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Prevalence and public health implications of mycotoxigenic fungi in treated drinking water systems

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ABSTRACT

Insufficient potable water resources and poorly treated drinking water quality are the world's number one cause for preventable morbidity and mortality from water-related pathogenic microorganisms. Pathogenic microorganisms, including mycotoxigenic fungi, have been identified in treated drinking water. This paper presents a review of mycotoxigenic fungi as a health risk to the public as these fungi are responsible for allergies, cancers and opportunistic infections mainly to immunocompromised patients. The exacerbating factors contributing to fungal presence in water distribution systems, factors that lead to fungi being resistant to water treatment and treated drinking water quality legislations are also discussed. This paper provides a review on the prevalence of mycotoxigenic fungi and their implications to public health in treated drinking water, and the need for inclusion in treated drinking water quality regulations.

Key words | fungi, mycotoxigenic fungi, mycotoxins, public health, treated drinking water

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INTRODUCTION

The environment is naturally rich in microbial diversity, most of which has not been studied yet and is currently unknown (Tekere *et al.* 2011; Fakruddin & Mannan 2013). Surface water, even when treated, has occasionally been associated with harmful infectious disease outbreaks, mostly caused by human and animal enteric pathogens (Bozzuto *et al.* 2010; Sibanda & Okoh 2013). Pathogenic microorganisms like bacteria, viruses and parasites are well known microbial water contaminants (Szewzyk *et al.* 2009). On the contrary, fungi have not been widely considered when discussing waterborne pathogens, but are now regarded as an emerging chronic water quality problem (Yamaguchi *et al.* 2007; Sonigo *et al.* 2011; Ashbolt 2015).

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Most countries' water quality legislations neither clearly address fungi, nor its perceived public health impacts or even set parameters for the control of fungi in treated drinking water (Oliveira *et al.* 2016; Babič *et al.* 2016, 2017). The European Union (EU) drinking water directive stipulates that treated drinking water should be 'free from any microorganisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health' (Babič *et al.* 2017). While the intentions of the directive are clear and meant to protect public health, the fact that even in contaminated water, fungi are not usually present in 'numbers or concentrations' characteristic of bacteria and other microbial contaminants, has led to the omission of fungi from the battery of routine drinking water quality monitoring tests. Consequently, this has led to the lack of information about the possible human health impacts of its presence in drinking water sources (Babič *et al.* 2016).

MICROBIAL QUALITY OF TREATED DRINKING WATER

Microbial quality of water is usually monitored by measuring microorganisms using indicator organisms such as *Escherichia coli* (Obi *et al.* 2008). However, over-reliance on indicator bacteria to determine the sanitary and public health safety of treated drinking water has its own challenges, including the fact that other pathogens like enteroviruses and protozoa are more resistant to disinfection than *E. coli*, such that a zero count of *E. coli* does not essentially indicate the absence of other microorganisms (WHO 2011a; Meals *et al.* 2013). Heterotrophic plate count is the only indicator method for fungi as it is used to indicate changes in microbial concentration that show entry or regrowth in treated drinking water (Zerzghi *et al.* 2010; Babič *et al.* 2017). The problem, however, is that there is no regulatory value with the heterotrophic plate count, leading to a conclusion of compliance that is defined as a 'no abnormal change' which may ultimately not indicate the presence or absence of fungi (Babič *et al.* 2017). The available methods may not be reliable to detect and/or quantify all the waterborne pathogens including fungi that are also known to resist disinfection.

The WHO has an international obligation of issuing guidelines of universal application (WHO 2017) that also entail the issuing of directives, determination of legislation, setting of recommendations, and requires the testing and monitoring of drinking water quality (Babič *et al.* 2016). A preventative approach that only monitors the quality of treated drinking water, the 'Water Safety Plans' was endorsed by the WHO (WHO 2017). The plan considers factors that may contribute to endangering the quality of water from the source of water to the end user (José Figueras & Borrego 2010). While the WHO did not include fungi in the routine battery of microbiological parameters used to determine the quality of treated drinking water, it has labelled fungi as nuisance organisms because of taste and odour problems (Kinsey *et al.* 2003; Hageskal *et al.* 2006, 2009; Sonigo *et al.* 2011).

