AN ASSESSMENT OF FUNGAL OCCURRENCE IN TREATED DRINKING WATER AND IMPLICATIONS TO PUBLIC HEALTH

Report to the
Water Research Commission

by

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WRC Report No. 2568/1/20
ISBN 978-0-6392-0149-8

May 2020

WATER
RESEARCH
COMMISSION

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GEZINA, 0031

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EXECUTIVE SUMMARY

BACKGROUND

Safe drinking water is of paramount importance in the protection of public health. Raw water is normally treated to produce drinking water that meets national standards, set according to the acceptable physical, chemical and biological properties. However, it is acknowledged that the final treated drinking water environment is non-sterile, and it hosts a diverse microbiome as total elimination of microorganisms from drinking water during treatment and distribution is impractical. Although the quality of final water leaving the treatment plant can be of acceptable standards, its quality can still deteriorate within the water distribution system. Microbial deterioration of treated drinking water can occur due to a number of reasons which include insufficient disinfectant residual, occurrence of microbial biofilms within the distribution system and contaminant intrusion during breakdown and maintenance of the systems. The presence of microscopic undesirable fungi in drinking water and distribution systems is deemed unfavourable because the fungi can potentially cause diverse effects on human health including the potential of producing mycotoxins. The presence of pathogenic and mycotoxic fungi in drinking water, although not specified in the national water quality standards, needs to be ascertained if their impacts on human health through the drinking water supplies route is to be established, studies of which have not yet been done in South Africa.

AIMS AND OBJECTIVES

In South Africa, provision of safe potable water is one of the main responsibilities of municipalities to users in their jurisdictions. This study focused on the assessment of fungal occurrence in treated drinking water in Johannesburg West as a case study and implications to public health as a means of strengthening existing water safety planning practices, as well as to provide the baseline information to regulatory authorities to consider working towards including fungi in the battery of drinking water microbial quality tests in South Africa to protect human health. The specific aims for the study were:

1. To determine the presence of fungi and total and faecal coliforms from selected sites along the treated drinking water distribution network in Johannesburg West.
2. To characterise fungal isolates to confirm identity and the presence of potentially mycotoxigenic fungi.
3. To analyse water samples for the presence of mycotoxins.
4. To determine statistically correlations between the presence of coliforms and fungi in treated drinking water and the potential health impacts.
5. Make output-dependent recommendations about monitoring and the potential health impacts of fungi in drinking water distributions.

METHOD

Water samples were collected from selected sites in the Johannesburg West areas, mainly in Roodepoort. Thirty sampling sites covering inlets and outlets to reservoirs, clinics, garages, schools and households, were identified along the distribution points in consultation with Johannesburg Water. Water samples were collected monthly for a period of 12 months. The samples were analysed for pH, total and free chlorine, the presence of fungi, mycotoxins and total and faecal coliforms. The presence of total and faecal coliforms was determined culturally on m-Endo and m-FC plates respectively while fungi were determined culturally on Potato Dextrose Agar (PDA). Molecular identification of axenic fungal cultures was also done by DNA analysis of the ITS gene using ITS1 and ITS4 universal primers. SPE-LC-MSMS was used in the analysis of the water samples for mycotoxins.

SUMMARY OF FINDINGS AND CONCLUSIONS

Analysis of the samples showed the presence of fungi in treated drinking water from all the sampling points studied. Fungi were more prevalent at monitoring sites located within informal settlements and such occurrence can be attributed to poor hygiene standards at the communal collection sites. The predominant fungal genera detected included Penicillium, Aspergillus, Cladosporium, Alternaria, Phoma, Epicoccum and Trichoderma species. Most of the identified fungi are known to produce mycotoxins and therefore potentially mycotoxigenic.

LC-MS analysis confirmed the presence of the following mycotoxins and concentrations: 15-acetyldeoxynivalenol (15.154 to 71.606 ng/Kg); nivalenol, tenuazonic acid, deoxynivalenol (8.405 to 96.139 ng/Kg), 3-acetyldeoxynivalenol (18.737 to 145.689 ng/Kg), aflatoxin G2, aflatoxin G1, aflatoxin M1, aflatoxin B1 (3.069 to 3.083 ng/Kg), and sterigmatocystin (0.223 ng/Kg). In the absence of defined standard limits for mycotoxins in water, the concentrations of the detected mycotoxin were compared to the South African acceptable maximum limits for food, foodstuffs and beverages. The concentration of the targeted mycotoxins from the analysed samples were below the South African acceptable maximum limits for food, foodstuffs and beverages, and consumption risk estimations against limits from literature showed that no risks to human health can be implied with the current results. Overall, the work done here shows that a wide range of potentially mycotoxigenic fungi are detectable in treated drinking water from Johannesburg West, while the observed levels were low (minimal risk), their presence however, may signal a potential water quality problem that requires monitoring.

Low total coliform counts were detected at a number of the sites but to a lesser extent compared to fungi, rarely were the faecal coliforms encountered. The recorded total coliform counts per site were low at 1-6 cfu/100ml. Almost all sites with fungi had bacteria but more sites had fungi and in higher counts than coliform bacteria. Statistically, no strong correlation was observed between total coliforms and fungi (r=0.4266). The fact that the total coliforms were detected across sampling sites at levels within SANS 241 (2015) limits of ≤10 and to a less extent as compared to the fungal incidence's points to a preliminary conclusion that the water is bacterial safe and that there is no correlation between faecal coliform and fungal occurrence in drinking water. Coliform indicator bacteria are therefore not a good indicator of fungi in drinking water.

RECOMMENDATIONS

As recommendations for future studies and water safety planning, research covering wider sampling areas and with more regular sampling is recommended so as to come up with concrete evidence for decision-making on the extent of the fungal occurrence and implication to human health. Also, since it has been shown in this study that fungi occur in the drinking water, further studies need to be done to establish the conditions that influence the establishment of fungi and the production of mycotoxins.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project.

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The University of South Africa Research Department is acknowledged for the role they played in hosting this research project. We acknowledge the administrative staff in the Department of Environmental Science, UNISA, and the laboratory technical staff of the College of Agriculture and Environmental Science for their supporting roles during the project. Many thanks to Prof M Nindi and Dr V Mhuka (UNISA Chemistry Department) for assisting with the mycotoxin analytics, Mr AC Greyling (Department of Geography), for assisting with the map and Dr H Ogola (Visiting researcher, Department of Environmental Science, UNISA), for input on the report writing and data analysis. We are grateful to the City of Johannesburg, Johannesburg Water for providing consent and access to the sampling sites.

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ACRONYMS & ABBREVIATIONS

ADD	Average Daily Dose
ARC	Agriculture Research Council
CFU	Coliform Forming Unit
DWS	Department of Water and Sanitation
ITS	Internal Transcribed Spacer
NMMP	National Microbial Monitoring Programme
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
SANS	South African National Standard
SPE	LC MSMS-solid phase extraction liquid chromatography – tandem mass spectrometry
TDI	Total Daily Intake
WHO	World Health Organisation
WRC	Water Research Commission
WSP	Water Safety Planning

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Ensuring that drinking water is of good quality and free of pathogenic microorganisms is paramount to human health protection and as a basic human right (WHO, 2011a; Pereira et al., 2010). Though drinking water is supposedly treated to meet the set standards before leaving the water treatment plants, it is acknowledged that drinking water distribution systems are a non-sterile water environment habitable by diverse microorganisms including fungi. Reasons being that treatment does not always completely eradicate all microorganisms and also often microbial re-growth and/or contamination occurs during distribution. Water distribution systems are expected to act as conduits that transport sufficient quantities of safe drinking water to consumers while creating a physical barrier against the external environment that can introduce pathogenic organisms (Van Zyl, 2014). Douterelo et al. (2014a) stated that some microorganisms may be able to survive disinfection in a drinking water treatment plant, and manifest in the distribution network and up to the consumer's point of use. In drinking water distribution systems, microbial growth is controlled by secondary disinfection, such as the addition of chlorine that remains as chlorine residual or free chlorine in the distribution system, limiting growth of microorganisms including fungi (Pereira et al., 2013). However, despite the presence of chlorine residual, microorganisms such as fungi, viruses and protozoa, have shown an ability to survive in the treated water systems (Douterelo et al., 2014b). Many studies have been carried out to assess the effectiveness of chlorine disinfection against bacteria (Murphy et al., 2008; Lee et al., 2010), protozoa (Corona-Vasquez et al., 2002), and viruses (Page et al., 2009; Lim et al., 2010), with satisfactory results against bacteria and viruses. However, Pereira et al. (2013) have reported that fungi demonstrate more resistance to chlorine inactivation compared to bacteria and viruses. The effectiveness of chlorine on fungal spores has also not been well established (Douterelo et al., 2014a).

Monitoring water quality during distribution and establishing appropriate remedial actions are therefore imperative in the framework of process control and risk management (El-Chakhtoura et al., 2015) and as part of the water safety planning process (WHO, 2011b). In South Africa, fungi are not part of the set drinking water quality standards (SANS 241: 2015) and as such are not part of the tests or requirements for compliance for drinking water (SANS 241: 2015). Lack of standard requirements for fungi have resulted in studies on fungal occurrence in drinking water being limited when compared to those on bacteria (Babič et al., 2017; Douterelo, et al., 2014b; Pereira et al., 2009;). This study was therefore set to investigate the occurrence of fungi, mycotoxins and their influence on water quality and human health from inlet and outlet of reservoirs, hospitals, households and schools along the treated water distribution networks in Johannesburg West, South Africa.

1.2 RESEARCH PROBLEM AND JUSTIFICATION

Drinking water quality guidelines require monitoring of microbiological properties to determine the quality against set standards (WHO, 2011a). It has been observed that although water can be treated to acceptable standards, its quality deteriorates in the water distribution systems (Hageskal et al., 2009). Fungi are known to occur widely in treated drinking water, but there has been little attention given to their existence and implications to human health (Babic et al., 2017). The World Health Organisation stated that the mortality rate of water-related diseases is over 5 million persons a year, above 50% being microbial intestinal infections (Cabral, 2010). Fungi are amongst pathogens that are believed to cause hostile infections that may contribute to the mortality rate (Arvanitidou et al., 1999; Sonigo et al., 2011). Water is one of the routes through which pathogenic fungi reach target individuals (Babic et al., 2017). There is

need to evaluate the extent of occurrence of fungi in treated drinking water distribution systems to determine the less studied mycological qualities of treated drinking water in South Africa.
Microbial water quality differs from area to area as it is affected by a wide range of natural and human influences (Manivanan, 2008; Babič et al., 2016). The types of fungi that produce mycotoxins in South Africa may be different from that of other countries and they need to be known. The occurrence of fungal species and potential to produce mycotoxins and their influence on water quality and human health in the treated drinking water distribution networks in Johannesburg West, South Africa was investigated. The study contributes to knowledge on fungi in drinking water and makes recommendations on the relevancy of fungi to water quality and consumer health.

1.3 PROJECT AIMS

The aim of this study was to assess fungal and mycotoxin occurrence in treated drinking water in Johannesburg West and its implications to public health. Project specific research objectives were:

1. To determine the presence of fungi and total faecal coliforms from selected sites along the treated drinking water distribution network in Johannesburg West.
2. To characterise the fungal isolates to confirm identity and the presence of potentially mycotoxigenic fungi.
3. To analyse water samples for the presence of mycotoxins.
4. To determine statistically if any correlations between the presence of coliforms and fungi in treated drinking water and the potential health impacts.
5. Make output-dependent recommendations about monitoring and the potential health impacts of fungi in drinking water distributions.

1.4 SCOPE AND LIMITATIONS

The study set to investigate the occurrence of fungi and detect mycotoxins and their implications on water quality and human health in the treated drinking water distribution network in Johannesburg West, South Africa. The study makes use of microbial cultural and molecular methods for coliform enumeration and in fungal diversity studies. Chemical detection and quantification of selected mycotoxin were done to evaluate the occurrence of these fungal metabolites and possible health risks likely to occur to humans through drinking water intake. The work was done on selected points of the drinking water distribution system and only looked at the water towers/reservoirs, water tanks, outside taps at households, garage, schools and clinics. Drinking water points inside consumer buildings were not evaluated as part of this study. In this study, sampling was limited to 30 sites dominantly in the Roodepoort area. This can be considered a smaller representative area and the sample number rather limited, and this was due to budgetary constraints.

A strict selection criterion was applied to ensure that diverse key sampling points were represented. Informal settlements, schools, clinics, garage and houses represent different consumers receiving points with different environmental settings for the treated drinking water and as such these were some of the selected sites. Water quality analysis was limited only to pH, residual chlorine levels, mycotoxins, bacterial coliforms and fungi. The number of physiochemical parameters to analysed was reduced so as to cut on the analysis cost and also since monitoring was done at sites mostly analysed by Johannesburg water, the project steering committee had recommended that extensive duplication of activities was to be avoided. However due to challenges in arranging for data access with Johannesburg municipality, it was not possible to obtain the monitoring data as had been set at the beginning of the project as well as the identity of the reservoir supplying the different consumer sampling points. Monitoring was also done only once a month, over a 12 months, critical incidences or changes in the drinking water system could have been missed

when no sampling was done. Raw water sources are known to influence the quality of the treated drinking water (Kanzler et al., 2007; Babič et al., 2017), however, as a limitation of this study it was not possible to obtain consent to sample the source water and treated water at the Rand Water treatment plant. Another limitation of the study was that it was not possible to do biofilm studies and fungal occurrences as influenced by microbial biofilms in the distribution system. Fungi are known to thrive and form an important part of the microbial biofilm community in drinking water distribution systems and as such to have a complete picture of fungal diversity and metabolites in drinking water, the inclusion of fungi would be highly recommended (Babic et al., 2017).

