplant, often result in more intensive immunosuppression for longer periods of time (Richardson, 2005). By contrast, the use of highly active antiretroviral therapy (HAART) has reduced the rate of fungal infections in HIV-positive individuals (Richardson, 2005).

Box 4 illustrates an example of a group of immunocompromised patients and how their immune deficiency affects the risk of developing fungal infections.

The risk of fungal infection for immunocompromised patients is predominantly in hospitals, where patients are being treated for diseases that reduce immunocompetency or are undergoing immunosuppressive therapies. Awareness of the risk of infection from water in hospitals is high, and guidelines exist for reducing the risk for immunocompromised patients (see sub-section 5.2.6. for more details). For example, it is recommended that such patients use sterile water during their stay in hospital (Anaissie et al., 2002a).

Box 4: Acute leukaemia: Immunodeficiency and risk of fungal infection

Patients with acute leukaemia are at increased risk of fungal infections due to neutropenia, a disorder caused by the leukaemia where the patient does not produce enough neutrophils (a type of white blood cell). Therapy to remove cancerous bone marrow prolongs the state of neutropenia. In such patients, incidence of invasive aspergillosis was 6.3-8% in a prospective study in the Paris area. Invasive pulmonary fungal infection is more common in patients with blood cancers than disseminated disease (i.e. where disease spreads from the initial site of infection to other body organs and systems), which is more common following haematopoietic stem cell transplants (Richardson, 2005).

- **Underlying conditions**

Those with underlying health problems, such as pulmonary disorders, cystic fibrosis and diabetes mellitus, are more at risk of invasive or systemic fungal infections than the general population (Denning, 2006). For example, patients with cystic fibrosis (CF) are at risk of allergic bronchopulmonary aspergillosis as their respiratory tracts are often colonised by *Aspergillus fumigatus*. Pulmonary aspergillosis (i.e. deeper in the lungs than bronchopulmonary aspergillosis) is a complication of lung transplants in CF patients due to colonisation of their airways prior to transplant. In one study, 53% of lung transplant recipients with CF were already colonised by *Aspergillus* spp. before the transplant (Helmi et al., 2003).

Treatment of systemic inflammatory diseases, such as Crohn's disease and rheumatoid arthritis, using agents that neutralise macrophage inflammatory cytokines also increases the risk of opportunistic fungal infections (Richardson, 2005).

- **Haemodialysis**

Contaminated dialysate is a potential source of fungal infection in dialysis patients when machinery malfunctions. A study in Greece found that fungi and yeasts were recovered from 77.7% and 12.9% of dialysate samples respectively from 85 haemodialysis units in Greece (Arvanitidou et al., 2000). Similar results were found in an analysis in Brazil, with filamentous fungi being found in tap water samples and yeasts found in dialysate samples (Pires-Gonçalves et al., 2008). Occasionally this contamination can lead to disease. For example, two patients who had dialysis from the same machine at a centre in Illinois, US, developed infections caused by *Phialemonium curvatum*. The fungus was isolated from both blood samples of the affected patients and the water used for dialysis. The problem arose due to malfunction and improper maintenance of the machine (Rao et al., 2009).

- **Very low birth weight babies and children**

Premature or very low birth weight (VLBW) babies are also at risk of fungal infections. For example, the incidence of such infections is estimated at 2-4% in VLBW infants and can rise to 10% in those babies with the smallest birth weight (McCrossan et al., 2007). This is because newborns tend to have weaker immune systems, and VLBW or premature babies may have indwelling catheters or be receiving broad-spectrum antibiotics. Other risk factors for infections in these babies include a gestational age of less than 32 weeks, an Apgar score of less than 5 at 5 minutes after birth, shock, presence of central venous catheters and a stay in intensive care of longer than 7 days before infection (Clark and Hajjeh, 2002). Mortality from systemic candidiasis is around 30% (Richardson, 2005).

Paediatric cancer patients can also experience invasive fungal infections. A retrospective study of the incidence of candidaemia in cancer patients found a variety of species were responsible for episodes of candidaemia. The rate of mortality from the infection was 21%, with *C. albicans* and *C. tropicalis* responsible for most of the deaths (Mullen et al., 2003).

Babies and infants are also more susceptible to mycotoxins (Paterson et al., 2009).

5.2.5. MORTALITY

Invasive systemic infections have high mortality rates, depending on the causal species; the characteristics of the host, such as the degree of immunocompetency; the timing of diagnosis; and the timing and effectiveness of therapy. Estimates of mortality differ and range from 50 to 100% (Warris, 2001); examples are provided in Table 5-2.

Table 5-2: Mortality rates from main systemic fungal infections (Pfaller et al., 2006)

Disease	Mortality rate
Invasive candidiasis	10 - 49% (excess attributable mortality rate)
Invasive aspergillosis	62 - >85%
Fusariosis	79-87%

5.2.6. MANAGING NEGATIVE HEALTH IMPACTS

The implications of fungi in drinking water for the general healthy public have not been thoroughly assessed (Hageskal et al., 2009). However, invasive fungal infections are rare in such individuals (Peter et al., 2002 and Pfaller et al., 2006).

Preventing individuals in at-risk groups from being exposed to fungi in water, particularly in hospitals, is important (Paterson et al., 2009). Various guidelines exist for this purpose. For example, in the UK the NICE guidelines (2003) recommend using cooled freshly boiled water or sterile water for mixing feeds or cleaning feeding tubes when caring for immunocompromised patients. Procedures for protective isolation, particularly of patients with immune deficiency, can also help to limit exposure to fungi. Specific policies vary slightly between hospitals. They may prohibit showering if

the water is thought to be contaminated or require sterile water for drinking[10]. Providing separate bathrooms for at-risk patients and thoroughly cleaning the walls and floor of showers before use is also recommended; this measure has been found to be effective in reducing exposure to fungi (Hayette et al., 2010). Point of use water filtration devices could also be added to taps and showers to prevent aerosolisation of fungi (Hageskal et al., 2009). However, this measure is relatively costly, due to the need to frequently replace the filters. An alternative would be to apply a thermal shock to water entering the hospital to remove heat-sensitive fungi (Hayette et al., 2010).

Amphotericin B is the standard therapy for invasive fungal infections, and has a success rate of between 25 and 34% (Warris, 2001). However, resistance to antifungal medication is increasing (Paterson et al., 2009); in particular, resistance to amphotericin B is common in many pathogenic species (Richardson, 2005), such as *Aspergillus* species (Pfaller et al., 2006). Resistance of *Candida* species has in some instances been associated with prophylactic use of fluconazole, an anti-fungal medication, although this has not been found in all hospitals in which prophylactic medication is used (McCrossan et al., 2007). However, in general, *Candida* species continue to be sensitive to common antifungal medication (Kibbler et al., 2003).

Managing risk of allergic disease in sensitive patients who are experiencing long stays in hospitals is also important, in order to avoid further complications to their condition (Hayette et al., 2010).