Other water quality legislations include those by the United State Environmental Protection Agency, an agency of the federal government of the United States created for the purpose of protecting human health and the environment by drafting and implementing regulations based on laws passed by Congress (Kanematsu & Barry 2016)). The EU Drinking Water Regulations (2014) recommend the application of water quality standards for Europe, European Union Countries and Northern Europe, and procedures for sampling frequency and methods of analysis (EU 2014). The above water quality legislative bodies also do not regulate the limits for fungi in treated drinking water, and the presence of fungi in tap water samples (Babič *et al.* 2016, 2017). The USEPA did look at the addition of microsporidia in drinking water regulations although it was later withdrawn from its 'Contaminant Candidate List' (Babič *et al.* 2017). Sweden is the only country that currently includes specific measures for the monitoring of micro-fungi in treated drinking water. The Swedish Drinking Water Guidelines specify a criterion of 100 CFU of micro-fungi per 100 mL in treated water as being fit for human consumption (Sammon *et al.* 2010).

The EU only issued a recommendation for mycotoxins to protect consumer's food and animal feeds from harmful effects of mycotoxins that became effective as legislation from the 1st October 2006 (Zain 2011). Mycotoxin regulations in food and feeds have been established in approximately 100 countries worldwide, with only 15 of these countries from Africa, to protect the consumer from harmful effects of these mycotoxins (Wagacha & Muthomi 2008). Ever since the 2003 FAO report which discovered that there were only aflatoxin regulations in Africa, there has been little or no progress regarding mycotoxin regulations in the continent (Misihairabgwi *et al.* 2017). Developing countries do not take into consideration mycotoxin regulations, predominantly due to lack of country-specific data of certain toxins, resources to obtain toxicological and exposure data and the analytical capacity to enforce the regulations (Wagacha & Muthomi 2008; Misihairabgwi *et al.* 2017).

OCCURRENCE AND PREVALENCE OF FUNGI IN TREATED DRINKING WATER

Fungi are natural inhabitants of composting plants, soil and water (Korzeniewska 2011; Calvo-Polanco *et al.* 2016).

Terrestrial fungi are capable of migrating from soil into fresh water systems through animals, plants and soil (Wurzbacher *et al.* 1998; Magwaza *et al.* 2017). Fungal species like *Fusarium* and *Aspergillus* species have been identified to multiply in water reservoirs where they have been implicated in waterborne infections (Kanzler *et al.* 2007). Fungi in treated drinking water was first noticed in the 1960s and 1970s due to taste and odour problems, but was little considered as it was not the main focus for the analysis (Hageskal *et al.* 2009), and also because the contributory connection between the occurrence of fungi and water quality was not yet understood (Doggett 2000). The consumption of drinking water polluted by fungi had not been associated with serious disease casualties until studies performed in the 1980s revealed a number of cases linked to fungal contaminated treated drinking water (Hageskal *et al.* 2009).

Fungal deposition in water distribution systems is attributed to spores and not hyphae growth, which raises concerns that mycotoxins' production of taste and odour problems suggest that vegetative growth occurs *in situ* (Doggett 2000). In the water distribution system, the biomass of microorganisms suspended in the water phase is the main site of the occurrence of fungi belonging to moulds, including pathogenic species that are harmful to humans (Grabińska-Łoniewska *et al.* 2007). Fungi in treated drinking water have been identified in most countries, as shown in Table 1.

Fungi in treated drinking water from the various investigations, as shown in Table 1, demonstrates that the most prevalent fungi are *Acremonium* sp., *Alternaria* sp., *Aureobasidium* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Epicoccum* sp., *Exophiala* sp., *Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Paecilomyces* sp., *Penicillium* sp., *Phialophora* sp., *Phoma* sp., *Rhizopus* sp., *Trichoderma* sp. and *Verticillium* sp. Most of the fungal genera described in the studies are dematiaceous fungi which are capable of secreting melanin or melanin-like pigment in their cell walls. This makes them thick-walled species with hydrophobic spores which give them the advantage to resist water treatment (Sonigo *et al.* 2011; Auwal & Taura 2013; Al-gabr *et al.* 2014; Babič *et al.* 2017). These persistent fungi normally originate from soil, wood and decomposing plant material (Fox *et al.* 2016), which explains why they end up in raw water. *Cladosporium* sp., *Penicillium* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Phoma* sp., *Epicoccum* sp., *Trichoderma* sp., *Acremonium* sp., *Exophiala* sp., *Alternaria* sp. and *Phialophora* sp. are capable of producing mycotoxins and other secondary metabolites that produce toxic chemicals which impair water quality and become a threat to humans and animals (Pitt *et al.* 2000; Pereira *et al.* 2010; Sonigo *et al.* 2011).