1.5 ETHICAL STATEMENT

Ethical clearance for the project was obtained from UNISA College of Agriculture and Environmental Science following college ethics procedures and, specifically covering the research of both the PhD and MSc student as part of the prerequisite for such a funded research project and research module. As part of the ethics clearance requirements, consent was sought from all participants including Johannesburg Water and all the other private participants.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

The natural environment harbors a variety of microbial diversity including many microorganisms that have not been studied and are not known (Tekere et al., 2011). Drinking water and different water sources have been described to contain a variety of fungal species (Gonçalves et al., 2006; Hageskal et al., 2006; Hageskal et al., 2007; Hageskal et al., 2009; Pereira et al., 2009; Ali et al., 2017; Babič et al., 2017; Siqueira et al., 2011; Oliveira et al., 2016). Both filamentous fungi and yeasts have previously been isolated in treated drinking water including; *Aspergillus, Cladosporium, Epicoccum, Penicillium, Trichoderma, Arthrinium phaeospermum, Aspergillus flavus, Cladosporium cladosporioides, Fusarium culmorum, Mucor hiemalis* and *Trichoderma harzianum* as predominant fungal genera and species found in treated and untreated water (Kinsey et al., 1998; Siqueira et al., 2011; Sonigo et al., 2011). Potentially pathogenic fungi are amongst these fungi found in aquatic ecosystems (Biedunkiewicz et al., 2014). Pathogenic fungi are known to cause hostile infections that may contribute to the mortality rate (Arvanitidou et al., 1999; Sonigo et al., 2011). Despite the widespread occurrence of fungi in aquatic environments, treated water contamination with fungi has received considerably minimal attention and may be considered to be an underestimated problem (Hageskal et al., 2009; Sonigo et al., 2011). While drinking water is considered an unnatural habitat for fungi, fungi do in fact, enter drinking water through various pathways (Hageskal et al., 2009; Sonigo et al., 2011). Fungi are now regarded as a potential prevailing problem (Hageskal et al., 2009; Doggett, 2000). Potentially pathogenic, allergenic and toxigenic fungal species like *Aspergillus fumigatus* were recovered from 49% of investigated hospital tap water samples in Oslo (Warris et al., 2002). Whereas fungal species are known to produce mycotoxins in foods and beverages (Kamili et al., 2012), concern is increasing over pathogenic fungi in drinking water. Paterson et al. (1997) detected aflatoxins produced by *A. flavus* from a cold-water storage tank and Mata et al. (2015), showed that *Cladosporium*, *Fusarium* and *Penicillium* frequently occurred in sampled bottled water.

2.2 DRINKING WATER QUALITY MANAGEMENT IN SOUTH AFRICA

Water quality management is defined as the maintenance of the fitness for sustainable use of water resources by striking an equilibrium between socio-economic development and environmental protection. In South Africa, the provision of safe and reliable water is the cornerstone of municipal services (DWS, 2015). The national government through the Department of Water and Sanitation has implemented regulations and policies to safeguard the provision of good quality and safe water to all (DWS, 2015). Some municipalities especially rural municipalities however, often struggle to meet these national set guidelines, leading the majority of rural populations to still rely on individual boreholes and other surface water sources (Rivett et al., 2012). Over the years, the South African water quality management has improved from a pollution control approach to the integrated water quality management which takes into account the receiving water users and aquatic ecosystem's water quality requirements (DWS, 2015). This new approach consists of measures and arrangements such as water resources protection, water use licensing and national pricing of waste discharge, that are provided for in the National Water Act, 1998 (Act No. 36 of 1998). In 2008, the Department of Water and Sanitation launched the Blue/Green Certification programme as an incentive for municipalities to improve the quality of water provided to their consumers (DWS 2015).

In the blue drop certification, requirements for monitoring water quality during distribution, and establishing appropriate remedial actions are imperative in the framework of process control and risk management (El-

Chakhtoura et al., 2015) and as part of the water safety planning process. In the blue-drop, the microbiological compliance for tap water is measured at 97.3% against the National Standard (SANS 241; 2015) and the City of Johannesburg always attains the blue drop status.

2.3 DRINKING WATER TREATMENT AND INACTIVATION OF FUNGI

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 destruction or inactivation of pathogenic microorganisms to prevent the spread of waterborne diseases (EPA 2013). According to Berry et al. (2006), the supply of clean drinking water is a major public health milestone. However, the increasingly demand of this scarce water caused by urbanization and industrialization must ultimately be matched by the increasing intensity of land-based treatment and recycle (Cosgrove & Loucks, 2015). Many studies have been done to guarantee the provision of good drinking water quality and face the challenge resulting from environmental pollution. Nevertheless, 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 (Sammon et al. 2011; Pereira et al. 2013). In general most water utilities treat water via coagulation, flocculation, sedimentation and filtration methods that are designed with the main objective of removing microbial pathogens using disinfectants such as chlorine as a significant component in the water treatment process (Adam et al., 1998).

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 however, remove most 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 eventually get 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) whereunto microorganisms can attach themselves. These suspended particles, depending on the quality of water, end up being transferred to the filtration process (Thupaki et al. 2013).

For effective disinfection of water, water turbidity must be reduced to less than one nephelometric turbidity units (1 NTU) (WRC 2002). Rapid sand filters are commonly used though they do not have enough retention time to remove all particle-adsorbed microorganisms from the water (O'Connor & O'Connorr 2001). Fungi can grow attached to a substrate and colonize filters in water treatment plants giving them an excellent 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).

The use of disinfection in water treatment as a public health measure has resulted in a major decline in people contracting water-related diseases from drinking water (EPA 2013). As a survival strategy, fungi and other bacteria often enter into a state of dormancy when conditions become hostile. However, when the conditions become favourable again, they get back to their vegetative state and start the process of spore germination (Luu et al. 2015). This phenomenon is popular with melanised thick-walled fungal species hence their resistance to water treatment and disinfection (Hageskal et al. 2012). When the turbidity of water is greater than 1 NTU, usually as a result of organic particles, microorganisms become protected from disinfection by being entrapped in the particles or adsorbed onto particle surfaces which then act as shields against the disinfectant (Al-berfkani et al. 2014; Spellman 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 most pathogenic organisms than chlorine, but their disadvantages are high costs and mostly the inability to have a residual concentration that persists long enough to prevent the re-growth of microorganisms in the distribution system (Freese & Nozaic 2004).

Ozone inactivates fungal species by causing irreversible cellular damage (Rojas-Valencia 2011). However, 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 cell surface (Hageskal et al. 2012). Additionally, fungi with pigmented spores such as *Aspergillus* and *Penicillium* have a 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 by exposing some species of fungi to ultraviolet light may seem futile as strongly melanised spores of *Aureobasidium pullulans* and *Aureobasidium melanogenum* have shown resistance to elongated radiation interactions (Castiglia & Kuhar 2015). The widely used chemical disinfectant is chlorine. However, despite its strong oxidant properties that make it reliable in removing or inactivating pathogenic microorganisms in water, 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).

2.4 FUNGI OCCURRENCE IN DRINKING WATER DISTRIBUTION SYSTEMS

Generally, local authorities and other assigned private and government entities are the custodians of bulk drinking water supplies where water is stored in reservoirs and towers for distribution to different users (Ayanshola et al. 2015; Earle et al. 2005). Water from treatment plants to consumers is transported for long distances via different engineered systems that include pipe networks of different materials, storage vessels, fittings and valves (Tinker et al. 2009). When water leaves the treatment plant the water quality may deteriorate in the distribution system (Douterelo et al. 2014). Some microorganisms, including fungi, have been linked to drinking water problems within distribution networks (Doull et al., 1982; Fish et al. 2015). Treated drinking water distribution systems have been identified to harbour both terrestrial and zoosporic fungi (Magwaza et al. 2017). Terrestrial fungal species often enter water bodies through dead animals, plants, soil and through litter that have been in contact with water (Nasser 2003). Fungi are members of a large group of eukaryotic organisms belonging to the kingdom Eumycota and can occur as unicellular yeast or filamentous and, multicellular moulds (Thliza et al. 2015). The group contains more than 70 000 species of fungi, of which less than 0.5% of them are of concern in human diseases and cause about 90% of all fungal infections (Hundalani & Pammi 2013). They are widely distributed in nature with some being known as aquatic fungi, adapted naturally to survive in water (Ali et al. 2017). Mitosporic fungi produce spores, which are released into their environment. Ecology of aquatic fungi has not attained the degree of importance as the ecology of soil fungi and the qualitative composition of the fungal population in water is now becoming fairly well known (Hundalani & Pammi 2013). Both forms of fungi, i.e., filamentous and yeasts have previously been isolated in treated drinking water (Siqueira et al. 2011; Sonigo et al. 2011). *Aspergillus, Cladosporium, Epicoccum, Penicillium, Trichoderma, Arthrinium phaeospermum, Aspergillus flavus, Cladosporium cladosporioides, Fusarium culmorum, Mucor hiemalis* and *Trichoderma harzianum* are the predominant fungal genera and species often found in treated and untreated water (Kinsey et al., 1998; Kamili et al. 2012).

When fungi enter the water distribution system, they can be harboured by places in the network like reservoirs that generates 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 for microbial growth placing these environments at potential high 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 like 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 get established into 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; Hurtado-McCormick et al. 2016; Douterelo et al. 2014). Fungi produce secondary metabolites such as organic acids which contribute to microbiological corrosion in water pipes (Grabińska-Łoniewska et al. 2007). The 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 maintained with chlorine residual or chloramines; microorganisms including fungi, viruses and protozoa, have been shown to thrive in pipe networks (Douterelo et al. 2014). 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).

Rand Water supplies treated drinking water in bulk to the City of Johannesburg (CoJ) who then distributes the water to consumers through Johannesburg Water. The water is transported over long distances to the receiving storage reservoirs for distribution and then sent via different engineered systems that include pipe networks of different materials, storage vessels, fittings and valves to the end users. The Mayor of Johannesburg in his State of the City Address for 2016 did recognise the ageing infrastructure of the water distribution system (Parks 2016). While water distribution systems are expected to act as a barrier for the treated water in order to protect the water against contamination (Speight, 2008), literature has shown that ageing infrastructure has been responsible in encouraging the growth of biofilms that harbour microorganisms, including fungi (Mulamattathil et al. 2014; Siqueira et al. 2011; Gonçalves et al. 2006). If biofilm has been formed in the water distribution system, fungi that produce mycotoxins can prevail and are protected against residual chlorine treatment (Oliveira et al. 2016). The City of Johannesburg conducts water quality monitoring in their treated water distribution system monthly and Rand Water also audits the water quality in the distribution network once a year and an independent third party is assigned for the audit. The audit results compare very well with the requirements set for the water quality standards (SANS 241: 2015). There is however a lack of information on fungal prevalence in drinking water distribution systems generally in South Africa and its implications to public health as it is not prescribed in routine monitoring.

Fungi in treated drinking water have been identified in many countries as shown in Table 2-1. Fungi in treated drinking water from the various investigations as in Table 2-1 shows that the most prevalent 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, that makes them to be thick-walled species with hydrophobic spores which give them an 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 that impair water quality and become a threat to humans and animals (Sonigo et al. 2011; Pitt et al. 2000a; Pereira et al. 2010).

Table 2-1: Conducted surveys for fungi in treated drinking water globally

Country and City	Year	Source of Samples	Fungal Isolates	Ref.
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., Emmosia sp., Epicoccum sp., Eurotium sp., Exophiala sp., Fusarium sp., Gliocladium sp., Mucor sp., Penicillium sp., Phialophora sp., Pyrenocheta 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., Aurebasidium sp., Basidiobolus sp., Botrytis sp., Chaetomium sp., Cladosporium sp., Cryptococcus sp., Curvularia sp., Doratomyces sp., Epicoccum sp., Eurotium sp., Fusarium sp., Geotrichum sp., Gliogladium sp., Helminthosporium sp., Microsporum sp., Monosporium sp., Mucor sp., Phoma sp., Pyrenocheta sp., Rhizopus sp., Scopulariopsis sp., Sepedonium sp.* and *Trichoderma sp.*	(Arvanitidou et al. 2002)
Poland	2000-002	Water distribution system	*Aspergillus sp., Cladosporium sp., Fusarium sp., Fusidium sp., Geotrichum sp., Gonatobotrys sp., Paeciliomyces 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)

Country and City	Year	Source of Samples	Fungal Isolates	Ref.
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)

2.5 OCCURRENCE OF FUNGI AND MYCOTOXINS IN DRINKING WATER

Fungal entrance into drinking water distribution systems can be attributed to contamination pathways that include; breakthrough during treatment, deficiencies in stored water facilities cross-connections, mains breaks and intrusions, and during maintenance of the mains (Sonigo et al. 2011). The fungi become established on the inner surfaces of pipes, can interact and react with sealing and coatings, and biofilms within distribution systems, or can be suspended in the water; the fungi and/or their metabolites can, in turn, reach the consumer. The presence of microscopic fungi in drinking water and distribution systems has been associated with its secondary contamination that results from damages to pipes caused by prolonged utilization and release of compounds that are substrates for growth and development of these fungi (Biedunkiewicz et al. 2014). Fungi can survive after filtration and are thus accounted for as a significant cause of post-treatment water pollution (Cabral & Fernandez 2002; Kirk et al. 2008).