5.3. FACTORS THAT COULD INFLUENCE SOME INDIRECT HEALTH IMPACTS

5.3.1. BIOCORROSION

Fungal species that have the potential to corrode pipes in the water distribution system include those species that are iron reducing, such as *Penicillium*, *Aspergillus* and *Rhizopus* (Emde et al., 1992).

Corrosion of pipes can lead to metal concentrations in the water rising above those recommended by drinking water quality guidelines, potentially leading to health implications and changes in water taste (Dietrich et al., 2004). The element vanadium is found in iron corrosion by-products, which can be released into drinking water when the by-products are disturbed. Vanadium has the potential to cause negative health impacts (Gerke et al., 2010). Furthermore, corrosion tubercles may provide a habitat for fungal species in treated water (Emde et al;, 1992).

[10] See for example Royal United Hospital Bath NHS Trust Isolation Policy. Available from: www.ruh.nhs.uk/about/policies/documents/clinical_policies/yellow_infection_control/Yellow_627_Isolation_Policy.pdf [Accessed 26/1/2011]

Corrosion inhibitors are applied to the water to minimise the release of corrosion by-products into the water and resultant health risks. However, the health risks arising from fungi-induced corrosion has not been well studied.

5.3.2. INTERACTIONS WITH OTHER PATHOGENS AND DISEASES

Both the ecology and virulence of pathogenic organisms can be affected by the presence of other microbes. For example, fungi and bacteria influence each other directly and indirectly through physical interactions and chemical exchanges, and via metabolic by-products, changes in the environment (e.g. pH) and alteration of the host's immune response. See section 4.2.1. for further discussions of the interactions between fungi and bacteria. In some cases, such as bacterial biofilms on the surfaces of fungal hyphae, the interactions reduce fungal viability. In other circumstances, interactions can be mutually beneficial. For example, mixed-species biofilms may infer greater protection against antimicrobial substances or host immune defences (Peleg et al., 2010).

Mixed-species infections have clinical implications. For example, colonisation of the respiratory tract with *Candida* spp. increases the risk of ventilator-associated pneumonia from *Pseudomonas aeruginosa* (Azoulay et al., 2006). Whether this is related to drinking water depends on the source of the fungi colonising the respiratory tract. Assessing the significance of mixed-species infections in humans is difficult. However, it has been observed that bloodstream infections of both *Candida* spp. and a bacterial species have a higher mortality rate than *Candida* spp. infection alone. In animal models it has been found that simultaneous infection with *C. albicans* and *Escherichia coli* killed the host more frequently than infection with either species alone. These species are frequent causes of hospital-acquired bloodstream infections (Peleg et al., 2010) and *C. albicans* has been found in drinking water (see Annex 1).

5.4. TASTE AND ODOUR ISSUES

Taste and odours are common water quality problems in many countries. The common problem includes i) chlorine taste and odour ii) rust and metallic tastes iii) musty, earthy and fishy tastes and odours and iv) rotten egg smells. Odour compounds may originate from industrial effluents or from the biological activities of the algae, cyanobacteria and heterotrophic microorganisms (Cees et al. 1974). The major odour compounds include naphthalene, 2-methylbenzthiazol, chlorinated organics such as bis(2-chloroisopropyl) ether, o-chlorophenol, dichlorobenzes and hexachlorobutadiene (Cees et al. 1974).

5.4.1. DETERMINING THE SOURCE OF TASTE AND ODOUR ISSUES

Occasionally, problems with the taste and odour of water arise due to contaminants within the water distribution system. Investigations of the source of such problems

usually happen on a case-by-case basis in response to a problem and in many earlier investigations, fungi were not the main focus of analysis. The source of the problem can be terrestrial or from microbial activity in biofilms, with the compounds then being washed into the water supply. In both cases, the microbes responsible will not necessarily be isolated from samples of the affected water. Conversely, detection of fungi in such samples cannot be taken to imply causality (Hageskal et al., 2009).

5.4.2. COMPOUNDS AND FUNGAL TAXA RESPONSIBLE FOR TASTE AND ODOUR ISSUES

The Actinomycetes have been found to be associated with the musty and earthy odours in water (Zaitlina and Watson, 2006). Musty/earthy odours are the second problems encountered by the water utilities besides chlorine (Suffett et al. 1996). The filamentous fungi and the actinomycetes in the water can produce volatile compounds like geosmin (Paterson et al. 2007). Many of the taste and odour compounds produced by bacteria are also found to be produced by filamentous fungi and significantly affect the effectiveness of chemicals used for disinfecting drinking water (Paterson et al. 2009). Fungi also produce their own compounds with distinctive off-odours and tastes. Some of the fungal isolates are capable of transforming 2,4,6-tricholorophenol to 2,4,6-trichloroanisol and that causes taste and odour problems in the distribution system (Paterson et al. 2009). Several of the fungi that have been isolated from drinking water are known to produce such compounds during their metabolism (see Annex 1), including Aspergillus spp., Acremonium spp., Phialophora and Penicillium spp. which produce geosmin (Kelley et al., 1997 and Hageskal et al., 2006).

During investigations of bad tasting water, the quantities of fungi present were found to be in the region of 102-103 CFU l^{-1}, which may represent a threshold level (Gonçalves et al., 2006). Fungi growing in localised pockets near the consumer end may be at the origin of taste and odour problems (Kelley et al., 1997).

5.4.3. PUBLIC PERCEPTION OF TASTE AND ODOUR ISSUES

Problems with the taste and odour of drinking water are frequently perceived by the consumer as being an indication that the water presents a health risk (Rogers, 2001). There is unlikely to be a strong link between health risk and off-tastes (Jardine et al., 1999), and perception of risk is modulated by a variety of other factors including external information (such as from water companies or the media), trust in water suppliers and previous experiences, particularly previous health problems (de França Doria et al., 2009). Reassurance from water companies may not be effective (Jardine et al., 1999 and McGuire, 1995). Therefore, minimising taste and odour problems, such as those arising from fungi, is important to maintain consumer confidence in high-quality drinking water (Rogers, 2001).

6. CONCLUSIONS

Fungi are a common component of the microflora in water distribution systems and in treated tap water. The specific community of fungal species found varies between systems, and may also vary over time. Some species are resident in the system while others are transient and do not become established. A number of species have been regularly isolated from different systems, including some that are known human pathogens. However, there are numerous issues with the methods used to sample, isolate, identify and quantify fungal species in water samples. Fungi are unevenly distributed in water due to being filamentous nature or held in biofilms. Therefore, it is difficult to obtain a representative sample. The species isolated is influenced by the method used for isolation and identification, which can itself select for some individual species. No international standard methodology is widely in use, which presents a significant hindrance to progressing in this field of research as it is not possible to compare results between studies (Kelley et al., 1997 and Paterson and Lima, 2005).