Treated drinking water distribution systems and microbial quality of water

There are a number of microorganisms that are a source of drinking water problems, and some of the problems have been linked to the presence of fungi in the distribution systems (Doull *et al.* 1982; Fish *et al.* 2015). When fungi enter the water distribution system, it can be harboured by places in the network such as reservoirs that generate stratification, stagnation, dead zones, depletion of residual disinfectants and biofilm formation (Oliveira *et al.* 2016). These conditions, together with chemical-physical characteristics like high turbidity and temperature, pH, total organic carbon (TOC) and dissolved oxygen (DO), are favourable to microbial growth, making these environments at potential risk of water quality degradation by fungi (Oliveira *et al.* 2016). Fungi have been shown to enter the water distribution system in many ways that may be unavoidable such as mains interruptions, installations and maintenance (Doull *et al.* 1982). Others may include treatment breakthrough, water storage problems and cross-connections (Gashgari *et al.* 2013). When introduced in water, fungal species become established in biofilms in the inner surfaces of pipes (Mains 2008).

Many water companies have encountered operational and technological challenges that have, at times, led to consumer complaints because of fungi related problems (Grabińska-Łoniewska *et al.* 2007; Douterelo *et al.* 2014a, 2014b; Hurtado-McCormick *et al.* 2016). Fungi also produce secondary metabolites that exude organic acids which contribute to microbiological corrosion in water pipes (Grabińska-Łoniewska *et al.* 2007). This corrosion inhibits proper disinfection as accurate concentrations of chlorine residual in the treated distribution water system are altered (Sonigo *et al.* 2011). Even though water distribution systems are expected to act as contamination barriers in protecting the public's health against contamination by microorganisms, fungi together with viruses and protozoa have been shown to

Table 1 | Conducted surveys for fungi in treated drinking water globally

Country and city	Year	Source of samples	Fungal isolates	Reference
Greece, Thessaloniki	1998	Tap water (hospital and community)	*Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Bipolaris* sp., *Chaetomium* sp., *Chrysosporium* sp., *Cladosporium* sp., *Curvularia* sp., *Doratomyces* sp., *Emmonsia* sp., *Epicoccum* sp., *Eurotium* sp., *Exophiala* sp., *Fusarium* sp., *Gliocladium* sp., *Mucor* sp., *Penicillium* sp., *Phialophora* sp., *Pyrenochaeta* sp., *Rhizopus* sp., *Scopulariopsis* sp., *Sepedonium* sp., *Stachybotrys* sp., *Trichoderma* sp., *Trichothecium* sp. and *Verticillium* sp.	Arvanitidou *et al.* (1999)
UK, USA	1996	Surface water and distribution systems	*Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Cladosporium* sp., *Epicoccum* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Phialophora* sp., *Pythium* sp. and *Trichoderma* sp.	Kinsey *et al.* (1998)
Greece	2000	Municipal water supplies of haemodialysis units	*Penicillium* sp., *Aspergillus* sp., *Verticillium* sp., *Actinomycetales* sp., *Trichothecium* sp., *Chrysosporium* sp., *Absidia* sp., *Acremonium* sp., *Alternaria* sp., *Aureobasidium* sp., *Basidiobolus* sp., *Botrytis* sp., *Chaetomium* sp., *Cladosporium* sp., *Cryptococcus* sp., *Curvularia* sp., *Doratomyces* sp., *Epicoccum* sp., *Eurotium* sp., *Fusarium* sp., *Geotrichum* sp., *Gliocladium* sp., *Helminthosporium* sp., *Microsporum* sp., *Monosporium* sp., *Mucor* sp., *Phoma* sp., *Pyrenochaeta* sp., *Rhizopus* sp., *Scopulariopsis* sp., *Sepedonium* sp. and *Trichoderma* sp.	Arvanitidou *et al.* (2002)
Poland	2000–2002	Water distribution system	*Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Fusidium* sp., *Geotrichum* sp., *Gonatobotrys* sp., *Paecilomyces* sp., *Penicillium* sp., *Phialophora* sp., *Sclerotinia* sp., *Sesquicillium* sp., *Stachybotrys* sp., *Trichoderma* sp. and *Verticillium* sp.	Grabińska-Łoniewska *et al.* (2007)
Germany, North Rhine-Westphalia	1998/9 (12 months)	Drinking water	*Phialophora* sp., *Acremonium* sp., *Exophiala* sp., *Penicillium* sp., *Verticillium* sp., *Fusarium* sp., *Phoma* sp., *Aspergillus* sp., *Cladosporium* sp., *Chalara* sp., *Paecilomyces* sp., *Mucor* sp., *Geomyces* sp., *Ochroconis* sp., *Conidiobolus* sp., *Humicoala* sp., *Myrothecium* sp., *Tilletiopsis* sp., *Plectosporium* sp. and *Volutella* sp.	Göttlich *et al.* (2002)
Portugal, Braga	2003/4	Tap water	*Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Penicillium* sp., *Phialophora* sp., *Rhizopus* sp. and *Mycelia sterilia* sp.	Gonçalves *et al.* (2006)
Pakistan	Year n/a once, 30 samples	Municipal water and fruit juice	*Aspergillus* sp., *Monodictys* sp., *Penicillium* sp., *Trichoderma* sp., *Drechslera* sp. and *Fusarium* sp.	Nazim *et al.* (2008)
Australia	2007/8	Municipal water	*Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Fusarium* sp., *Pithomyces* sp., *Alternaria* sp., *Paecilomyces* sp., *Acremonium* sp., *Epicoccum* sp., *Curvularia* sp. and *Asporogenous* sp.	Sammon *et al.* (2010)
Saudi Arabia, Jeddah City	Year n/a (once)	Treated water from hospitals and private houses	*Alternaria* sp., *Aspergillus* sp., *Acremonium* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizopus* sp., *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp.	Gashgari *et al.* (2013)