Pathogenic microorganisms like bacteria, viruses and parasites are well known as water contaminants (Hageskal et al. 2009) but fungi, however, have not been considered for years when discussing water quality. Pathogenic fungi are believed to cause hostile infections that may contribute to the mortality rate (Arvanitidou et al., 1999; Sonigo et al. 2011). Some fungal species and their metabolites are known and /or allergens (Sonigo et al. 2011). The study by Memon (2012) reveals that the incidence of fungal species from the samples of drinking water in the distribution system of the city of Hyderabad (Pakistan) mostly contained more than one species. It has also been observed that *A. flavus, A. fumigatus* and *A. Niger* were the most frequently isolated species. *Penicillium* and *Aspergillus* species have been shown to have both high resistance to disinfection and ability to produce mycotoxins (Sonigo et al. 2011; Babič et al. 2016). These species of fungi and many others have been implicated in waterborne infections (Kanzler et al. 2007). Similar to human cells, infections from mycotoxigenic fungi are a challenge to diagnose and difficult to cure as they are eukaryotes (Yamaguchi et al. 2007). Mycotoxins have severe and chronic effects on humans and animals, as many of them are believed to be carcinogenic, cytotoxic, and mutagenic and have immunosuppressive complexes (Arroyo-Manzanares et al. 2015).

The occurrence of fungi in treated drinking water may cause adverse effects on human health as they have the potential of producing mycotoxins (Biedunkiewicz et al. 2014). The concentrations of these mycotoxins may increase during storage of water due increase in the population of the fungi species, and daily intake of such water containing mycotoxin could result in bioaccumulation in the body which could be hazardous to human health (Biedunkiewicz et al. 2014). An assessment for the presence of yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies in Brazil by Yamaguchi et al. (2007) showed the presence of both yeasts and filamentous fungi in both types of water samples. Despite of their wide occurrence in treated water, there has been little attention given to their existence and implications for human health (Yamaguchi et al. 2007). Worldwide, water quality legislations do not discuss about fungi or even set parameters for the control of fungi in treated water, their omission from the battery of microbial water quality parameters routinely done in water quality testing laboratories lead to the lack of information about the possible impact in human health (Babič et al. 2016). Monitoring fungi, their metabolites and potential risks is being considered in this study as part of monitoring studies for emerging pathogens in drinking water and domestic water distribution systems. On the other hand, establishing correlation between fungi and indicator bacteria is essential to understand if the presence and abundance of indicator bacteria can be used to accurately predict the presence of fungi in drinking water. To date literature presents conflicting results on the correlations that exist between fungi and bacteria in drinking water (Sonigo et al. 2011).

With clear evidence that the occurrence of fungi in drinking water distribution systems is a reality that cannot be ignored, this study endeavoured to address the need for monitoring the prevalence of fungi

in water distribution systems to determine the less studied mycological qualities of treated drinking water as part of a broader initiative to safeguard public health in South Africa. Mycotoxins are secondary metabolites that are produced by fungi and they are hazardous to humans (Zain 2011). Fungi that produce mycotoxins have also been reported in treated drinking water (Paterson & Lima 2015) and 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. Mycotoxins have serious and chronic effects on humans, as many mycotoxins are carcinogenic, mutagenic, cytotoxic, and have immunosuppressive complexes (Arroyo-Manzanares et al. 2015).

Although water in distribution networks contains some residual chlorine, microorganisms including fungi have shown to thrive in the networks (Grabińska-Łoniewska et al. 2007; Douterelo et al. 2014a). Fungi have been shown to survive still and reproduce in distribution systems biofilms, producing metabolites of concern in water (Kinsey et al. 2003), and some fungi have shown higher resistance to chlorine disinfection than *E. coli* (Pereira et al. 2013). Biofilms are colonization sites that play a key role in the dissemination of these species to public drinking water (Göttlich et al. 2002). Maintenance including frequent scouring of pipes is important as the sediments in the water supply systems are a potential site for the proliferation of fungal population. Fungal mycelia growing on this substrate is dense, and sporulation is more prolific than the growths of fungi observed in pipe wall samples (Sammon et al. 2011). Several fungal mycotoxins have been described. Table 2-2 gives some examples of mycotoxin toxicity and their food sources. Table 2-3 shows some toxic effects of some of the mycotoxins and producing genera.

Table 2-2: Relative toxicity and known sources of some of the mycotoxins in foods.

Mycotoxin	**Major foods**	**Species**	**Health effects**	**LD50 (mg/kg)**
Aflatoxins	Maize, groundnuts, figs, tree nuts (Aflatoxin M1 (secreted by cow after metabolism of aflatoxin B1), milk, milk products	*Aspergillus flavus* *Aspergillus* *parasiticus*	Hepatotoxic, carcinogenic	0.5 (dog) 9.0 (mouse)
Cyclopiazonic acid	Cheese, maize, groundnuts, Rodo millet	*Aspergillus flavus Penicillium aurantiogriseum*	Convulsions	36 (rat)
Deoxynivalenol	Cereals	*Fusarium graminearum*	Vomiting, food refusal	70 (mouse)
T-2 toxin	Cereals	*Fusarium sporotrichioides*	Alimentary toxic aleukia	4 (rat)
Ergotamine	Rye	*Claviceps purpurea*	Neurotoxin	-

Data sources from http://www.who.int/ceh/capacity/mycotoxins.pdf (accessed 0/12/2018)

Table 2-3: 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	(Bloom 2008; Šegvić et al. 2013; Campbell et al. 2004; (Mukherjee 2012); WHO 2011b; Shadanaika 2005)
Fumonisins	*Fusarium sp.*	Oesophageal cancer	(Bennett, 1987; Shadanaika 2005; Pitt 2000b)
Citrinin	*Aspergillus sp.*, *Penicillium sp.* and *Monascus sp.*	Nephrotoxic, teratogenic	(Shadanaika M. 2005)
Ochratoxin A	*Aspergillus sp.* and *Penicillium sp.*	Nephrotoxic, hepatotoxic, teratogenic and carcinogenic	(Shadanaika 2005; Dao et al. 2005; Abbott 2002)
Patulin	*Aspergillus sp., Penicillium sp., Paecilomyces sp.* and *Byssochlamys sp.*	Immune toxicity, cytotoxic; tremorgenic and pulmonary oedema	(Bloom 2008; Shadanaika 2005; Campbell et al. 2004; Puel et al. 2010)
Sterigmatocystin	*Aspergillus sp.*	Carcinogenic	(Shadanaika 2005; Coelho et al. 2010)
Cyclopiazonic acid	*Aspergillus sp.* and *Penicillium sp.*	Convulsions	(WHO 2011b; Shadanaika 2005)
Trichothecenes	*Fusarium sp. Trichoderma sp.*	Toxic aleukia	(Shadanaika M. 2005)
Deoxynivalenol	*Fusarium sp.*	Anorexia, nausea, vomiting, headache, abdominal pain, diarrhoea, chills, giddiness and convulsions	(WHO 2011b; Pitt 2000a,b)
T-2 toxin	*Fusarium sp.*	Alimentary toxic aleukia	(WHO 2011a)
Zearalenone	*Fusarium sp.*	Carcinogenic	(Kuhn & Ghannoum 2003)

Mycotoxin	**Genera**	**Health effects**	**Reference**
Ergot Alkaloids	*Cladosporium sp.*	Gangrenous and convulsive forms of ergotism in humans	(Abbott 2002; Hussein & Brasel 2001)
Penicillic acid	*Penicillium sp.*	Carcinogenic	(Abbott 2002)
Tenuazonic acid, alternariol, altenuene, and altertoxin-1	*Alternaria sp.*	Inhalation allergy problems and mycotoxicoses	(Volk 2013; Shadanaika 2005; Buse et al. 2013)
Ergotamine	*Claviceps purpurea*	Neurotoxins (poisonous to nerves)	(WHO 2011a)
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	(Partida-Martinez et al. 2007; Abbott 2002)

2.6 PUBLIC HEALTH IMPLICATIONS OF FUNGI AND MYCOTOXINS IN TREATED DRINKING WATER

The biggest fear for public health regards the consumption of treated drinking water contaminated with pathogenic microorganisms (Hageskal et al. 2009). The presence of fungi in treated drinking water and its health impacts became a major issue of concern after cases of fungal contaminated water were reported in Finland and Sweden during the 1980s and 1990s (OECD & WHO 2003; Boe-Hansen et al. 2003). Several 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). This has raised human health concerns as fungi have been proven to resist water treatment processes, especially the melanised fungal species (Awopetu et al. 2013; Obi et al. 2008). Fungal infections were quite low from the late 1950s and early 1960s even in immunocompromised patients, yet over the past two decades fungal infections have drastically increased as they are easily diagnosed and individuals whose immune response is inadequate have increased (Khan et al. 2010). Most of the fungi that were identified in Table 2-1 are dematiaceous and are characterized by their pale brown to dark melanin-like-pigment in the cell walls.

Dematiaceous fungi are responsible for causing a number of cutaneous and subcutaneous infections in immunocompetent people and invasive or disseminated infections in both immunocompetent and immunocompromised patients (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 especially to those people with these underlying conditions. 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 patients having an impaired immune system as their immune effector cells become compromised allowing fungi to colonize and attack the human tissues leading to more complications (Oliveira et al. 2013).

Fungi have been implicated in a number of diseases including allergies, respiratory illness, cutaneous infection and life-threatening meningitis (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), *Cryptococcus* and *Candida* typically cause meningitis (Black & Baden 2007) with the *Candida* species responsible for cutaneous infections (Volk 2013). Taste and odor problems in water are caused by *Aspergillus sp., Acremonium sp., Phialophora* sp. and *Penicillium* sp. (Sonigo et al. 2011; Hageskal et al. 2006). 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). 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 depends on the type of mycotoxin, the concentration and length of exposure; as well as age, health, and sex of the exposed individual (Bennett et al. 2003). Mycotoxins found in water may be extremely diluted and may not be of major concern, their concentrations may increase resulting in hazardous levels to human health particularly when water is stored in reservoirs for longer periods (Siqueira 2011). Table 2-3 gives some of the mycotoxin producing fungi and the health effects. The absence of toxigenic fungi in treated drinking water may not give an assurance that the water is free of mycotoxins, as mycotoxins may persevere long after the fungi had died (Pitt et al. 2000a). 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 also that the number of cases of infection and antifungal resistance are also getting high, and the control of antifungal disease does not indicate any possibilities of being achieved soon (Araj et al. 2015; Meirelles et al. 2017; Pellon et al. 2018).

2.7 FUNGAL-BACTERIAL INTERACTIONS AND CORRELATIONS IN WATER DISTRIBUTION SYSTEMS

Bacteria and fungi exist and interact in many environments as they often share common substrates. The interaction of fungi with bacteria ranges from disorderly polymicrobial assemblies to closely related symbiotic associations of fungal hyphae and bacterial cells (Frey-Klett et al. 2011). Bacteria are the ones 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 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 *P. 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). It is vital 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 the drinking water standards. 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 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). Since a single indicator or even ranges of indicators are unlikely to be appropriate for every occasion, it can be beneficial to modify indicator organisms to specific conditions when developing national standards. Some researchers (Ashbolt et al. 2001) argue 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 indicators. However, a point worth noting is that fungi often colonise pre-established bacterial biofilms and as such the correlations deductible in biofilms is not necessarily the same as for water samples. Some of the reported correlations reports between fungi and bacteria are shown Table 2-4.

Table 2-4: Some reported correlations between fungi and bacteria

Positive correlations	Negative correlations	No correlation
A positive correlation was found between yeasts and total heterotrophic bacteria in tap water (Brazil) (Yamaguchi et al. 2007)	A negative correlation has been observed between fungi and bacteria in samples of high bacterial biomass (Germany) (Göttlich et al. 2003)	No correlation was found between fungal and bacterial biomass in unchlorinated 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 *E. coli* and *Enterococcus* (untreated water) (Pereira et al. 2009).	-	-

2.8 SUMMARY

Water treatment is critical in controlling pathogenic microorganisms from reaching consumers. The literature review as presented here have shown that fungi are microorganisms of concern in treated drinking water and there need for more studies to provide for information upon which monitoring decisions can be made. In South Africa there are standards 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 (SANS 241:2015). Fungi have been left out from the battery of drinking water quality compliance monitoring parameters yet scientific literature has proven the presence of fungi and its mycotoxins in treated drinking water. Melanised and slimy conidia have been found in treated water distribution systems with the prevalence of *Cladosporium, Phoma, Alternaria, Aspergillus, Penicillium, Exphiala Fusarium, Acremonium, Exophiala* and *Phialophora* that has a general capacity to resist disinfection regimes (Göttlich et al. 2002; Babic et al., 2018). The potential health impact of waterborne fungi is still not clear whilst precautions are often needed in hospitals for high-risk patients. Monitoring and keeping the number of fungi under surveillance after water treatment and in distribution systems is fundamental in guarding against potential harm to human health, and also to improve the aesthetic quality of water in relationship to taste and odour. Although examination of fungi can be difficult as cautious and experienced personnel is required, nevertheless this cannot be disregarded anymore as fungi influence water quality in many ways.