Water treatment and disinfection processes are effective in reducing the number and diversity of species found in the raw source water, although fungi are not completely removed and may be only partially inactivated. Secondary contamination via mains breaks, maintenance and low/negative pressure events is a potentially significant but poorly understood contamination pathway. A number of procedures are already in place to reduce the risk of secondary introduction of contaminants, although their effectiveness in reducing fungal contamination is not well known. Residuals of chemical disinfectants are maintained in distribution systems to maintain the microbiological quality of the water, which will also inactivate fungi within the system.

Once in the distribution system, fungi are capable of establishing and multiplying, particularly in biofilms, particles, and water with a long residence time in dead ends, tidal points and oversized pipes. A number of biotic and abiotic factors influence the ecology of fungi in drinking water distribution systems, including water temperature and flow rate, material of pipes and interactions with bacteria and protozoa. Knowledge on some specific aspects of the ecology of fungi in these environments is lacking. For example, the relationship between bacteria and fungi in drinking water is not well understood, as indicated by the lack of agreement between studies regarding correlations between them. Further work is needed to characterise this relationship in order to determine if and how the bacterial content of water is associated with its fungal content.

Fungi are responsible for a range of infections and allergies. In healthy populations, superficial or localised fungal infections, for example of the skin, are relatively common and can be treated. Allergic disease caused by fungi may also be of relevance in this

population. More severe invasive fungal infection is limited to at-risk individuals, such as those with immune deficiency or underlying conditions such as cystic fibrosis.

Measures are in place in high-risk locations, such as hospitals, to manage risk of fungal infection via airborne spores or hyphal fragments. Despite such measures, however, incidence of infection in at-risk individuals is continuing to increase. This has led researchers to investigate alternative sources of infection. Species known to be pathogenic, such as *Aspergillus* spp., have been isolated from drinking water, and therefore the potential exists for patients to be exposed to fungi via drinking water. In a small number of cases, water has been confirmed as the source of fungi following genotyping of isolates from the patient and from the environment. Monitoring of fungi in drinking water linked to an alert system for outbreaks of fungal infection would help in identifying the environmental source of infection. Pathways of exposure to fungi in drinking water include ingestion of drinking water, inhalation of spores that have become aerosolised from running the shower or tap or using saunas, skin contact with fungi in water, or introduction via wounds or the conjunctiva when bathing or showering. A significant knowledge gap concerns the quantity of fungi in water acceptable and the threshold level for infection or allergic response (Hageskal et al., 2007). However, this may depend on individual host factors.

Opinions among researchers as to whether fungi in drinking water are a significant source of fungal infections in vulnerable patients are contradictory, leading to debate about whether further information is required before action taken (Hageskal et al., 2009). However, risk of severe invasive fungal infections for healthy individuals is low, regardless of the environmental source of the pathogenic fungi (Anaissie et al., 1989, Chen et al., 2001, Walsh et al., 2004). Therefore, precautionary measures beyond normal water treatment and disinfection may not be needed for this group, particularly given the need to avoid causing alarm amongst the public (Hageskal et al, 2009). Further studies to more precisely evaluate this risk would be helpful.

Applying the precautionary principle and given the high mortality rate from invasive fungal infections amongst high-risk patients, preventative measures for this group would be warranted. A number of measures are already in place in hospitals, such as preventing vulnerable patients from showering. Evidence of which exposure pathways are most significant for such patients would enable appropriate mitigation measures to be put in place. Furthermore, more studies that investigate the environmental source of hospital-based fungal infections would be beneficial to determine the degree of risk from water relative to other sources.

6.1. FUTURE PERSPECTIVES

The number of people in at-risk groups continues to increase due to HIV/AIDS, advances in medical treatment of conditions such as cancer that prolong immunosuppression, increases in transplant numbers and medical advances in keeping

extremely low birth weight babies alive. Therefore, monitoring and control of fungi in the hospital environment, including in water, is vital to avoid greater numbers of severe infections with a high mortality rate.

Climate change should also be considered in its potential to alter the abundance and species composition of fungi in water supplies. For those taxa that exhibit seasonal variation, it would be important to assess how warmer and wetter weather in the UK alters their numbers and habitats. For example, O'Gorman and Fuller (2008) found that levels of airborne spores of *Cladosporium* were positively correlated to temperature and that spores of *Penicillium* and *Aspergillus* were positively correlated with relative humidity. Climate change may also increase exposure to fungi. For example, floods are expected to increase in frequency in the future, leading to increased numbers of people inhabiting water-damaged buildings. Therefore, risk of being exposed to aerosolised fungi can increase, as was found following the New Orleans flooding (Ahikari et al., 2009).

6.2. POTENTIAL IMPROVEMENTS TO THE WATER SYSTEM

Standard treatment procedures for drinking water have been shown to be effective in removing many of the species and reducing the number of fungal CFUs (Kinsey et al., 2003).

A number of other measures in addition to treatment have been identified to control microbial growth, particularly in biofilms, within water distribution systems. These are presented in Table 6-1. It should be noted that these measures are intended to control microorganisms in the distribution system and not specifically fungi, and represent normal good practice for water suppliers in the UK.

Table 6-1: Measures for controlling microbes in drinking water distribution systems

Measure	Description
Mains flushing and cleaning	Biofilms, particles and tuberculation (deposits of corrosion products on inner surfaces of pipes) affect the systems hydraulics. Regular flushing and cleaning removes such deposits, enabling water to flow better through the system. Maintaining positive pressure throughout the system is also important. Storage facilities should also be flushed or cleaned and then disinfected at regular intervals.
Maintenance of disinfectant residuals	Ensuring sufficient concentrations of disinfectants throughout the distribution system reduces the contamination of treated water for example by microbes in biofilms in the system. It also can inactivate pathogens and suppress microbial and biofilm growth.
Mains repair and replacement	Sections of the distribution system with frequent leaks or contamination problems are sometimes replaced rather than repairing the problem or flushing the system. Other devices such as valves may also be replaced when they fail.
Flow management and	There should be sufficient turnover of water in storage

Measure	Description
minimising dead ends	facilities and areas of low flow to avoid long residence times and particle accumulation. This can be done by exercising valves and avoiding excess storage. Proper network design should also minimise the number of dead ends.
Corrosion control	Controlling corrosion can reduce biofilm development as corrosion inhibitors also inhibit biofilm formation and prevent biofilms from sloughing off by coating the inner surface of the pipe.
Control of nutrient concentrations	Control of nutrients, particularly carbon, occurs during treatment through techniques such as coagulation, membrane filtration, granular activated carbon and biological treatment (microbial activity at the point of treatment).
Reduction of cross-connections and backflow	Installing and inspecting backflow prevention devices reduce the intrusion of microbes from cross-connections.
Control of contamination from materials and equipment	Disinfection and high pressure washing of tools can reduce the microbes found thereon. Following maintenance procedures, it is important to thoroughly disinfect and flush the system (in one direction to avoid removing biofilms) before the system becomes operational. Repairing mains breaks involves isolating the affected system before carrying out disinfection and flushing.