thrive in pipe networks (Speight 2002; Douterelo et al. 2014a, 2014b). Different types of materials including stainless steel, cast iron galvanised steel, copper and polyethylene have been used to manufacture water distribution pipes, and these materials often favour the formation of biofilm in the water distribution systems (Mulamattathil et al. 2014).

Fungi and microbial biofilms in water distribution systems

Microorganisms in the environment survive mainly by attaching themselves into and growing onto living and non-living surfaces from natural biotopes to non-natural appliances (Donlan 2001; Lahaye et al. 2016). Microorganisms produce extracellular polymers that facilitate attachment and matrix formation that help them attach and grow on surfaces (Donlan 2001). The micro-colonies of organisms that exist within extracellular matrices are called biofilms. A biofilm is described 'as a layer of microorganisms in an aquatic environment held together in a polymetric matrix attached to a substratum such as pipes and sediment deposits' in the water distribution system (Momba et al. 2000). Biofilms have a dynamic process of development on surfaces that entail attachment, growth, motility and extracellular polysaccharide production (Feng et al. 2015). Biofilms are formed inside the drinking water distribution systems the same way as plaque that forms on teeth (Mains 2008). Biofilms in water distribution systems provide a good habitat for microorganisms as they accumulate better in solid–liquid interfaces that enable them to be embedded in the gelatinous matrix of extracellular polymers excreted by the microorganisms leading to resistance of microbes from environmental stresses (Feng et al. 2015).

Microorganisms enter the distribution system after surviving water treatment processes, and due to recontamination and re-growth (Liu et al. 2016). Bacteria first colonise the pipe surfaces producing a slime layer that helps trap organic particles that can be used for food and energy. Many microorganisms including protozoa, algae, viruses, helminths and fungi then become attached to the biofilm (Donlan 2001; Mulamattathil et al. 2014). Fungal colony forming units (CFUs) held in biofilms have been shown to be 1,000 to 5,000 times greater than in water, although the density of fungal species in biofilms may vary according to locations (Sonigo et al. 2011). Filamentous fungi have also been isolated in biofilm samples in drinking water distribution systems in Colombian drinking water networks (Hurtado-McCormick et al. 2016).

To further protect fungi and other pathogenic microorganisms from destruction or elimination in water pipelines, bacterial populations in biofilms produce persister cells that neither grow nor die, and display resistance to antimicrobial agents (Berry et al. 2006; Feofilova et al. 2012; Scorzoni et al. 2017). The cells provide barriers that prevent disinfectants from penetrating as the disinfectant binds to the extracellular polymeric matrix rather than reacting with the cells. The enzymes in the matrix are also capable of biodegrading disinfectant residuals (Fish et al. 2016). A study by Sammon et al. (2011) showed reduced vulnerability of microorganisms in biofilms due to formation of a protected subpopulation of persister cells. Persister cells in fungi indicate that when the biofilm is destroyed by antimicrobial agents, a new biofilm, with a new sub-population of persisters is formed, which is, in reality, a modified phenotype, and that adhesion to a surface initiates dormancy (Ramage et al. 2012). Chlorine residual does not infiltrate thick biofilms and sediments as it may be biodegraded by the enzymes present (Mains 2008). To reduce the build-up of biofilms that harbour stubborn pathogens, maintenance of the water distribution systems must take into account flushing, pigging or water/air scouring of the pipes to flush out sediments that encourage proliferation of fungal populations (Alegre et al. 2010). Fungal mycelia growing on substrate are dense and sporulation is more prolific than the growths of fungi observed on pipe wall samples (Sammon et al. 2011). Biofilm formation not only affects the quality of water by reducing the effectiveness of chlorine residual but raises the costs of maintenance for the distribution system (van der Kooij 1998; Boe-Hansen et al. 2003). Biofilm recalcitrance towards disinfection may contribute to the dissemination of fungi to public drinking water (Göttlich et al. 2002).