CHAPTER 3: EXPERIMENTAL DESIGN AND METHODS

3.1 STUDY SITES AND IDENTIFICATION OF SAMPLING SITES

The city of Johannesburg is located at 26.2044° S, 28.0456° E in Gauteng Province, South Africa covering an area of 1,645 km² and with a population of about 4.1 million. The study was conducted in Roodepoort, Johannesburg West. The population of Roodepoort is 11.6% of the city's population (City of Johannesburg 2013). The treated drinking water distributed by Johannesburg water is received from Rand Water (Zuikerbosch treatment plant in Vereeniging), sourced from Vaal Dam and Zuurbekom. The bulk treated water is distributed through pipes to distribution reservoirs/towers in Johannesburg. Water samples were collected from selected sites in the Johannesburg West mainly in Roodepoort (Figure 3-1). Sampling sites included communal taps, clinic taps, school taps, household taps, filling points, garages and quality monitoring points (reservoir inlets/outlets and towers outlets) along the treated drinking water distribution network. Permission and assistance with site identification were obtained from Johannesburg Water. Thirty (30) sampling sites were chosen based on security, ease of access, and the cooperation of landowners. Twenty-five sampling sites were from the Johannesburg Water monitoring sites. Table 3-1 shows a detailed description of the sampling sites.

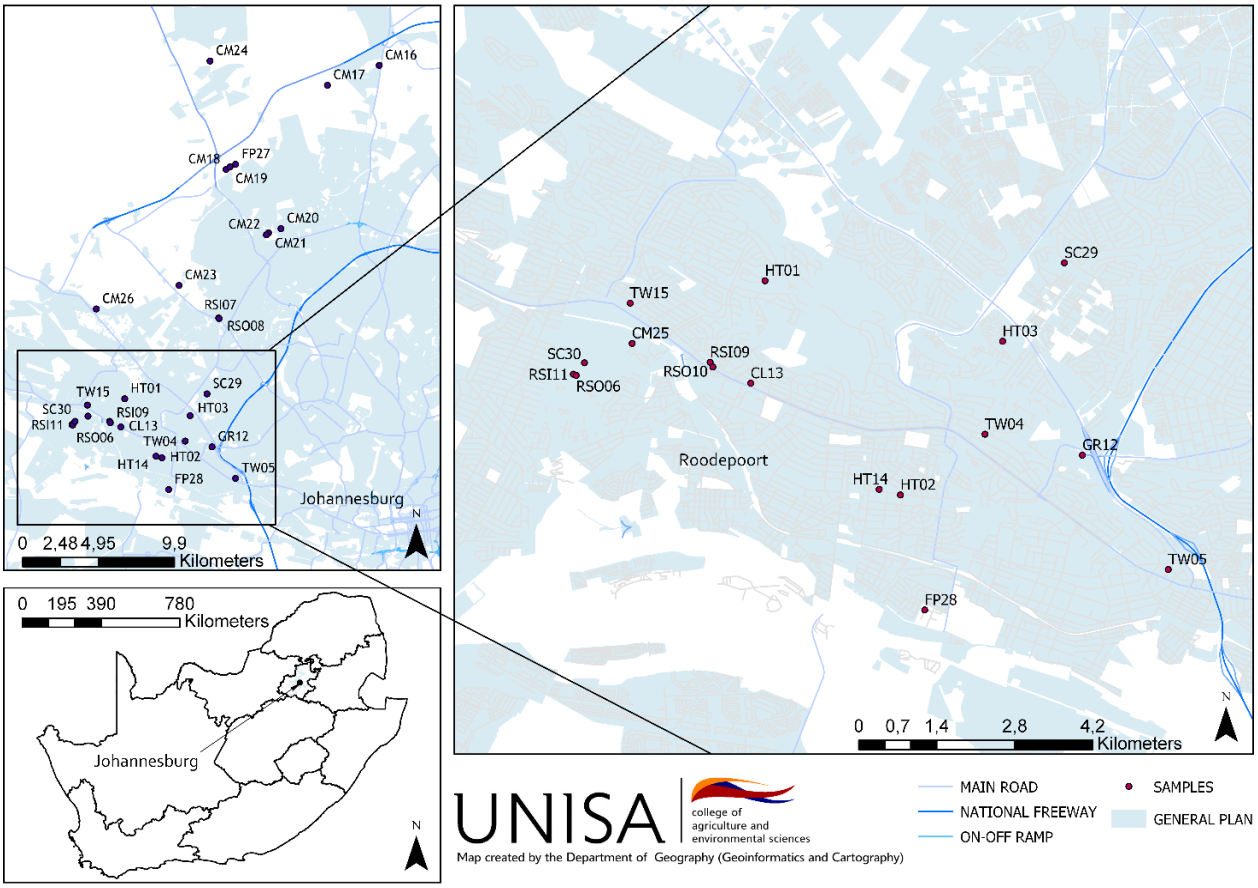

Figure 3-1: Location of selected study sites in Roodepoort, Johannesburg West

Table 3-1: List of sampling sites in Roodepoort areas, Johannesburg West

Code	Source	Description of the sample site
FP28	Filling point	Water was collected from a yellow threaded pipe that hanging on a steel pipe. The surrounding area was covered by grass next to a tarred road.
GR12	Garage tap	The tap was behind the garage above the concrete ground next to a bin.
TW04	Tower	The tap is inside a locked metal cage, with less external interferences.
TW05	Tower	The tap is inside a locked metal cage, with less external interferences.
RSI07	Reservoir Inlet	The tap is inside a locked metal cage, with less external interferences.
RSO08	Reservoir Outlet	The tap is inside a locked metal cage, with less external interferences.
CM23	Communal tap	Water was collected from a JoJo storage tank (10,000 Litres), approximately one meter above the ground level. There is low grass around the tank.
HT03	Household tap	The water tap is mounted on a concrete wall next to a bin and low grass.
CM16	Communal tap	Tap is on a concrete wash basin almost one meter above ground. Close to the toilets near the road and houses.
CM17	Communal tap	Several taps connected in a pipeline about a meter above concrete ground. Some of the taps were leaking most of the time and there were pools of dirty water as people are always washing and discharging water close to the tap.
CM20	Communal tap	Several taps connected on a pipeline about a meter above concrete ground, with pools of water and moderate grass around it.
CM21	Communal tap	Several taps connected on a pipeline about a meter above concrete ground. Next to a dump site close to the houses.
CM22	Communal tap	Several taps connected on a pipeline about a meter above concrete ground, with pools of water due to people washing close to the tap. There are municipality toilets at about five meters away.
CM24	Communal tap	Several taps connected on a pipeline about a meter above concrete ground. Dump site on the opposite side of the road.
FP27	Filling point	Water was collected from a yellow threaded pipe that laid on the grassy ground. There were pools of water around.
CM18	Communal tap	The tap is next to a gravel road on dry ground.
CM19	Communal tap	Several taps connected on a pipeline about a meter above concrete ground. There was a constant stream of wastewater flowing on the road next to the taps.
SC30	School tap	Tap is mounted on the wall, and the ground is concrete and dry.
CM26	Communal tap	Several taps connected on a pipeline about a meter above concrete ground.
RSI09	Reservoir Inlet	The tap is inside a locked metal cage, with less external interferences.
RSO10	Reservoir Outlet	The tap is inside a locked metal cage, with less external interferences.
CL13	Clinic tap	Tap is mounted on the wall, and the ground is concreted and dry. Tap is under a tree.
TW15	Tower	The tap is inside a locked metal cage, with less external interferences.
HT14	Household tap	Retirement village, water was collected from an outside tap about 0.5 meters high on dry concrete ground.
RSI11	Reservoir inlet	The tap is inside a locked metal cage with less external interferences.

Code	Source	Description of the sample site
RSO06	Reservoir outlet	The tap is inside a locked metal cage with less external interferences.
CM25	Communal tap	Water is collected from a JoJo storage tank (10,000 Litres), approximately one meter above the ground level. There is low grass around the tank.
SC029	School tap	Tap is mounted on the wall, and the ground is concreted and dry.
HT01	Household tap	Tap is mounted on the wall, and the ground is concreted and dry. Next to a bin.
HTO2	Household tap	Tap about half a meter above grassy ground.

3.2 SAMPLE COLLECTION

Water for all microbial analysis was collected using 500mL sterile bottles containing 1 ml of 1.8 % m/v sodium thiosulphate solution. Autoclaved 500mL glass bottles were used to collect water for mycotoxin analysis. Five litre sterile plastic bottles were used to collect water for metagenomics analysis. Before sample collections, the tap was flushed for 3 minutes and bottles filled up and tightly capped following the Johannesburg Water sampling protocol. All the samples were kept in cooler boxes containing ice bricks and transported to the Environmental Sciences laboratory at UNISA (Florida Campus, Johannesburg) for analysis within 24 hours, except for the mycotoxin analysis.

3.3 SAMPLE ANALYSIS

The collected water samples were analysed for pH, total and free chlorine, the presence of fungi, total and faecal coliform. Microbiological analysis was done within 24 hours of sampling. Figure 3-2 shows the scheme of work for the analysis of samples.

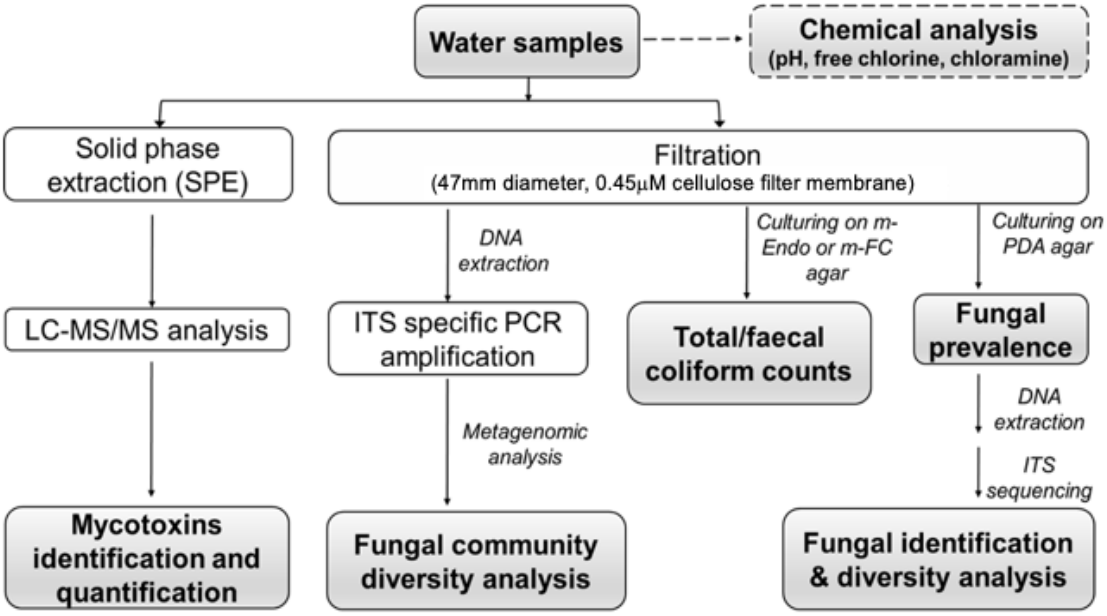

Figure 3-2: Scheme of work for the analysis of the samples.

3.3.1 pH, total and free chlorine analysis

Free chlorine, total chlorine and pH were measured and recorded on site using Lovibond apparatus with chlorine disc No.3/40A, chlorine disc3/40S and pH disc2/1J with cuvettes and colorimeter. DPD 1 and 3 tablets for chlorine and total chlorine were used respectively, and phenol red tablets were used for pH in a pH colorimeter.

3.3.2 Total and Faecal coliform

Total and faecal coliforms were analysed for according to standard procedures (Method 9132; EPA 2002) using m-Endo Agar (Sigma, South Africa) and m-FC agar (Sigma, South Africa) respectively. The membrane filters were placed grid side up onto petri dishes containing the m-Endo agar for total coliform enumeration and m-FC agar for faecal coliform enumeration and incubated at 37°C for 18-24 hours and 44.5°C for 48 hours respectively. All colonies exhibiting a greenish-gold metallic sheen on m-Endo were enumerated as CFU of total coliforms per 100 ml while blue colonies that developed on m-FC agar were enumerated as CFU of faecal coliforms per 100 ml.

3.3.3 Determining the presence of fungi

To determine the presence of fungi, 100 ml of water was filtered through 47 mm diameter; 0.45µm pore size Millipore HA-type cellulose filter membranes (Merck-Millipore, RSA) in duplicate and placed on potato dextrose agar (PDA). The potato dextrose agar (PDA) plates with the filter membranes were incubated at 27°C and observed after 48 hours for the growth of fungal colonies with continuous monitoring for 4-7 days. The fungal cultures were purified by cutting approximately 1cm^2 fungal plugs and transferring them onto freshly prepared PDA plates until axenic cultures were obtained.

3.3.4 Molecular analysis and identification of fungal isolates

DNA was extracted from axenic fungal cultures using ZR Quick-DNA™ Fungal/Bacterial DNA MiniPrep™ Kit (Inqaba Biotech, RSA) following the manufacturer's protocol. Fungal universal primers sets ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used to amplify the extracted DNA using PCR. For PCR, 25 µl reaction volumes comprising of 12.5 µl 2X PCR Master Mix (Inqaba Biotech, Pretoria, RSA), 0.5 µl each of the forward and reverse primers, 6 µl DNA template and 5.5 µl nuclease free water were used. The cycling program was set as follows: initial denaturation step at 95°C for 5 minutes, followed by 32 cycles melting at 95°C for 30 seconds, annealing at 55°C for 30s, and elongation at 72°C for 1 minute and a final elongation step of: 72°C for 10 minutes. The PCR amplicons were purified and sent to Inqaba Biotech (Pretoria, South Africa) for sequence analysis after which a FinchTV1.4.0 software (Geospiza, PerkinElmer, Inc.) was used to manually correct the chromatograms. The resultant sequences were subjected to BLAST analysis to compare the identity of the isolates. Finally, all the sequences obtained in this study were submitted to GenBank to obtain the accession numbers.