Other options to reduce the fungal contamination of drinking water are to implement control measures at the point of use. Such measures include installing filters on taps and showers, and using treatment/disinfection methods such as copper and silver ionisation in hospitals and other high-risk locations (Hageskal et al., 2009).

6.3. RESEARCH NEEDS

There are a number of aspects regarding fungi in drinking water that have not been well studied, or for which considerable uncertainty or contradiction still exists. Once the risk posed by fungi in drinking water has been has been better established, the costs and benefits of additional treatment and control measures should be determined. Specific research needs to achieve this are presented in Table 6-2 by priority level.

Table 6-2: Research needs

Research need	Significance
Medium priority	
Importance of drinking water as an environmental source of fungal infections in at-risk patients	A small number of studies have genotyped fungal isolates from infected patients and various environmental sources of fungi. The importance of *Candida* species in drinking water is particularly unknown, and pertinent given the relative importance of *Candida* as a pathogen.

Research need	Significance
Relative importance of ingestion as an exposure pathway for fungi in drinking water (compared to inhalation or skin contact).	To determine whether control measures for at-risk individuals should include drinking sterilised water, reducing risk of aerosolised spores, avoiding bathing in unsterilised water, etc. Knowing the most common pathways of exposure will ensure that mitigation methods are targeted appropriately.
Effects of analytical methods on results regarding fungal species and quantities	Greater understanding of how the method chosen can affect the results and development of a standard methodology will allow facilitate many of the other research needs.
Low priority	
Interactions with bacteria	To determine if numbers of pathogenic fungi correlate with standard parameters of drinking water or whether additional monitoring is needed for locations with high-risk people such as hospitals.
Relative proportions of fungi and biofilms in distribution systems compared to in consumer-side installations	To determine if measures to reduce fungi in the distribution system are needed or whether consumers, particularly hospitals and individuals in at-risk groups, should be provided with information on how to reduce fungal prevalence. A better understanding of fungal regrowth within distribution systems will also allow assessment of the relative effectiveness of water treatment and disinfection procedures.
Risks associated with secondary contamination pathways	To better understand and quantify the risks from secondary contamination pathways. However, control measures are already in place for reducing secondary contamination with other microbes and pollutants.

There are a number of other aspects of the ecology of fungi in drinking water that are not fully understood or have not been well researched. A greater understanding of these issues will not affect assessment of the level of risk but may be beneficial for a greater academic knowledge of the subject. These are presented in Table 7-3.

Table 6-3: Areas for potential future research

Research area	Description
Effects of nutrient levels on fungal ecology in distribution systems and competition between fungi and bacteria	Nutrient levels likely to be less influential for fungi than bacteria, given that many fungi can grow in low-nutrient environments. Determining the nature of competition for nutrients may help to better understand fungi-bacteria interactions.
Interactions with viruses	Infection of *Aspergillus* with mycoviruses appears to reduce fungal viability, and hence such interactions may reduce rather than raise risk of fungal infection from drinking water. This hypothesis should be tested however.
Interactions with algae	Interactions have only been studied in marine environments but appear to have little relevance for risk of fungal infection.

Research area	Description
Fungi-induced corrosion of pipes	The importance of fungi in microbially-induced corrosion is not well known. However, corrosion inhibitors are applied when appropriate to reduce release of corrosion by-products and health impacts.
Effect of pipe material on establishment of fungal biofilms or fungal colonisation of existing biofilms and rate of detachment	May be an important consideration for future pipe replacement. However, impacts of material on pathogenic bacteria, by-products, etc. may be more important than effects on fungi and many other factors will affect the decision of pipe material.
Clarification of effect of water flow rate on biofilms and fungi in biofilms, and biofilm detachment	May be a consideration in designing future water distribution networks although generic guidance already exists.
Interactions with protozoa	Fungal replication inside protozoa has occurred but the significance of this as a means by which fungi are protected from treatment and disinfection is not known. Co-associations between pathogenic protozoa and fungi may also be significant.
Impacts of climate change on fungal numbers and ecology in drinking water	Changing fungal numbers or ecology may increase risks for certain population groups and therefore require different control measures.
Adequate monitoring plans and methods	In response to potential future risk (e.g. from climate change) research into optimal monitoring plans, combined with monitoring for other pathogens, would ensure that changing risk can be ascertained.
Concentrations of mycotoxins in drinking water and significance of drinking water as an exposure pathway	While it is not thought that mycotoxins have caused acute disease in the UK or US, it would be useful to determine the concentrations of mycotoxins in drinking water, particularly in relation to chronic exposure.

Box 5: Summary of conclusions

- Fungi present in drinking water may cause severe fungal infections in immunosuppressed patients. In a small number of studies, drinking water supplies have been found to be the source of infection, although the pathway of infection (drinking vs. inhalation of aerosolised spores while showering) is uncertain
- Additional research would be required to further investigate the link between fungi in drinking water and infections in immunosuppressed patients, address its frequency from an epidemiological viewpoint and determine the fungal species and quantity in water to cause such infections.
- The present risk of health impact for the general population is thought to be low based on current knowledge. Therefore current procedures for water system maintenance or water monitoring and treatment might be sufficient.
- The literature should be reviewed periodically in order to take account of potential environmental or procedural changes, such as climate change or altered water treatment processes.
- If future scientific works suggests an increase in risk, pilot epidemiological studies and surveillance may be justified.
- Further research and monitoring (if needed) would be facilitated by the use of a simpler and quicker method of fungal quantification and identification than culture.
- Greater knowledge of the associations between fungi and bacteria would help to ascertain whether commonly measured bacterial parameters of water quality correlate with fungi presence.

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7. GLOSSARY

Biofilm: microbial populations enclosed in a matrix which are adherent to each other and/or surface, i.e. biofilms are not single cells dispersed in a fluid (Stoodley et al., 1997).

Conidia: asexual fungal spores produced by mitosis, non-motile.

Eutrophic: aquatic habitats with high concentration of organic compounds (nutrients) and low dissolved oxygen content.

Filamentous fungi: fungi that grow in multi-cellular colonies.

Heterotrophic: organisms that do not produce their own food, and hence require organic carbon from external sources for growth.

Invasive infection: an infection that spreads from the initial site of infection to the surrounding tissues.

Melanised fungi: fungal species which are encapsulated in a layer of melanin pigment. This is thought to protect them from particular stressors, including the immune system of the hosts of pathogenic fungi (Mednick et al., 2005, and others).

Mycotoxin: a toxic secondary metabolite produced by a fungus.

Oligotroph: organisms that live in low-nutrient environments.

Oligotrophic: aquatic habitats with low concentration of organic compounds (nutrients) and high dissolved oxygen content.