PUBLIC HEALTH IMPLICATIONS OF MYCOTOXIGENIC FUNGI IN TREATED DRINKING WATER

The presence of fungi in treated drinking water and its health impacts were not taken seriously until cases caused by fungal contaminated water were reported in Finland and Sweden during the 1980s and 1990s (Dufour *et al.* 2003; Boe-Hansen *et al.* 2003). Waterborne filamentous fungi are known to act as pathogens or allergens that have adverse impacts on human health, and mostly on immune-compromised patients (Oliveira *et al.* 2016). Transmission of pathogenic microorganisms by drinking water has continued to be a major cause of water-related illnesses, as confirmed by the frequencies of outbreaks reported around the world (WHO 2011b). Fungal infections are a challenge to cure as fungal cells are eukaryotic, just like human cells (Yamaguchi *et al.* 2007). Pathogenic fungi are believed to have caused hostile infections that have contributed to high mortality rates (Arvanitidou *et al.* 1999; Khan *et al.* 2010; Mayer *et al.* 2013; Tsui *et al.* 2016; Pal 2017).

Fungal infections were quite low from the late 1950s and early 1960s, yet over the past two decades, fungal infections have dramatically increased as they are easily diagnosed (Khan *et al.* 2010). Most of the fungi that were identified in Table 1 are dematiaceous fungi responsible for causing a number of cutaneous and subcutaneous infections including invasive and contagious infections (Pfaller & Diekema 2004). A significant proportion of waterborne illnesses related to fungi are likely to go undetected by the communicable disease surveillance and reporting systems. The possible health impacts caused by fungi in treated water are still not well documented, although protective measures are recommended for people who are at high risk (Hageskal *et al.* 2009), especially the increasing population of patients having an impaired immune system, as their immune effector cells become compromised allowing fungi to colonise and attack the human tissues, leading to more complications (Oliveira *et al.* 2013).

Fungi have been implicated in a number of diseases causing allergies, respiratory illness, cutaneous infection and life-threatening meningitis (Pfaller & Diekema 2004; Sulaiman *et al.* 2014). *Alternaria* sp., *Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. have been linked to allergies and respiratory illness (Korzeniewska 2011; Máiz *et al.* 2018). *Cryptococcus* and *Candida* cause meningitis (Black & Baden 2007), with the *Candida* species responsible for cutaneous infections (Khan *et al.* 2010; Volk 2013). Taste and odour problems in water are caused by *Aspergillus* sp., *Acremonium* sp., *Phialophora* sp. and *Penicillium* sp. (Hageskal *et al.* 2006; Sonigo *et al.* 2011). Fungi such as *Rhizopus, Fusarium, Alternaria, Aspergillus* and *Penicillium* produce mycotoxins that are harmful to public health as these mycotoxins are carcinogenic and have the ability to impair the immune system (Bhat *et al.* 2010; Sonigo *et al.* 2011; Magwaza *et al.* 2017). Mycotoxins of great concern for public health include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids (Zain 2011). The types of infections caused by mycotoxigenic fungi depend on the type of mycotoxin, the concentration and length of exposure; as well as age, health, and sex of the exposed individual (Bennett & Klich 2003). Mycotoxins found in water may be extremely diluted and may not be of major concern, but their concentrations may increase resulting in hazardous levels to human health, particularly when water is stored in reservoirs for longer periods (Siqueira 2011). The absence of toxigenic fungi in treated drinking water may not provide assurance that the water is free of mycotoxins, as mycotoxins may persevere long after the fungi has died (Pitt *et al.* 2000). Mycotoxins have serious and chronic effects on humans and animals, as many of them are believed to be carcinogenic, cytotoxic, mutagenic and may lead to immunosuppressive complexes (Arroyo-Manzanares *et al.* 2015). Although now there are reports regarding advances in antifungal therapy, it is worth noting that the number of cases of infection and antifungal resistance are also alarmingly high, and the control of antifungal disease does not indicate any possibilities of being achieved soon (Araj *et al.* 2015; Meirelles *et al.* 2017; Rodrigues *et al.* 2017; Pellon *et al.* 2018). Treated drinking water quality without pathogenic microorganisms including fungi is very critical for the health of all humans and mostly those with immunodeficiency conditions. Table 2 shows the toxic effects of some of the mycotoxins, producing genera and health effects.