3.3.5 Metagenomics analysis

Genomic DNA was sent to Inqaba Biotechnical Industries, a commercial NGS service provider, for sequencing. The genomic DNA samples were PCR amplified using a universal primer pair (ITS1f and ITS4). Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon. Following quantification, the samples were individually indexed, and another purification step was performed. Amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. 20Mb of data (2x300bp long paired end reads) were produced for each sample.

The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

3.3.6 Analysis of water samples for mycotoxins

3.3.6.1 Standard preparation

LC-MS grade standards purchased from Sigma Aldrich, South Africa for each mycotoxin were used in the quantification and identification of mycotoxins. The selected mycotoxins for analysis are listed in Table 3-2. These were selected based on the diversity of fungi detected in the water samples from the regular sampling conducted as well as informed by the literature on the common mycotoxins in water that are produced by the prevalent fungi. Ochratoxin, though an important mycotoxin produced by some of the fungi that prevail in the drinking water, was not analysed due to unavailability of the necessary standards at the time of analysis. Individual stock standard solutions were prepared following the method by Mata et al. (2015), where each standard was dissolved in acetonitrile. Standards were later mixed to a solution consisting of Sterigmatocystin 600 µl, B-Trichothecene 300 µl, Gliotoxin 300 µl, Patulin 300 µl, Fumonisin (FB_1 and FB_2), Tenuazonic acid 30 µl, Aflatoxin B_1 30 µl, Aflatoxin M_1, Aflatoxin G_1 30 µl and Aflatoxin G_2 30 µl. The solution was then topped up with acetonitrile to a volume of 3000 µl. To evaluate the linearity of the method, mixed standard solutions of all mycotoxins at a concentration range from 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 250, 500 and 1000 ppb were prepared using acetonitrile/water (1:3, v/v). All the standard solutions were stored at -20°C in amber glass vials after preparation.

Table 3-2: List of analysed mycotoxins and producing fungi

Mycotoxin	Fungi
Aflatoxins (B_1, G_1, G_2, M_1)	*Aspergillus*
Citrinin	*Penicillium, Aspergillus* and *Acremonium*
B-Trichothecene (Deoxynivalenol (DON), Nivalenol (NIV), 3-Acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON))	*Fusarium, Trichoderma*
Fumonisin (FB1 and FB2)	*Fusarium*
Patulin	*Penicillium, Aspergillus* and *Paecilomyces*
Sterigmatocystin	*Aspergillus*
Tenuazonic acid	*Phoma and Alternaria*
Gliotoxin	*Trichoderma*

3.3.6.2 Sample preparation

The water samples were prepared following the procedure by (Mata et al. 2015) using solid phase extraction (SPE) on Oasis HLB 6cc (200mg) extraction cartridges (Waters – Microsep (PTY) LTD, South Africa). These cartridges were chosen because of their wide application range and the high capacity to retain a large number of both hydrophilic and hydrophobic compounds (Mata et al. 2015). Water samples (500 mL) used in the analysis were selected from sites where potentially mycotoxigenic fungi were identified the most and one site with limited mycotoxigenic fungi. The Dionex Auto Trace SPE instrument (ThermoFisher Scientific, USA) was used for the sample's preparation. The cartridges were conditioned with 5 mL methanol and 5 mL UHP grade water into aqueous waste. The water samples were then passed through the cartridges at a flow rate of 10 mL per min. The retained compounds were eluted with 5 mL of

methanol, dried up under a nitrogen gas stream and reconstituted with 500 µL of acetonitrile for chromatographic analysis.

3.3.6.3 LC-MS and data analysis

The targeted mycotoxins were analysed using an LC-Quadrupole Orbitrap Mass Spectrometry as described by Lehner et al. (2011) with slight modifications. Briefly, the chromatographic separation of individual mycotoxin from samples was conducted using UHPLC system (Accela, Thermo Fisher Scientific, San Jose, CA, USA) equipped with a reversed-phase Gemini C_{18} analytical column, 150 x 2.0 mm i.d., 5 µm particle size, set at a temperature of 20°C and connected with a C_{18} 4 x 2 mm i.d. security cartridge (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% (v/v) formic acid and 5 mM ammonium acetate in water (A) and 0.1% (v/v) formic acid with methanol (B). The analysis was done using a gradient separation starting with 100% of eluent A. Eluent B was increased to 20% in 2 min and increased further to 95% in 11 min. This composition was held for 3 min and returned to initial conditions in 0.1 min, followed by a re-equilibration time of 5 min (total running time = 18 min) before the next injection. The flow rate was 0.6 mL/min and the column temperature were set at 30°C. The HPLC system was coupled to a Thermo Fisher Scientific Orbitrap mass spectrometer (Q Exactive Plus) with a heated electrospray interface (HESI). Standard MS source conditions compatible with the flow rate were used (capillary temperature, 290°C; sheath flow, 55; spray voltage, 3500 V; auxiliary temperature, 450°C). Analysis was performed in full MS SIM in positive mode over a scan range from *m/z* 53.4 to 800 with a mass accuracy of <5 ppm. The mass resolution was set to 140 000, AGC (automatic gain control) target was set at 3.0×10^6 with a maximum injection time (IT) of 100 ms. Generated data were processed using Trace Finder EFS Software Version 3.2 (Thermo Scientific). Parameters set in the software used to identify and quantify target analytes were the presence of the protonated molecule at accurate mass and retention time. Linear regression analysis and linearity were qualified by the linear correlation coefficient, r^2.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 INTRODUCTION

From public health perspective, treated drinking water supply system should be safe and always free from any pathogenic microorganism (WHO 2004). Water may be polluted at its sources by excreta or sewage, and the presence of faecal coliforms indicates potential public health risk of contamination by pathogenic microorganisms (WHO 2017; DWAF, 1996). Though the distribution system is a pressurised and closed system to protect supplied water from potential contamination, water pipes often, burst and allow contamination of drinking water. If total coliforms are identified in treated drinking water distribution systems and stored water supplies, it often indicates that there was regrowth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants (WHO 2017; DWAF, 1996).

4.2 TOTAL AND FAECAL COLIFORM COUNTS

Looking at seasonal variation, temperature was found to have no significant effect on the coliform concentrations (Plummer et al. 2014). There were no detectable seasonal trends for total coliform that were apparent in this study as shown by the occurrences of total coliform throughout the different seasons. This may be partly due to that treated water in the distribution system is transported in subsurface, pressurized and closed water distribution pipelines that do not allow atmospheric temperatures to have much effect on the water as attested by Plummer et al. (2014). From this study only RSI11 had no total coliforms detected at any point during the study period. Figure 4-1 shows that coliforms were present at all other site and the counts were variable throughout the sampling.

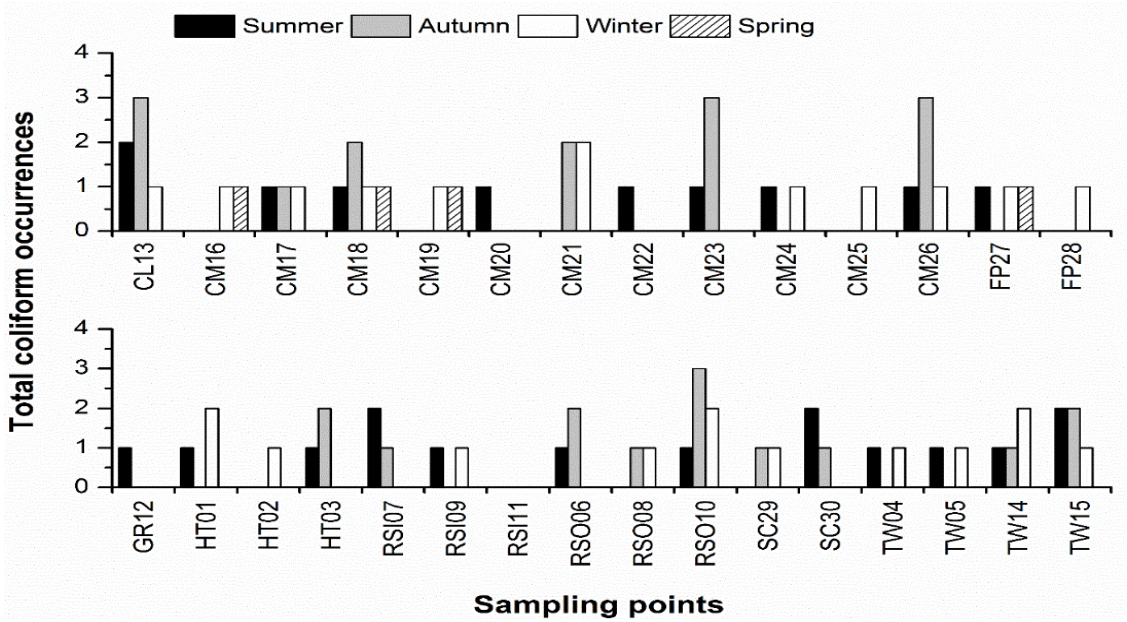

Figure 4-1: Total coliform occurrences (number of months with coliforms) per site per season over the sampling period (November 2016 to October 2017).

Overall, the recorded total coliform counts were < 10 cfu/100ml at all the sites, and were within the target for treated drinking water quality range set in SANS 241 (2015) of ≤10 per 100m/L. This indicated that according to the set water quality standards, there is negligible risk of microbial contamination effects to public health from the treated drinking water (DWAF, 1996). In contrast to total coliforms that were reported at almost all sampling sites, faecal coliforms were only detected at four sites in three months within the study period. The sampling sites CM 25 and CM 26 had the highest faecal coliform counts of 8 cfu/100ml in March and the sites are communal taps in informal sites where hygienic conditions are often low and high populations exists. The target set for faecal coliforms for domestic water quality in SANS 241 (2015) is 0 cfu/100 ml. The range 0-10 cfu/100 ml is considered a slight risk of microbial contamination. In this study, faecal coliform presence was sporadic in occurrence at each site at < 10 cfu/100ml. The results therefore indicate that risk of faecal coliform contamination detected was negligible, within the slight risk range, and in compliance with water quality standards. Although the source of faecal contamination of the treated drinking water in these sites could not be conclusively determined, ingression during pipe bursts and maintenance coupled with associated lower residual chlorine concentration in the distribution system might have contributed to the occurrence of faecal coliforms. To this effect an analysis of the chlorine levels occurring in the drinking water system were determined.

4.3 FREE CHLORINE, CHLORAMINE AND PH

Chlorination is the most widely used method for disinfecting water supplies worldwide. However, maintenance of residuals chlorine levels throughout the water distribution systems on a continuous basis is generally challenging due to degradation of chlorine (Housewright et al., 1982). During the chlorination process, aqueous chlorine reacts with ammonia and forms chloramines, either mono-, di- and trichloramines, but only monochloramine has useful disinfection effect. The set national standards for minimum monochloramine concentration in treated drinking water is ≤ 0.3 mg/L at any point of delivery and a daily intake of <3 mg/day is recommended (SANS 241: 2015). Also according to the (SANS 241: 2015), free chlorine level of ≤ 5 mg/L is sufficient residual to maintain the quality of treated water through the distribution network. In this study, the results for the average free chlorine and residual monochloramine concentration detected during summer (Nov-Jan), autumn (Feb-Apr), winter (May-Jul), and spring (Aug-Oct), were between 0.09-0.26 (Figure 4-2) and 0.50-1.52 mg/ml (Figure 4-3) respectively. These results show that free chlorine and chloramine levels found in the treated water distribution network of Johannesburg West, Roodepoort areas were within the acceptable set standard range (SANS 241: 2015). Average higher free chlorine concentration (>0.2 mg/ml) were observed for sampling sites RSI09, RSI07, RS010, CM26 and FP28, while lower concentration (~0.10 mg/ml) was reported for sites CL13 and SC29. Despite the residual monochloramine and free chlorine concentration levels being within the acceptable range, fungi were still prevalent in the analysed samples and to a less extent, coliforms. Literature has shown that some fungal species like *Aspergillus* and *Penicillium* resist chlorine disinfection (Sisti et al., 2012; Pereira et al., 2017; Ali et al., 2017). A study conducted by Pereira et al. (2017) on inactivation of fungi in treated surface water by chloramination, showed that at concentrations below 4 mg/L residual concentration, *Penicillium* and *Aspergillus* species were more resistant to chloramines inactivation than the *Cladosporium* and *Phoma* species.