Opportunistic infection: an infection caused by a microorganism in an immunocompromised host that is not normally pathogenic in a healthy host.

Psychrophile: organism that thrives at cold temperatures (i.e. close to 0°C), does not have temperature regulation mechanisms, and cannot develop at warmer temperatures (Feller and Gerday, 2003).

Secondary metabolites: Products of metabolic processes that are not directly associated with universal biochemical processes (i.e. protein formation, DNA replication, etc.) (Paterson and Lima, 2005).

Yeast: primarily single-celled fungi the vegetative growth of which is by budding or fission. Their sexual states are not enclosed in fruiting bodies (Furtzman and Fell, 1998).

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9. ANNEXES

ANNEX 1: FUNGAL TAXA IDENTIFIED IN TREATED DRINKING WATER AND IN WATER DISTRIBUTION AND STORAGE SYSTEMS

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
Absidia spp. (ff)	4 CFU/100 ml[11]	Surface		Norway[12]	*A. corymbifera:* An infrequent opportunistic pathogen (Larone, 2002)	Hageskal, 2006
	-			UK, US		Kinsey, et al., 1997
Acremonium spp. (ff)	1.4 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Many spp. opportunistic pathogens (Guarro et al., 1997, and others). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	132 CFU	Unknown	Water from the tap (treated)	Portugal		Gonçalves et al., 2006
	12.1%	Groundwater	Raw water, waterworks, water networks, house installation, newly laid pipes (unchlorinated)	Germany		Göttlich et al., 2002
	3-40 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006

[11] In cases where species were listed separately in the minimum CFU count per 100 ml for each of the species of the same genus was summed, and then the maximum count was summed to give a range. In cases where the minimum and maximum counts were the same, only one figure is given.

[12] Both treated and untreated water was investigated in this study, and the results do not differentiate between those species found in each water type. However, it is stated that a similar species diversity was found in both treated and untreated water, and therefore all species isolated in this study are considered as being likely to occur in treated water.

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	25.6% of samples positive	Groundwater	Tap water/ groundwater	Austria		Kanzler et al., 2007[13]
Altenaria spp.(ff)	3.8 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Can cause upper respiratory tract infections and asthma (Salo et al., 2006), some species opportunistic pathogens (Vermeire et al., 2010). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	1 CFU	Unknown	Water from the tap (treated)	Portugal		Gonçalves et al., 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	2.6% samples positive	Groundwater	Groundwater/ tap water	Austria		Kanzler et al.,2007
Arthrinium spp. (ff)	2 CFU/100 ml	Surface		Norway	Produce mycotoxins (Magan and Olson, 2004)	Hageskal, 2006
	-			UK, US		Kinsey, et al., 1997
Ascochyta spp. (ff)	-			UK	No reports of pathogenicity in humans. Can produce	International Mycological Institute, 1996

[13] Samples from this study were taken from both groundwater and tap water, the taxa found in each source were not differentiated.

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
					mycotoxins (Betina, 1993).	
Aspergillus spp. (ff)	3.9-7.1 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Some species causes invasive aspergillosis (Larone, 2002) and some are allergens (Banerjee and Kurup, 1998). Mycotoxins are also produced (Fox et al., 2004). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	1 CFU	Unknown	Water from the tap (treated)	Portugal		Gonçalves et al., 2006
	2%	Groundwater	Raw water, waterworks, newly laid pipes unchlorinated)	Germany		Göttlich et al., 2002
	-	Surface water	Surface source waters, after different treatment stages, water pumped to supply network	Poland		Grabinska-Loniewska et al., 2007
	5-20 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-					International Mycological Institute, 1996
	15.4% samples positive, 5.1% positive for *A. terreus*	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Aureobasidium spp. (yeast)	1.3-3.1 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	*A. pullulans* is a: rare pathogen – causes phaeo-hyphomycosis (Larone, 2002).	Doggett, 2000
	1-3 CFU/100 ml	Surface water		Norway		Hageskal, 2006.

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	5.10% samples positive	Groundwater	Groundwater/ tap water	Austria		Kanzler et al., 2007
Beauveria spp. (ff)	2-15 CFU/100 ml	Surface water		Norway	Reported pathogenicity (Henke et al., 2002)	Hageskal, 2006.
	-			UK, US		Kinsey, et al., 1997
	2.6% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Botrytis spp. (ff)	2-3 CFU/100 ml	Surface water		Norway	No reports of pathogenicity in humans	Hageskal, 2006.
	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Byssochlamys spp. (ff)	1-2 CFU/100 ml	Surface water		Norway	Produces a mycotoxin (patiulin) (Moulé and Hatey, 1977). No reports of pathogenicity in humans.	Kinsey, et al., 1997
Candida spp. (yeast)	4.8-6.3 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Pathogenic (Calderone and Fonzi, 2001).	Doggett, 2000

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	-		Treated tap water	Brazil	Produce compounds causing off tastes (Kelley et al., 1997).	Yamaguchi, 2007
Ceratocystis spp. (ff)	1-3 CFU/100 ml	Surface water		Norway	No reports of pathogenicity in humans. Can produce mycotoxins (Betina, 1993).	Hageskal, 2006
Chaetomium spp. (ff)	2 CFU	Unknown	Water from the tap (treated)	Portugal	Pathogenic (Guppy et al., 1998)	Gonçalves et al., 2006
	2-6 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Chrysonilia spp. (ff)	1 CFU/100 ml	Surface and groundwater		Norway	Reports of allergenicity (Francuz et al., 2010). Not reported as being pathogenic.	Hageskal, 2006
Chrysosporium spp. (ff)	1 CFU/100 ml	Surface water		Norway	Produces a mycotoxin (Betina, 1993). A rare pathogen (Chabasse et al., 1989 and Roilides et al., 1999)	Hageskal, 2006
Cistella spp.	2.6% samples positive	Groundwater	Groundwater/tap water	Austria	Not reported as being pathogenic in humans.	Kanzler et al., 2007
Cladosporium spp. (ff)	1.5 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Skin and toenail infections, sinusitis, pulmonary infections (Tamiska et al., 2006).	Doggett, 2000
	12 CFU	Unknown	Water from the tap (treated)	Portugal	Produce compounds causing off tastes (Kelley et al., 1997).	Gonçalves et al., 2006

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	2%	Groundwater	Waterworks, house installation, newly laid pipes (unchlorinated)	Germany		Göttlich et al., 2002
	-	Surface water	Source water from river	Poland		Grabinska-Loniewska et al., 2007
	3-17 CFU/100 ml	Surface water		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
				UK		International Mycological Institute, 1996
				Austria		Kanzler et al., 2007
Cordyceps spp.	2.6% samples positive	Groundwater	Groundwater/tap water	Austria	Not reported as being pathogenic in humans.	Kanzler et al., 2007
Cryptococcus spp. (yeast)	7.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	*C. neoformans:* opportunistic infections (Walsh and Groll, 1999). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	-			US		Kinsey, et al., 1997
Dactylaria spp.	2.6% samples positive	Groundwater	Groundwater/ tap water	Austria	*D. constricta* has caused subcutaneous and disseminated infections in immunocompromised patients (Larone, 2002).	Kanzler et al., 2007
Dendryphion spp.	1.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Not reported as pathogenic	Doggett, 2000