Occurrence and concentration of mycotoxins in treated drinking water

Mycotoxins are fungal poisons that are produced as secondary metabolites by the mycelial structure of filamentous fungi as well as spores (Gupta *et al.* 2015). Not all fungi produce mycotoxins, as most mycotoxin producing species are

Table 2 | Fungal mycotoxins, producing fungal genera and health effects

Mycotoxin	Genera	Health effects	Reference
Aflatoxins	*Aspergillus* sp.	Human carcinogenic and others are hepatotoxic, aflatoxicosis, aspergillosis, nephropathy, teratogenic effect and decreases resistance and susceptibility to HIV, TB, and other opportunistic infections	Campbell *et al.* (2004), Shadanaika (2005), Bloom (2008), Mukherjee (2012) and Šegvić et al. (2013)
Fumonisins	*Fusarium* sp.	Oesophageal cancer	Bennett (1987), Pitt (2000) and Shadanaika (2005)
Citrinin	*Aspergillus* sp., *Penicillium* sp. and *Monascus* sp.	Nephrotoxic, teratogenic	Shadanaika (2005)
Ochratoxin A	*Aspergillus* sp. and *Penicillium* sp.	Nephrotoxic, hepatotoxic, teratogenic and carcinogenic	Abbott (2002), Shadanaika (2005) and Dao *et al.* (2005)
Patulin	*Aspergillus* sp., *Penicillium* sp., *Paecilomyces* sp. and *Byssochlamys* sp.	Immune toxicity, cytotoxic; tremorgenic and pulmonary oedema	Campbell *et al.* (2004), Shadanaika (2005), Bloom (2008), and Puel *et al.* (2010)
Sterigmatocystin	*Aspergillus* sp.	Carcinogenic	Shadanaika (2005) and Coelho *et al.* (2010)
Cyclopiazonic acid	*Aspergillus* sp. and *Penicillium* sp.	Convulsions	Shadanaika (2005) and WHO (2011b)
Trichothecenes	*Fusarium* sp. *Trichoderma* sp.	Toxic aleukia	Shadanaika (2005)
Deoxynivalenol	*Fusarium* sp.	Anorexia, nausea, vomiting, headache, abdominal pain, diarrhoea, chills, giddiness and convulsions	Pitt (2000) and WHO (2011b)
T-2 toxin	*Fusarium* sp.	Alimentary toxic aleukia	WHO (2011b)
Zearalenone	*Fusarium* sp.	Carcinogenic	Kuhn & Ghannoum (2003)
Ergot alkaloids	*Cladosporium* sp.	Gangrenous and convulsive forms of ergotism in humans	Hussein & Brasel (2001) and Abbott (2002)
Penicillic acid	*Penicillium* sp.	Carcinogenic	Abbott (2002)
Tenuazonic acid, alternariol, altenuene, and altertoxin-1	*Alternaria* sp.	Inhalation allergy problems and mycotoxicoses	Shadanaika (2005), Volk (2013) and Buse *et al.* (2013)
Ergotamine	*Claviceps purpurea*	Neurotoxins (poisonous to nerves)	WHO (2011b)
Brefeldin	*Phoma* sp.	An antibiotic, responsible for reducing cancer stem cell activities, and inhibiting migration ability in human breast cancer	Abbott (2002)
Rhizonin	*Rhizopus* sp.	Hepatotoxic	Abbott (2002) and Partida-Martinez *et al.* (2007)

filamentous ascomycetes, basidiomycetes and Deuteromycetes with *Penicillium, Aspergillus* and *Fusarium* being the most mycotoxin-producing genera (Abbott 2002). These mycotoxins do not have any biochemical implications in fungal growth and their development (Hussein & Brasel 2001). Fungal growth and mycotoxin production are the consequence of an interaction among the fungus, the host and the environment (Atanda *et al.* 2011). The right combination of these factors determines the amount of colonisation of the substrate, the type and amount of mycotoxin produced (Pitt *et al.* 2000). The synthesis of any particular mycotoxin depends not only on the species but also on the strain (Pitt *et al.* 2000). Although the chemical structures of mycotoxins vary significantly, they are generally low molecular mass organic compounds. Due to the fact that mycotoxins are minute and fairly stable molecules, they become very problematic to eliminate and this makes it easy for them to enter the food and feed chain while keeping their toxic properties (Omar 2013). Mycotoxins may be ingested through food or water containing poisonous fungi (Volk 2013) or ingested as mycotoxins secreted by fungi without eating the fungus itself. If ingested, mycotoxins cause tissue destruction, angioinvasion, thrombosis, infarction and other manifestations of mycosis (Campbell *et al.* 2004).

Mycotoxigenic fungi have been reported in treated drinking water (Paterson & Lima 2008; Al-gabr *et al.* 2014). While mycotoxin concentrations can be low in water because of dilution, water retention in storage tanks, the long distances water travels in distribution systems, depletion of chlorine residual and the resistance of some of the fungi to disinfection can cause mycotoxin concentrations to increase to unsafe amounts in drinking water (Barriga *et al.* 2012). Parameters such as temperature and pH also encourage growth and persistence of fungi in water (Hussain *et al.* 2011). Small amounts of mycotoxins consumed regularly could be harmful to human health (Hageskal *et al.* 2009).