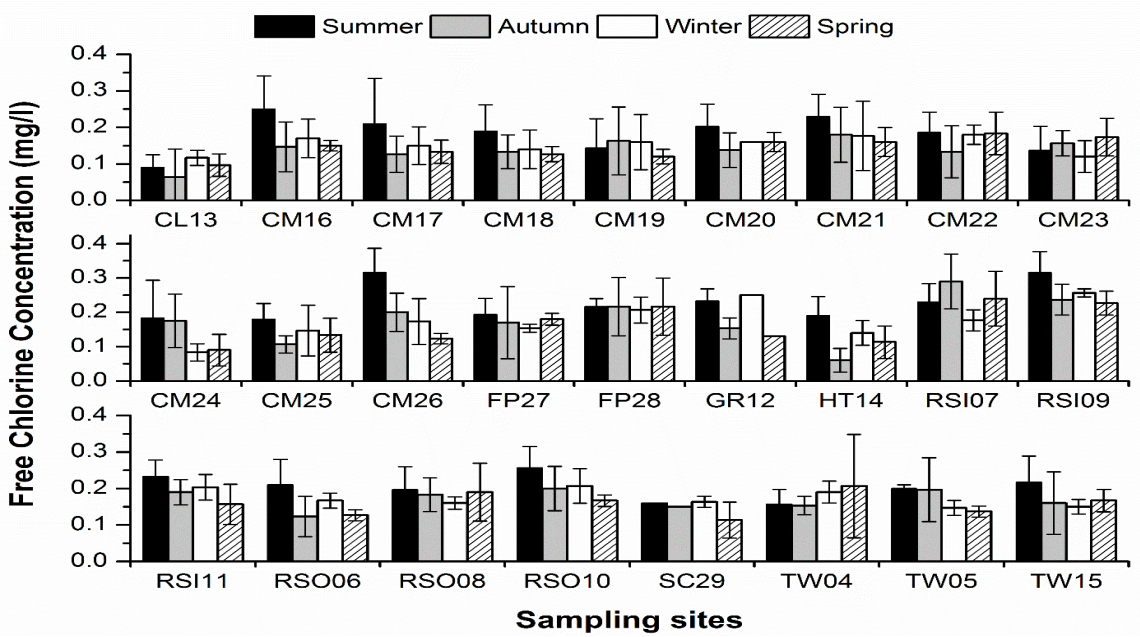

Figure 4-2: Average seasonal free chlorine residual concentration/site from November 2016 to October 2017. Error bars represent standard deviation of the averages for the sampling seasons.

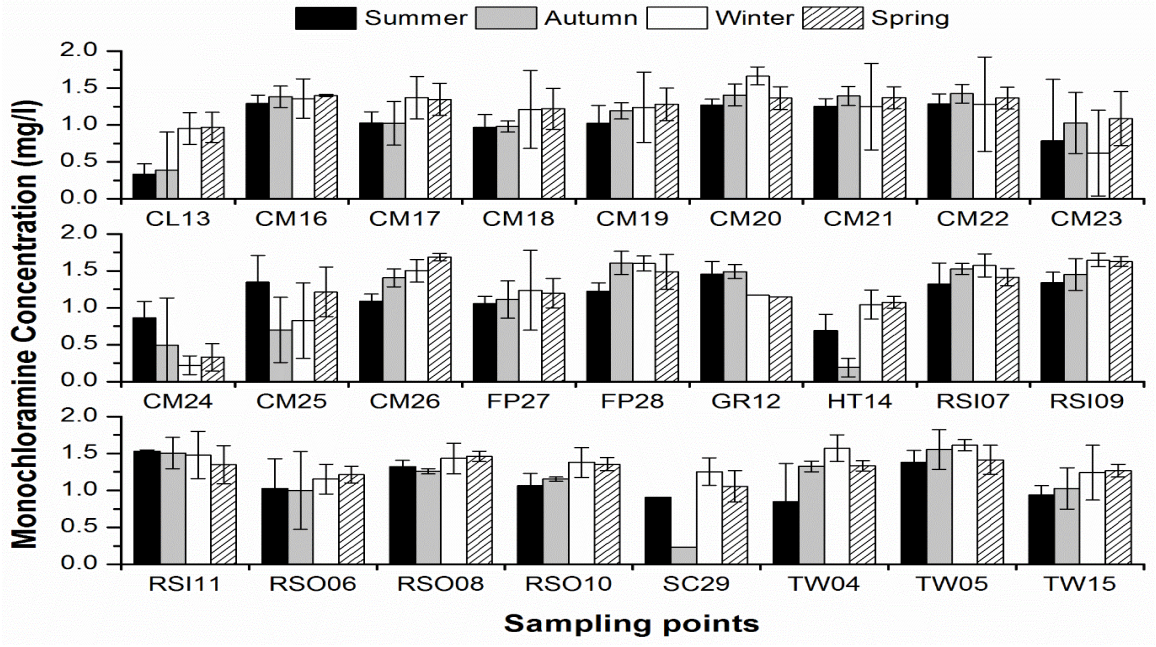

Figure 4-3: Average seasonal monochloramine concentration per site in drinking treated water for the period November 2016 to October 2017. Error bars represent the standard deviation of the averages for the sampling period seasons.

The pH of the treated drinking water samples was also assessed, and the average seasonal results for the different sampling sites are presented in Figure 4-4. All sampling sites had pH values between 7.9 and 8.3. These values are within the set standards of acceptable pH range of 5-9.7 (SANS 241; 2015). The pH of water is known to have an important role on fungal presence, their growth and bioremediation processes (Babič et al., 2017). A positive correlation was observed between the growth of aquatic hyphomycetes and pH between 5 and 7 (Babič et al., 2017). As fungi are known to often prefer acidic pH for their growth, the slight alkaline pH reported for water samples in this study indicates that treated water conditions maybe inhibitory to fungal growth and helps in reducing their prevalence in the distribution system.

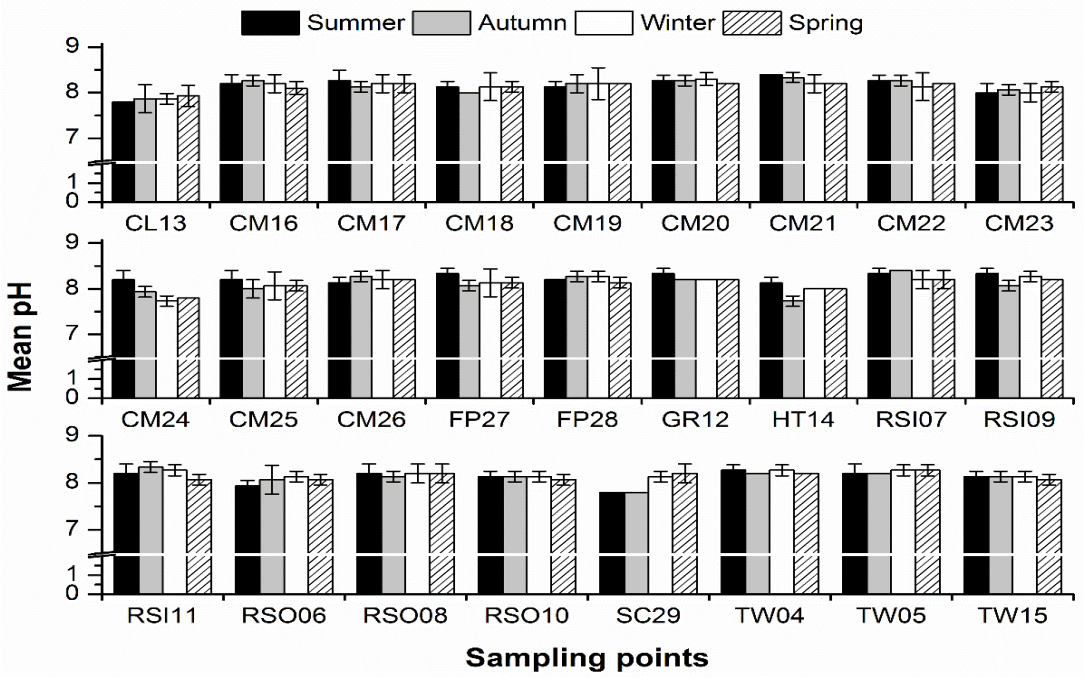

Figure 4-4: pH determined per season per sampling sites from November 2016 to October 2017. Error bars represent standard deviation of the seasonal averages for the sampling period.

4.4 OCCURRENCE OF FUNGI IN RELATIONSHIP TO TOTAL COLIFORM

It is very important to understand the interactions between bacteria and fungi in water in order to validate if the bacterial presence in water can be used as an indicator of fungal content (Gonçalves et al., 2006). Coliform indicator bacteria are widely accepted as standard indicator parameter for microbial quality of water (SANS 241; 2015). Coliform organisms, however have been shown to be an unreliable indicator for most other organisms including *Giardia* or *E. histolytica* in drinking water (Goncalves et al., 2006). Enteroviruses and protozoa are more resistant to disinfection than *E. coli*, such that even under conditions where *E. coli* has been killed or inactivated such as by disinfectants, a zero total coliform count does not necessarily indicate that resistant microorganisms are absent (WHO, 2017). This may be the case with some of the fungi that are capable of resisting disinfection (Frey-Klett et al., 2011). Bacteria and fungi have been shown to exist and interact in many environments as they often share a common substrate (Frey-Klett et al., 2011). Both fungi and total coliforms are both likely to be introduced to drinking water systems during maintenance procedures or enter during low and negative pressure events. Figure 4-5 shows the sites where there was the detection of both the fungi and total coliforms. Communal taps and reservoir samples had the highest occurrence of both fungi and coliforms, with fungi occurring in higher numbers and months, than coliforms throughout the sampling period.

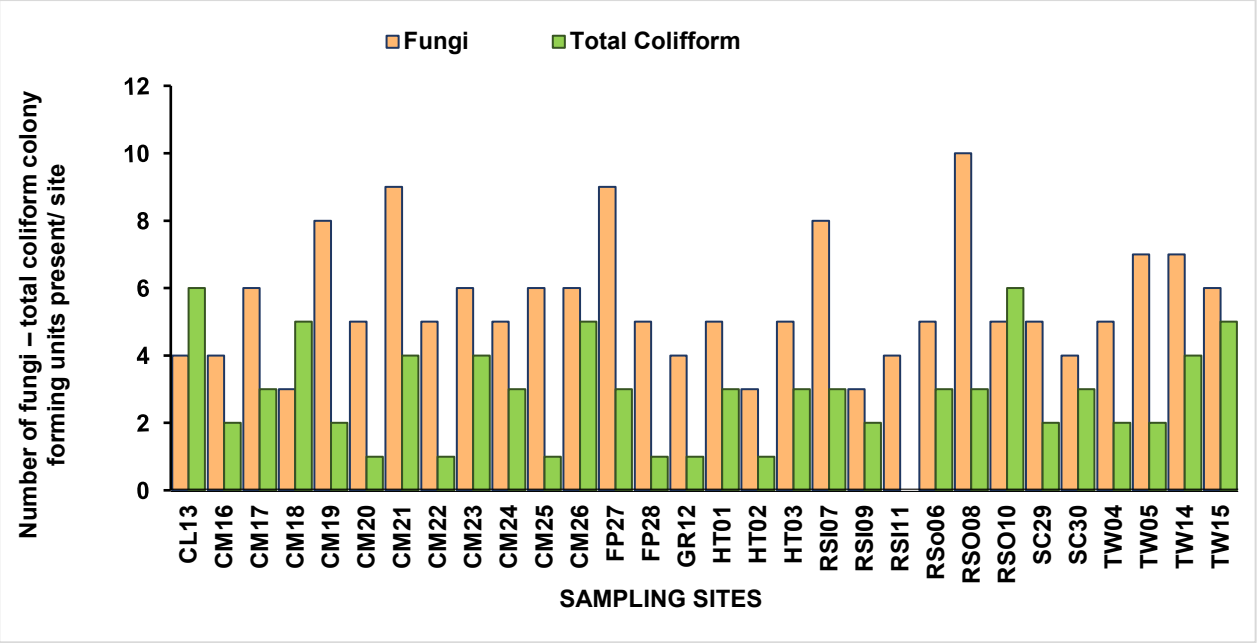

Figure 4-5: Total number of occurrences of both total coliforms and fungi at the study sampling sites during the sampling period (October 2016-November 2017).

To establish if there was a relationship between the total coliforms, fungal incidences and residual chlorine concentration of treated drinking water samples, correlation analysis of the results was undertaken using PAST (v3.0). Pearson's correlation coefficient was used to determine whether statistically significant correlation exists between cultured microbes and measured residual chlorine. Pearson's correlation coefficient and obtained values are shown in Table 4-1. There was a weak correlation between fungi and residual chlorine (r = 0.1937), which indicates that fungal species are highly resistant than bacteria (r= 0.8941) to free-chlorine treatment and can survive in treated water and it have potential to colonize the distribution systems. According to Pereira et al. (2013), chlorination effectiveness also depends on the chlorine concentration, matrix parameters such as organic matter, suspended solids and exposure conditions such as pH and temperature.

Table 4-1: Pearson correlation coefficient (at $p < 0.05$) of residual chlorine concentration, fungal prevalence and total coliform counts in treated drinking water samples[a]

	Parameter	1	2	3
1.	Fungi	-		
2.	Total coliform count	0.4266	-	
3.	Residual chlorine	-0.1937	-0.8941	-

Fungi prevalence in treated drinking water system [a] weak correlation ($0 < |r| < 0.3$), moderate correlation ($0.3 < |r| < 0.7$) and strong correlation ($|r| > 0.7$).