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
Doratomyces spp. (ff)	1.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Allergen (Fischer and Dott, 2003)	Doggett, 2000
Epicoccum spp. (ff)	1-2 CFU/100 ml	Surface and groundwater		Norway	Not reported as pathogenic (Lorone, 2002).	Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	5.1% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Eupenicillium spp. (ff)	1 CFU/100 ml	Surface water		Norway		Hageskal, 2006
	-			UK		Kinsey, et al., 1997
Exophialia spp. (yeast-like)	9.5%	Groundwater	Raw water, waterworks, water networks, house installation, newly laid pipes (unchlorinated)	Germany	Some species pathogenic (Lorone, 2002).	Göttlich et al., 2002
	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Fusarium spp. (ff)	3.5%	Groundwater	House installation, newly laid pipes (unchlorinated)	Germany	Some species produce mycotoxins such as fumonisins and trichothecenes (Betina, 1993), some opportunistic pathogens, causing eye infections and disseminated systemic infections (Lorone, 2002).	Göttlich et al., 2002
	-	Surface water	After different stages of treatment of river water, river water sedimentation basin source water	Poland		Grabinska-Loniewska et al., 2007
	102-107 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	-		Treated water	UK, US	Produce compounds causing off tastes (Kelley et al., 1997).	Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	2.6% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Geotrichum spp. (ff)	-	Surface water	Source water from river water sedimentation basin, after different stages of treatment of this water	Poland	Pathogenic (Sfakianakis, et al., 2007 and Kelley et al., 1997). Produce compounds causing off tastes (Kelley et al., 1997).	Grabinska-Loniewska et al., 2007
	1-2 CFU/100 ml	Surface water		Norway		Hageskal, 2006
	-			UK, US		Kinsey, et al., 1997
Gliocladium spp. (ff)	1.0 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Not been reported as being pathogenic. Produces mycotoxins (Betina, 1993).	Doggett, 2000
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Graphium spp.	2.6% samples positive	Groundwater	Groundwater/tap water	Austria	*G. basitruncatum* very rare pathogen – observed once in patient with acute leukaemia (Kumar et al., 2007).	Kanzler et al., 2007
Lecythophora spp. (ff)	1-3 CFU/100 ml	Surface and groundwater		Norway	Rare pathogen (Marriott et al., 1997)	Hageskal, 2006

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	12.8% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Leptodontidium spp. (ff)	-		Treated water	UK, US	Not been reported as being pathogenic.	Kinsey, et al., 1997
Leptosphaeria spp.	25.6% samples positive	Groundwater	Groundwater/tap water	Austria	Not been reported as being pathogenic.	Kanzler et al., 2007
Leucostoma spp. (ff)	1-4 CFU/100 ml			Norway	Not been reported as being pathogenic.	Hageskal, 2006
Mauginiella spp. (ff)	-		Treated water	UK	Not been reported as being pathogenic.	Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Microdochium spp. (ff)	-		Treated water	US	Not been reported as being pathogenic.	Kinsey, et al., 1997
	2.6% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Microsphaeropsis spp. (ff)	-		Treated water	UK, US	Pathogenic (Shah et al., 2001)	Kinsey, 2003
Monascus spp. (ff)	1-5 CFU/100 ml	Surface water		Norway	Produces mycotoxins (Betina, 1993)	Hageskal, 2006
Mortierella spp. (ff)	-		Treated water	UK, US	Not been reported as being pathogenic.	Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Mucor spp. (ff)	2.7-3.5 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after	US	Pathogenic: occasionally	Doggett, 2000

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
			treatment		causes zygomycosis (Lorone, 2002). Allergen (Corey et al., 1990). Produce compounds causing off tastes (Kelley et al., 1997).	
	4-9 CFU/100 ml	Surface water		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Nectria spp. (ff)	2.8 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Unknown	Doggett, 2000
	-			US		Kinsey, et al., 1997
Paecilomyces spp. (ff)	2.0 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Some species pathogenic (Walsh and Groll, 1999). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	2%	Groundwater	Raw water, newly laid pipes (unchlorinated)	Germany		Göttlich et al., 2002
	--	Surface water	After different stages of treatment of infiltration intake of river water	Poland		Grabinska-Loniewska et al., 2007
	7-16 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-			UK, US		Kinsey, et al., 1997
	5.1% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
Papulaspora spp. (ff)	0.84-1.1 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US		Doggett, 2000
Penicillium spp. (ff)	6.5-12.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Implicated in a range of diseases but causal significance unknown (Lorone, 2002). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	138 CFU	Unknown	Water from the tap (treated)	Portugal		Gonçalves et al., 2006
	7%	Groundwater	Raw water, waterworks, house installation, newly laid pipes (unchlorinated)	Germany		Göttlich et al., 2002
	-	Surface water	Infiltration intake of river water and after different treatment stages of this water	Poland		Grabinska-Loniewska et al., 2007
	48-136 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	48.7% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Pestalotiopsis spp. (ff)	-		Treated water	US		Kinsey, et al., 1997
Phaeosphaeria spp.	2.6% samples positive	Groundwater	Groundwater/tap water	Austria	Not reported as being pathogenic in humans.	Kanzler et al., 2007
Phialophora spp. (ff)	14 CFU	Unknown	Water from the tap (treated)	Portugal	Some species pathogenic (chromoblastomycosis,	Gonçalves et al., 2006