FUNGAL–BACTERIA INTERACTIONS AND CORRELATIONS IN WATER DISTRIBUTION SYSTEMS

It is key to ascertain the prevalence of fungi and make deductions as to the interactions and correlations between fungi and bacteria and any need to include fungi in drinking water standards. Bacteria and fungi exist and interact in many environments as they often share a common substrate. Fungal interaction with bacteria range from disorderly polymicrobial assemblies to closely related symbiotic associations of fungal hyphae and bacterial cells (Frey-Klett *et al.* 2011). Bacteria are responsible for the initial construction of biofilms while fungi colonise pre-established bacterial biofilms, which is a form of commensalism as one benefits while the other is unaffected due to different ecological requirements of the two organisms (Sonigo *et al.* 2011).

Fungi and bacteria are believed to positively use their competitive interactions during fungal decomposition of unmanageable organic matter (De Boer *et al.* 2005). Fungi produce most enzymes because they have higher biomass and bacteria benefit from the enzymatic capacity of fungi, in particular when it comes to enzymes involved in degrading plant polymers (Mille-lindblom 2005). However, some fungal species tend to suppress bacterial growth through production of antibacterial substances, for example, penicillin from the fungus *Penicillium notatum* (Mille-Lindblom *et al.* 2006). Studies have found different relationships between fungi and bacteria depending on bacterial and fungal species compositions and biological mechanisms affecting the relationship (Sonigo *et al.* 2011). Understanding the interactions between bacteria and fungi in water will give an insight as to whether the presence of certain bacterial species in water can be used as an indicator of its fungal content (Gonçalves *et al.* 2006). To date, no conclusive correlation has been found between indicator organisms such as *E. coli* and other coliforms to fungi in treated drinking water systems (Oliveira *et al.* 2016). This is because fungi can resist disinfection while coliform bacteria would be eradicated (Kinsey *et al.* 2003). This lack of correlation between coliforms and fungi presence in drinking water distribution systems may mean that there is a possibility for bacteriologically safe water to contain some pathogenic fungi (Sonigo *et al.* 2011). Ashbolt *et al.* (2001) argued that with the change in monitoring standards, more indicators of process efficiency are required rather than the reliance on the 'old-style' *E. coli* as an indicator. However, a point worth noting is that fungi often colonise pre-established bacterial biofilms and, as such, the correlations deductible in biofilms are not necessarily the same as for water samples.

WATER TREATMENT PROCESSES AND INACTIVATION OF FUNGI

Water is crucial for the sustenance of life and, therefore, access to safe drinking water is necessary for human health and for economic development (WHO 2008; UN Water 2015). The objectives for water treatment are not only to produce water that is acceptable in terms of being aesthetically pleasing in appearance (clearness) and taste and odour (Momba *et al.* 2008), but the initial aim is the destruction or inactivation of pathogenic microorganisms to prevent the spread of waterborne diseases (EPA 2013). In general, most water utilities treat water via coagulation, flocculation, sedimentation and filtration, that are designed with the main objective of removing microbial pathogens with the addition of disinfection such as chlorine as a significant component in water treatment (Adam *et al.* 1998). Literature has revealed that water treatment plant processes do not completely remove all pathogenic microorganisms, including fungi in water that end up in treated water distribution systems to the consumer point of use (Sammon *et al.* 2011; Pereira *et al.* 2013).

Coagulation and flocculation

Coagulants aid in destabilising colloidal particles in water to promote agglomeration to form larger sized particles known as flocculants (flocs) which can be effectively removed by sedimentation or flotation (Al-mamun *et al.* 2016). Sedimentation and flotation processes remove a great many of the microorganisms including fungi as they are trapped within the particles and settle to the bottom in sedimentation tanks or float out in flotation tanks, where they are eventually disposed of with the sludge (Kinsey *et al.* 2003). Most of the time there are light broken flocs or non-flocculated colloidal particles that are suspended in water (Jun *et al.* 2009; Oyegbile *et al.* 2016; Rasteiro *et al.* 2016; Marques *et al.* 2017), to which, microorganisms have the ability to attach themselves. These suspended particles, depending on the quality of water, end up being transferred to the filtration process (Thupaki *et al.* 2013).