A variety of fungal species belonging to the genera *Acremonium, Alternaria, Aspergillus, Aureobasidium, Beauveria, Botrytis, Candida, Chaetomium, Cladosporium, Epicoccum, Exophiala, Fusarium, Geotrichum, Gliocladium, Mortierella, Mucor, Naganishia, Ochroconis, Paecilomyces, Penicillium, Phoma, Rhizopus, Rhodotorula, Sarocladium, Sporotrichum, Sporothrix, Stachybotrys* and *Trich*

oderma have been cultivated from chlorinated water, pointing out possible resistance to the regular chlorination process (Babič et al., 2017). Also, recent research confirmed that fungi could survive treatment and disinfection methods and most of the single water treatment trials are not effective against all fungal species (Ali et al., 2017). The results also showed that there was an overall moderate positive correlation (r=0.4266) between the total coliform counts and fungal prevalence (Table 7). However, no statistical correlation between faecal coliforms and fungi was conducted due to sporadic detection of faecal coliforms within the study period. It has been shown that the obligatory microbial drinking water standards (*E. coli*, faecal coliforms or *Clostridia i.*e. total coliforms) have no indicative value for fungal contamination (Babič et al., 2017). Although there was moderate correlation between fungi and total coliform per site, the presence of both the bacteria and fungi might be indicative of poor hygiene standards at the sampling sites or contamination of the distribution systems. The less prevalence of coliforms compared to fungi, indicate that there is the likelihood that the water is bacteriologically safe but has potentially pathogenic fungi. Use of indicator coliform therefore is not necessarily an applicable quality measure for eukaryotic fungi.

4.5 FUNGI PREVALENCE IN TREATED DRINKING WATER SYSTEM

4.5.1 Isolation, Identification and diversity of the fungal isolates

Fungi were positively detected at most of the sampling sites for the duration of the sampling period. For identification, fungal isolates obtained from plating were characterized by DNA sequencing of the internal transcribed spacer (ITS). The partial ITS sequences of isolates obtained were further subjected to hierarchical clustering to pick operational taxonomic units (OTUs) for diversity analysis. The ITS region sequences were conducted against the UNITE Database (https://unite.ut.ee/analysis.php) which allowed identification to the genus level of 282 isolates. Based on a taxa cut-off set at 97% similarity, the sequences were grouped into 31 OTUs (Table 4-2). The Shannon diversity index and chao1 estimator of species diversity for each sampling source cluster are given in Table 4-2. The results show that species diversity was higher for communal tap (CM), reservoir outlet (RSO), tower (TW), school tap (SC) and reservoir inlet (RSI) samples compared to garage tap and clinical tap water samples (Figure 4-6). The lowest species diversity was observed in the garage tap water. However, it should be noted that the number of sequenced isolates also varied with sample type, thus the OTUs identified also followed same trend.

Table 4-2: Analyses of fungal isolates libraries from all treated municipal water sources

Sample source	No. of sequenced isolates	No. of OTUs[b]	H'[a]	Chao1
Community tap water (CM)	107	24	2.725	26.14
Reservoir outlet (RSO)	45	16	2.226	43.50
Household tap water (HT)	30	11	2.039	13.50
Tower (TW)	24	12	2.301	15.75
School tap water (SC)	23	12	2.255	17.50
Reservoir inlet (RSI)	20	11	2.221	14.75
Filling point (FP)	16	9	2.133	9.50
Clinic tap water (CL)	13	8	1.925	11.33
Garage water (GR)	4	4	1.386	10.00

[a] Shannon-Weaver diversity index ($H' = \sum P_i \log P_i N$). [b] Hierarchical clustering of sequences (at 97% similarity).

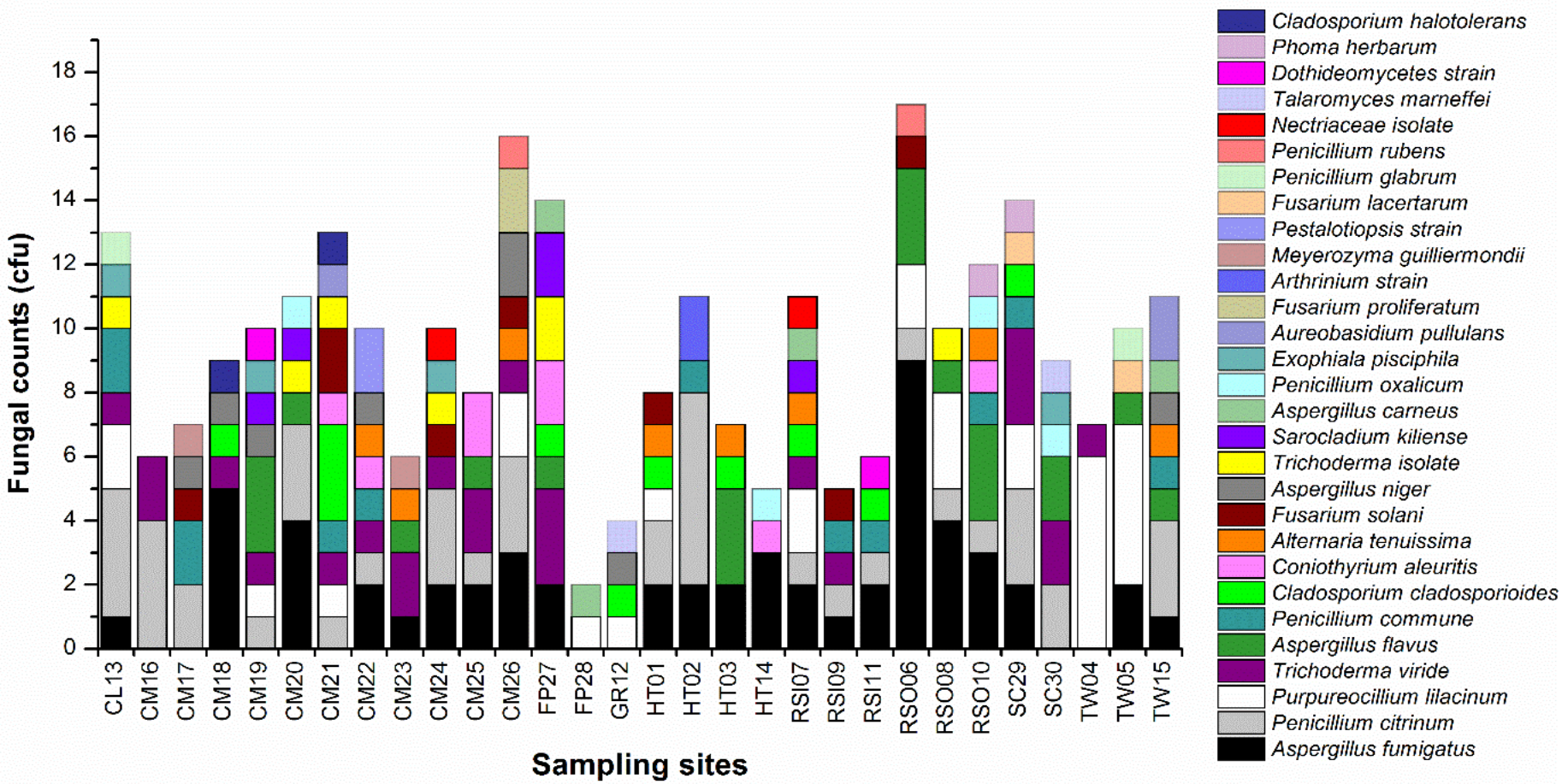

Figure 4-6: Total number of different fungal species that were identified at each site from November 2016 to October 2017.

Figure 4-6 shows the total number of times, and the different types of fungi detected at each site over the sampling period. Site RSO06 (reservoir outlet) had the most occurrences of fungi with 17 counts followed by sites CM21 (communal tap) and the lowest occurrences were reported for FP28. In terms of fungal diversity, site CM21 (community tap) had the most diverse fungal genera with 10 different genera isolated followed by site CM26 (communal tap), RS107 (reservoir inlet), and SC29 (school) with nine different genera each. Sites CM16 (communal tap), TWO4 (tower), and FP28 (household tap) had the least diversity of fungi (Figure 4-6). *Penicillium citrinum* and *Aspergillus fumigatus* dominated the identified fungi across the sampling sites. *Purpureocillium lilacinum* was detected most at TW04 and TW05 which are water towers.

The communal tap cluster of samples followed by reservoirs and then households were dominating in fungal occurrence (Figure 4-7). The detected fungi are common in terrestrial and aquatic environments. Large numbers of people assess communal taps and this often introduces reduced hygienic conditions thus external environmental input of fungi is likely. Niaz et al. (2012) showed that microbial incidences are high in overpopulated areas with poor hygienic standards. All isolates characterized were from phylum *Ascomycota* and grouped into 17 genera; the most common genera being *Aspergillus* (20% of isolates), *Penicillium* (16% of isolates), *Trichoderma* (9% of isolates), and *Purpureocillium* (10% of isolates) (Figure 4-7). Other genera isolated included *Fusarium*, *Alternaria*, *Coniothyrium*, *Cladosporium*, *Sarocladium*, *Exophiala*, *Auerobasidium*, *Arthrinium*, *Meyerozyma*, *Phoma*, *Talaromyces*, and unclassified *Nectriaceae* and *Dothidomycetes*. A study by Niaz (2012) revealed that the incidence of fungal species from samples of drinking water tested from the distribution system of the city of Karachi (Pakistan), was characterised by the presence of more than one species at a site. Similar to their finding, most sites in this study had more than four different types of fungi genera isolated during the sampling period.

4.5.2 Seasonal distribution of fungi over the sampling period

The monthly distribution of the fungal isolates for all sampling sites during the study period is shown in Figure 4-8. The most fungal occurrences were reported in April (15 different genera), followed by June (14 different genera), August (13 different genera), December and March with 10 different genera. The lowest occurrences were observed in February (2 genera). The occurrence does not seem to be in any way tied up to seasonal periods and it can be concluded that fungal occurrence varies within the distribution system with time independent of seasons. The most occurring genera throughout the sampling period were *Aspergillus, Penicillium* and *Trichoderma*.

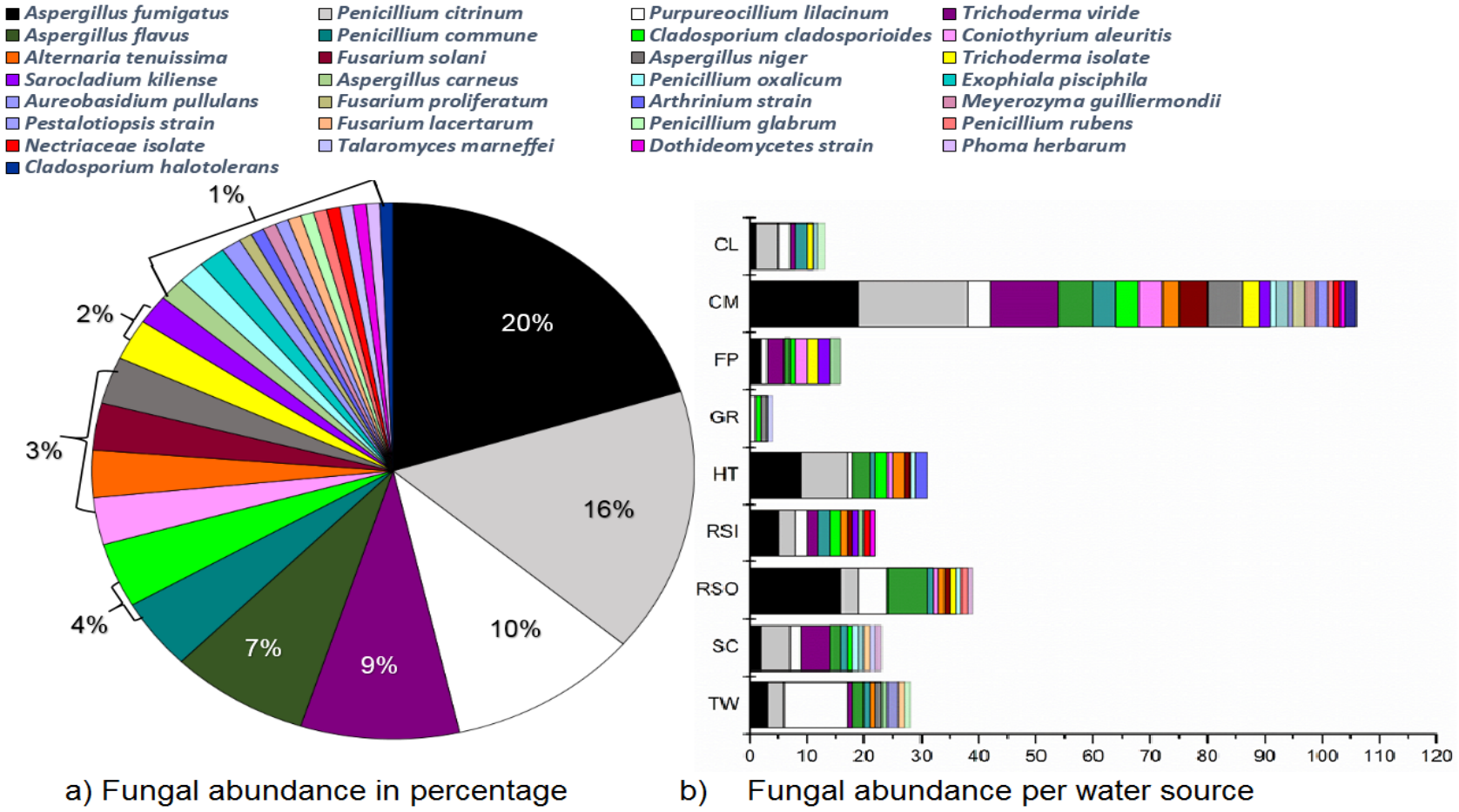

Figure 4-7: Spatial profile of the fungal community structure based on ITS gene sequences of isolates from different drinking water sources and treated water infrastructure. a) Relative abundance of all sequenced isolates as grouped into different OTUs at 97% similarity. b) Taxon abundance of the OTUs according to sampling sources.