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	32.7%	Groundwater	Raw water, waterworks, water networks, house installation, newly laid pipes (unchlorinated)	Germany	phaeohypthomycosis, cutaneous and nail infections) (Lorone, 2002). Produce compounds causing off tastes (Kelley et al., 1997).	Göttlich et al., 2002
	-	Surface water	After different treatment stages of infiltration intake of river water	Poland		Grabinska-Loniewska et al., 2007
	10-19 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	20.5% samples positive *P. malorum*, 2.6% positive *P.* spp.	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Phoma spp. (ff)	4.3 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Occasionally causes phaeo-hypthomycosis (Lorone, 2002). Allergen, subcutaneous and respiratory infections. Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	2.5%	Groundwater	Networks, newlylaid pipes (unchlorinated)	Germany		Göttlich et al., 2002
	2-18 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
	7.7% samples positive	Groundwater	Groundwater/ tap water	Austria		Kanzler et al., 2007
Phomopsis spp. (ff)	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	2.6% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al.,2007
Pithomyces spp. (ff)	-		Treated water	UK, US		Kinsey, et al., 1997
Pseudogym-noascus spp. (ff)	1 CFU/100 ml	Surface water		Norway		Hageskal, 2006
Rhizoctonia spp. (ff)	2.8 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Unknown	Doggett, 2000
Rhizopus spp. (ff)	10 CFU	Unknown	Water from the tap (treated)	Portugal	*Rhizopus spp.* pathogenic: commonly cause zygomycosis (Lorone, 2002).	Gonçalves et al., 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Rhodotorula spp. (yeast)	6.1-8.2 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Opportunistic pathogen (Lanzafame et al., 2001 and Neofytos et al., 2007). Produce compounds causing off tastes (Kelley et al., 1997).	Doggett, 2000
	10.3% samples positive	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
Scopulariopsis spp. (ff)	4 CFU/100 ml	Surface water		Norway	Pathogenic: causes nail infections and occasionally subcutaneous and invasive infection (Lorone, 2002).	Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
Sesquicillium spp. (ff)	-	Surface water	After different stages of treatment of infiltration intake of river water	Poland		Grabinska-Loniewska et al., 2007
	-		Treated water	UK		Kinsey, et al., 1997
Sporotrichum spp. (dimorphous)	2.0-2.8 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Has been associated with respiratory disorders (Lorone, 2002). Some/all species pathogenic e.g. *S. schenckii*	Doggett, 2000
	-			UK		Kinsey, et al., 1997
Sporothrix spp. (dimorphous)	1.0-1.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Some/all species pathogenic e.g. *S. schenckii*	Doggett, 2000
	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Stachybotrys chartarum (ff)	2.8-4.8 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Produces mycotoxins, potentially pathogenic (Lorone, 2002).	Doggett, 2000
	-	Surface water	Source water from infiltration intake and sedimentation basin from river	Poland		Grabinska-Loniewska et al., 2007

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
Staphylotrichum spp. (ff)	2 CFU/100 ml	Surface water		Norway		Hageskal, 2006
Stereum spp. (ff)	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
Stysanus spp. (ff)	2.9-4.7 CFU cm^{-2}	Groundwater	Biofilms on iron pipe surfaces of water distribution system after treatment	US	Unknown	Doggett, 2000
Trametes spp.	5.1% of samples positive	Groundwater	Groundwater/tap water	Austria	Not reported as being pathogenic in humans.	Kanzler et al., 2007
Trichoderma spp. (ff)	-	Surface water	Source river water sedimentation basin and after different stages of treatment of this basin	Poland	Produce compounds causing off tastes (Kelley et al., 1997).	Grabinska-Loniewska et al., 2007
	1-12 CFU/100 ml	Surface water		Norway		Hageskal, 2006
	-		Treated water	UK, US		Kinsey, et al., 1997
	-			UK		International Mycological Institute, 1996
	2.6% samples positive *T.viride*, 2.6% positive *T.* sp,	Groundwater	Groundwater/tap water	Austria		Kanzler et al., 2007
Truncatella spp.	-		Treated water	UK		Kinsey, et al., 1997
	-			UK		International Mycological

Taxon	Prevalence	Raw source water (ground/ surface)	Where in the distribution and storage systems it has been isolated	Where it has been isolated	Known pathogenicity/ risk	References
						Institute, 1996
Verticillium spp. (ff)	4%	Groundwater	Raw water, newly laid pipes (unchlorinated)	Germany	Reported as a possible cause of keratitis (Sutton et al., 1998) Produce compounds causing off tastes (Kelley et al., 1997).	Göttlich et al., 2002
	-	Surface water	Source infiltration intake river water and after different treatment stages of this water.	Poland		Grabinska-Loniewska et al., 2007
	1-2 CFU/100 ml	Surface and groundwater		Norway		Hageskal, 2006
	-			UK, US		Kinsey, et al., 1997

ANNEX 2: DIRECT AND INDIRECT HEALTH EFFECTS AND TASTE/ODOUR IMPACTS

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
Pathogenic moulds in hospital water distribution systems – clinical implications for patients with hematologic malignancies	Water, water surfaces, air and other environmental sources from bone marrow trans-plantation unit	Moulds (*Aspergillus* and other fungal species) were recovered in 70% of the water samples , 22% of the swabs from plumbing structures and environmental surfaces and 83% of the air samples	Direct impact – aerosolisation of fungal spores and potential exposure to patients. Hospital water systems serve as a potential reservoir of *Aspergillus* and other fungal species.	USA	Anaissie et al. (2003), Blood 101: 2542-2546.
High level of recovery of fungi from water and dialysate in haemodialysis units (Yeasts and filamentous fungi were investigated)	Municipal water (feed water) supplies of haemodialysis centres, treated water and dialysate.	Out of 255 samples, 209 (82.0%) samples were positive for filamentous fungi and 21 (8.2%) for yeasts. Filamentous fungi and yeasts were isolated from 69 (81.2%) and 3 (3.5%) of feed water samples, from 74 (87.1%) and 7 (8.2%) of treated water samples, 66 (77%) and 11 (12.9%) dialysate samples, respectively.	Direct impact – The occurrence of high percentage of filamentous fungi and yeasts from haemodialysis aqueous environments indicates a potential risks for haemodialysis patients.	Greece	Arvanitidou et al. (2000), Journal of Hospital Infection 45: 225-230.
Possible linkages between lignite aquifers, pathogenic microbes, and renal pelvic cancer (RPC)	Residential drinking water wells and dewatering well of lignite mine; surface waters of coal mine.	Samples were tested for presence of fungi, for metal, trace metal and other physic-chemical parameters. Significant associations were observed between cancer rates and the presence of fungi	Direct impact – the presence of pathogenic microbes are associated with high risks of renal pelvic cancer (RPC)	USA	Bunnell et al. (2006). Environmental Geochemistry and Health 28:577-587.

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
		Zygomycetes, organic compounds, some nutrients and chemical elements. Human pathogenic leptospires was detected in 50% of the surface water sites sampled.			
Occurrence and hygienic relevance of fungi in drinking water	Drinking water and ground water samples (Wells, water tanks and tap water)	Highest fungal concentrations in elevated water storage tanks and the lowest after UV-disinfection. 32 different taxa of fungi were found and isolated in all samples tested. *Cladosporium* spp. (74.6%), Basidiomycetes (56.4%) and *Penicillium* spp. (48.7%) were observed more frequently. Pathogenic fungi like *Aspergillus* spp. or *Fusarium* spp. were found.	Direct impact: Drinking water serves as a reservoir for opportunistic infections in hospitals because of the increasing number of immune-suppressed patients. Aerosolisation during showering is a major problem as compared to drinking of the water.	Austria	Kanzler et al. (2007) Mycoses 51, 165–169.
Occurrence of fungi in water used at a haemodialysis centre	Samples in the hydraulic circuit for the distribution of the water, dialysate samples and samples of sterilisation solution from dialysers.	116 isolates of fungi were recovered from 89% of all water samples collected. Prevalence of moulds in tap water samples and yeasts in dialysate samples. *Fusarium* spp. was the most abundant genus found. *Candida parapsilosis* was	Direct impact: Recovery of fungi from aqueous haemodialysis environments implies a potential risk for haemodialysis patients.	Brazil	Pires-Goncalves (2008), Lett Appl Micro 46: 542-547.