Filtration

It is very important that all the particles are removed to maintain turbidity (particles suspended in water) of less than one nephelometric turbidity units (1 NTU) for effective disinfection of water (WRC 2002). Rapid sand filters are commonly used although they do not have enough retention time to remove all microorganisms from water which are hidden in the particles (O'Connor & O'Connor 2001). Fungi can grow attached to a substrate and colonise filters in water treatment plants giving them a good opportunity to resist water treatment (Hageskal *et al.* 2009). If fungi survive sedimentation and flocculation, rapid sand filtration does not become an effective treatment for fungi (Kinsey *et al.* 2003) as these filters have been shown to partially remove microorganisms, especially fungi that end up in the distribution system (Kinsey *et al.* 2003). After filtration, the final and most trusted treatment process for destroying pathogenic microorganisms is disinfection (Tellen *et al.* 2010).

Disinfection

The use of disinfection in water treatment as a public health measure has shown a major decline in people contracting water-related diseases from drinking water (EPA 2013). Fungi and other bacteria have the ability to become dormant in order to preserve their lives when conditions are no longer conducive. But when the conditions become favourable again, they return to their normal state, recovering their metabolic activity and starting the process of spore germination (Luu *et al.* 2015). This is the phenomenon with the melanised thick-walled fungal species that are more resistant to water treatment and disinfection (Hageskal *et al.* 2012). When the turbidity of water is greater than 1 NTU usually with organic particles, the particles interfere with disinfection as microorganisms get entrapped in the particles or adsorbed onto particle surfaces (Spellman 2014), resulting in microorganisms being protected from disinfection that is required for their elimination (Al-berfkani *et al.* 2014). Different fungal species vary in their resistance to disinfection. *Penicillium* and *Aspergillus* species are more resistant to chlorine disinfection than the *Cladosporium* and *Phoma* species (Pereira *et al.* 2013). Ozone and UV radiation are more capable in the destruction of many pathogenic organisms than chlorine, but their disadvantages are high costs and mostly the inability to have residual that persists long enough to prevent the re-growth of microorganisms in the distribution system (Freese & Nozaic 2004).

Ozone

Ozone inactivates fungal species by causing an irreversible cellular damage (Rojas-Valencia 2011). There are resistant species to ozone like *Trichoderma viride* that is slightly affected only in elevated concentrations and *Penicillium* *spinulosum* which is the most resistant due to its hydrophobic surface (Hageskal *et al.* 2012).

UV radiation

Fungi with pigmented spores such as *Aspergillus* and *Penicillium* have better defence against radiation and are not responsive to UV treatment (Hageskal *et al.* 2009). The radiation cannot destroy fungal species even in slightly turbid water as the fungi tend to be harboured within the particles and escape disinfection (EPA 2013). Furthermore, disinfection methods by exposing some species of fungi to UV light may seem futile as strongly melanised spores of *Aureobasidium pulullans* and *Aureobasidium melanogenum* have shown resistance to elongated radiation interactions (Castiglia & Kuhar 2017).

Free chlorine

Chlorine is the mainly used form of disinfection in most water treatment plants in many countries. Although chlorine is widely used as a strong oxidant, that is reliable in removing or the inactivation of many harmful microorganisms in water when evaluated against other microorganisms, fungi were discovered to be more resistant to chlorine inactivation than the commonly used indicator organism *E. coli* (Luyt *et al.* 2012; Oliveira *et al.* 2013; Al-berfkani *et al.* 2014).

CONCLUSION

Water treatment is very important in controlling pathogenic microorganisms from reaching consumers. There are guidelines in place to test and monitor pathogenic microorganisms like bacteria, viruses and parasites in treated drinking water from the water source to the final drop of the tap. Fungi have been left out of the battery of drinking water quality monitoring parameters, yet scientific literature has proven the presence of fungi and their mycotoxins in treated drinking water. Melanised and slimy conidia have been found in treated water distribution systems with a prevalence of *Cladosporium, Phoma, Alternaria, Aspergillus, Penicillium, Exophiala, Fusarium, Acremonium, Exophiala* and *Phialophora* and that has the general capacity to resist disinfection regimes (Göttlich *et al.* 2002). The growing list of documented pathogenic fungi from treated drinking water can no longer be ignored as water contaminants. The potential health impact of waterborne fungi is still not clear, while precautions are needed for high-risk patients.

In many countries, little focus has been centred on the occurrence of fungi and their mycotoxins in treated drinking water supply networks. Monitoring and keeping the amount of fungi under surveillance after water treatment and in distribution systems is fundamental in guiding against harm to human health, and also to improve the aesthetic quality of water in relationship to taste and odour. This can also include the need to evaluate the removal of secondary metabolites by drinking water treatment processes. Although routine examination of fungi can be difficult as cautious and experienced personnel are key, nevertheless, this cannot be disregarded anymore as fungi influence water quality in many ways.

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