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
		the predominant yeast species found.			
Contaminated product water as the source of *Phialemonium curvatum* bloodstream infection (BSI) among patients undergoing hemodialysis	Bloods samples of person who underwent dialysis were tested positive for *Phialemonium curvatum* on culture. Water, surface, and dialysate samples were also tested by culture.	Two patients with BSI due to *P. curvatum* was identified. *P. curvatum* was identified from the product water used for dialysis at 2 of 19 treatment stations, one of which was the implicated station.	Direct impact: First report of patients acquiring a mould BSI from contaminated product water. The source of *P. curvatum* was likely the water distribution system.	USA	Rao et al. (2009) Infect Control Hosp Epidemiol 30: 840-847
Diversity and significance of mold species in Norwegian drinking water	Samples of raw water, treated water, and water from private homes and hospital installations were collected and the total fungal count and diversity was determined.	94 mould species belonging to 30 genera were identified. Species of *Penicillium*, *Trichoderma*, and *Aspergillus* were dominated and some of them found throughout the drinking water system.	Direct impact/ taste & odour problems: Many species isolated from water may have the potential to cause allergic reactions or disease in humans. Some species are contaminants of food and beverages. Some may cause unwanted changes in the taste or smell of water.	Norway	Hageskal et al. (2006) AEM, 72:7586–7593.
Enhancement of formation of the esophageal carcinogen benzylmethylnitrosamine from its precursors by *Candida albicans*	Pure culture of *Candida albicans* was used to study the formation of the carcinogen benzylmethylnitrosamine (NBMA; N-nitroso-N-methylbenzylamine).	Significant increase in the amount of NBMA formed in the cultures, compared to precursors-only controls. Exponentially growing cultures were also able to cause NBMA formation.	Indirect impact: Formation of nitrosamine could result in a concentration sufficient to initiate tumourigenesis. It may also cause hygiene related cancers, such as those of the penis and	China	Hsia et al. (1981) PNAS, 78:1878-881.

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
	Stationary *C. albicans* cultures were incubated with the precursors.		uterine cervix.		
Initial investigation of microbially influenced corrosion (MIC) in a low temperature water distribution system	Treated and untreated water samples were collected and analyzed for chemical and microbial constituents. A section of corroded pipe, carrying treated water was removed and included for microbial analysis.	Results showed that potentially corrosive microorganisms were present in untreated supply water, treated water and corrosion tubercles. Besides bacteria (Sulfite-reducers, sulphate-reducers, iron-reducers, sulphur-oxidizers), sulfate-reducing actinomycetes and iron-reducing fungi *(Penicillium. Rhizopus, Aspergillus)* were found in the samples.	Indirect impact/ taste & odour problems: Corrosion tubercles may serve as a habitat for certain taste and odour-producing actinomycetes and fungi in treated water supplies.	Canada	Emde et al. (1992). Wat Res: 26:169-175.
Health and immunology study following exposure to toxigenic fungi *(Stachybotrys chartarum)* in a water-damaged office environment	The health status of office workers after exposure to fungal bio-aerosols and its toxigenic metabolites (satratoxins) was studied. Exposure characterization and quantification were performed using microscopic, culture, and	Widespread fungal contamination of water-damaged, primarily cellulose material with *Stachybotrys chartarum* was found. *S. chartarum* produced macrocyclic trichothecene, satratoxin H, and spirocyclic	Direct Impact: The prolonged exposure to toxigenic *S chartarum* and other fungi was associated with reported disorders of the respiratory and central nervous, mucus membranes and immune system.	USA	Johanning et al. (1996) Int Arch Occup Environ Health 68:207-218

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
	chemical techniques.	lactones. Strong association with exposure indicators and employees/controls were found for respiratory, dermatological, eye and other chronic symptoms.			
Culturable mould in indoor air and its association with moisture related problems and asthma and allergy among Swedish children	Case control study: Relationship between mould spore exposure indoor and mouldy odour, visible signs of dampness and diagnosed asthma and allergy was studied with 198 children with asthmatic and allergic cases and 202 healthy controls.	No association was found between the indoor fungal spore concentration and mouldy odour and visible dampness in the homes. No association was found between the fungal spore concentration in indoor air and asthma/allergy in the children.	No impact?: The study suggests that, there is no reason for on-time air sampling of mould CFU in indoor air to identify the risk factors for asthma/allergy in children living in Scandinavian countries.	Norway	Holme et al. (2010). Indoor Air 20: 329–340.
Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children	Case study: Airborne samples of total and viable fungal spores were collected from bedrooms, living rooms, kitchens and outdoors. 80 households with 148 children between 7 and	The fungal concentration was more associated with musty odour, water intrusion and high indoor humidity. *Penicillium* – risk factor for asthma *Aspergillus* – risk factor for atopy.	Direct impact/risk: The exposure to certain fungal spores is found to be risk factor for asthma, atopy, respiratory symptoms in children. However, no association was observed between total/viable fungal spores and child health.	Australia	Garrett et al. Clinical and Experimental Allergy 28: 459-467.

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
	14 yrs involved in the study.	Fungal allergy is more common among children's exposed to *Cladosporium* / *Penicillium* and respiratory symptoms were common with exposure to *Cladosporium*.			
Growth and metabolites production by *Penicillium brevicompactum* in yoghurt	The growth study and the production of volatile organic compounds (VOC) and mycophenolic (MPA) was conducted using the fungus *P. brevicompactum*, which was previously isolated from contaminated yoghurt.	*P. brevicompactum* produced different metabolites in yoghurts. Sweetened yoghurts are considered an excellent medium for fungal growth. The occurrence of mycophenolic acid production under refrigeration temperatures has been found.	Indirect effect: Yoghurt may serve as a potential vehicle for production of toxic compounds by fungi growing at low temperature.	Italy	Ndagijimana et al. (2008) Int. J Food Micro 127: 276–283

Topic/Title	Type of samples Examined	Main findings from the study	Health impacts / risks	Country	Reference
Zearalenone (ZEN) production and growth in drinking water inoculated with *Fusarium graminearum*	The production of the mycotoxin ZEN was examined in drinking water inoculated with *F. graminearum*. This strain was isolated from a drinking water distribution system in US.	The results showed that the extracellular yield of ZEN was 15.0 ng per litre. Ergosterol was obtained an average of 6.2 µg per litre.	Indirect impact/toxin production: ZEN was produced readily in water by *F. graminearum*. It is recommended to monitor mycotoxin level in water as a standard method.	Portugal	Russell and Paterson (2007). Mycol Progress 6:109–113

ANNEX 3: PRIORITY PAPERS

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