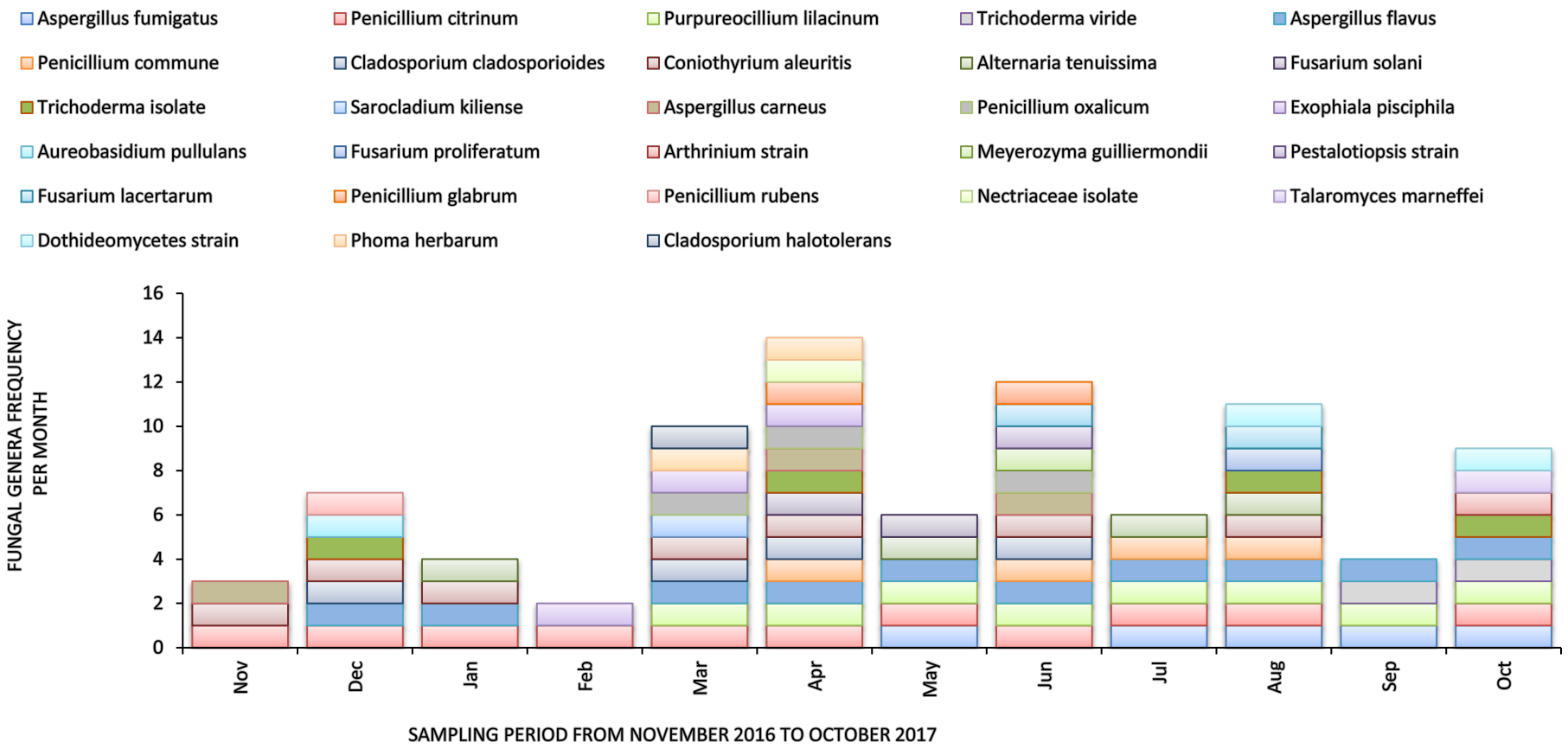

Figure 4-8: Monthly frequency of fungal occurrence during the study period.

Comparatively, *Aspergillus* followed by *Trichoderma* species constituted the majority of the fungi isolated from all sampling sites in September and October (Spring); these accounted for approximately two-thirds of the fungal species during this period. *Penicillium* species were the most abundant fungal isolates in April (Autumn) as shown in Figures 4-9 to 4-11. The study area experience wet seasons towards end of Spring, in Summer and beginning of Autumn. The results show that there were more occurrences of fungi in Autumn, Winter and Spring. Fungal occurrences were less in summer. The results are in agreement with the results obtained by Okpako et al. (2009) that showed that *Aspergillus, Fusarium, Trichoderma* and *Penicillium* were the most occurring genera in the rainy season than in the dry season. *Aspergillus* species grows well in warm temperatures and tends to dominate other fungal species (Milani, 2013). Although *Aspergillus* species were identified throughout the sampling period, more isolates were recorded in September and October, which are in warm and wet season in the study area (Figure 4-9 to 4-11).

Seasonal trends for other fungal species was not that much apparent in this study, the fungi occurred throughout the season. From the results in Figure 4-9 to 4-11, it can be concluded that autumn and spring favoured proliferation of fungi at the different sampling points. Noting that disturbances in the distribution systems such as maintenance episodes and re-contamination, and poor hygienic practices could be the main sources of fungi in the treated drinking water, fungal species dominating the external environment as influenced by seasons would also be expected to find their way into the system. This is supported by the reason that treated water in the distribution system is conveyed in closed water distribution pipelines that are laid underground preventing atmospheric temperatures and other environmental changes from causing impacts that affect the water distribution system (Plummer et al., 2014).

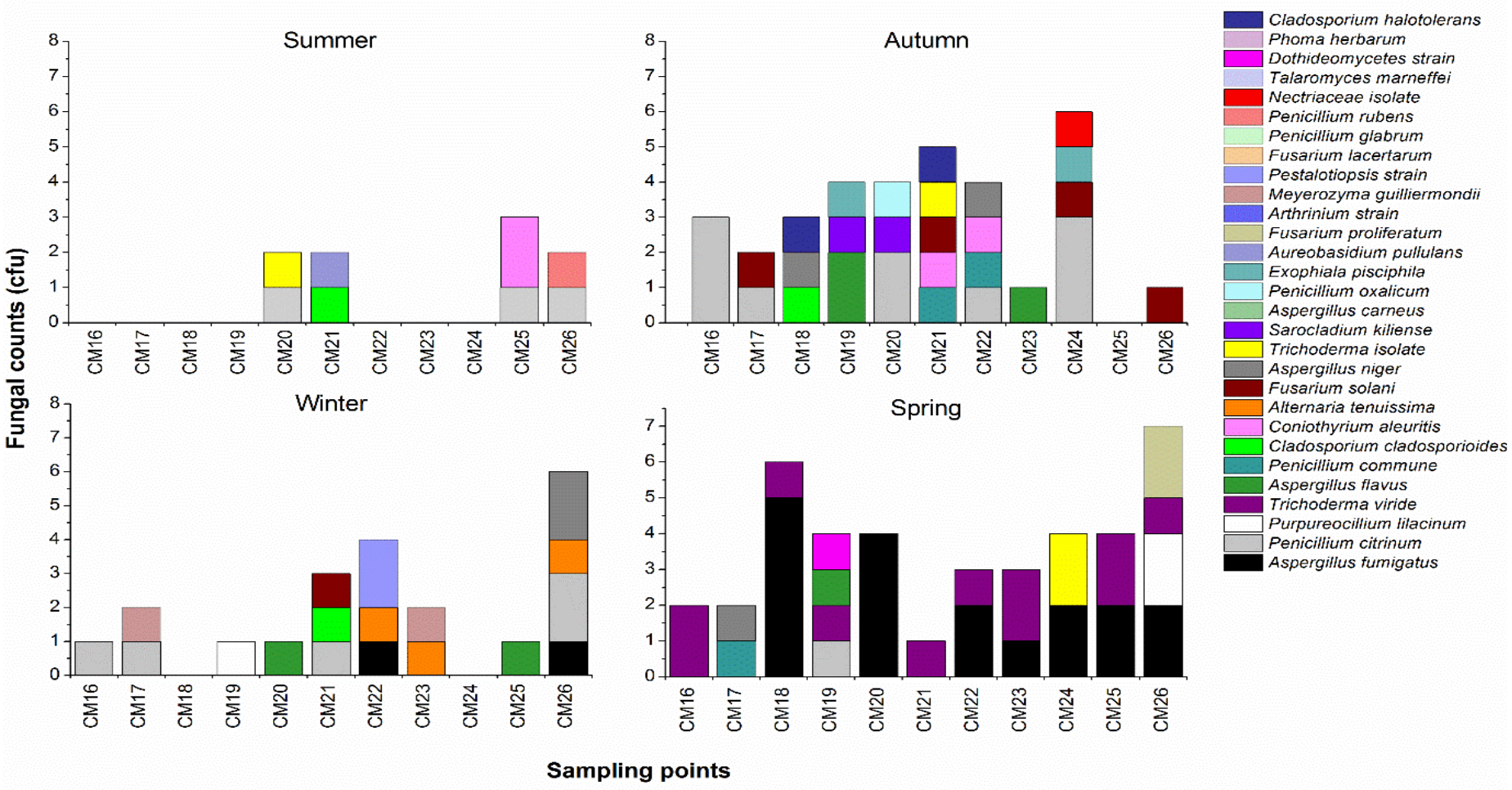

Figure 4-9: Stacked bar charts of the seasonal distribution of the fungal ITS gene clusters of all sequenced isolates.

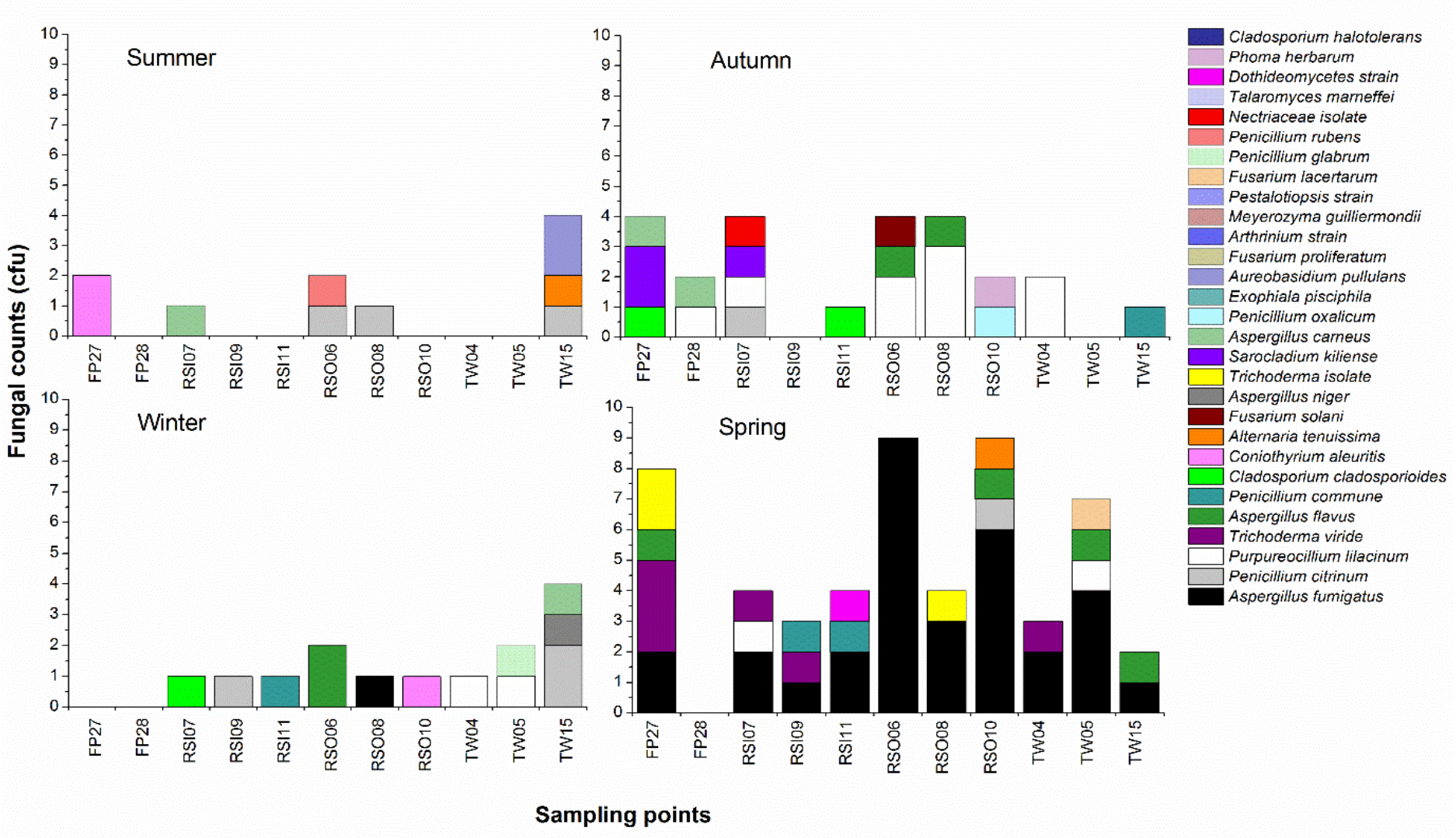

Figure 4-10: Stacked bar charts of the seasonal distribution of the fungal ITS gene clusters of all sequenced isolates.

c)

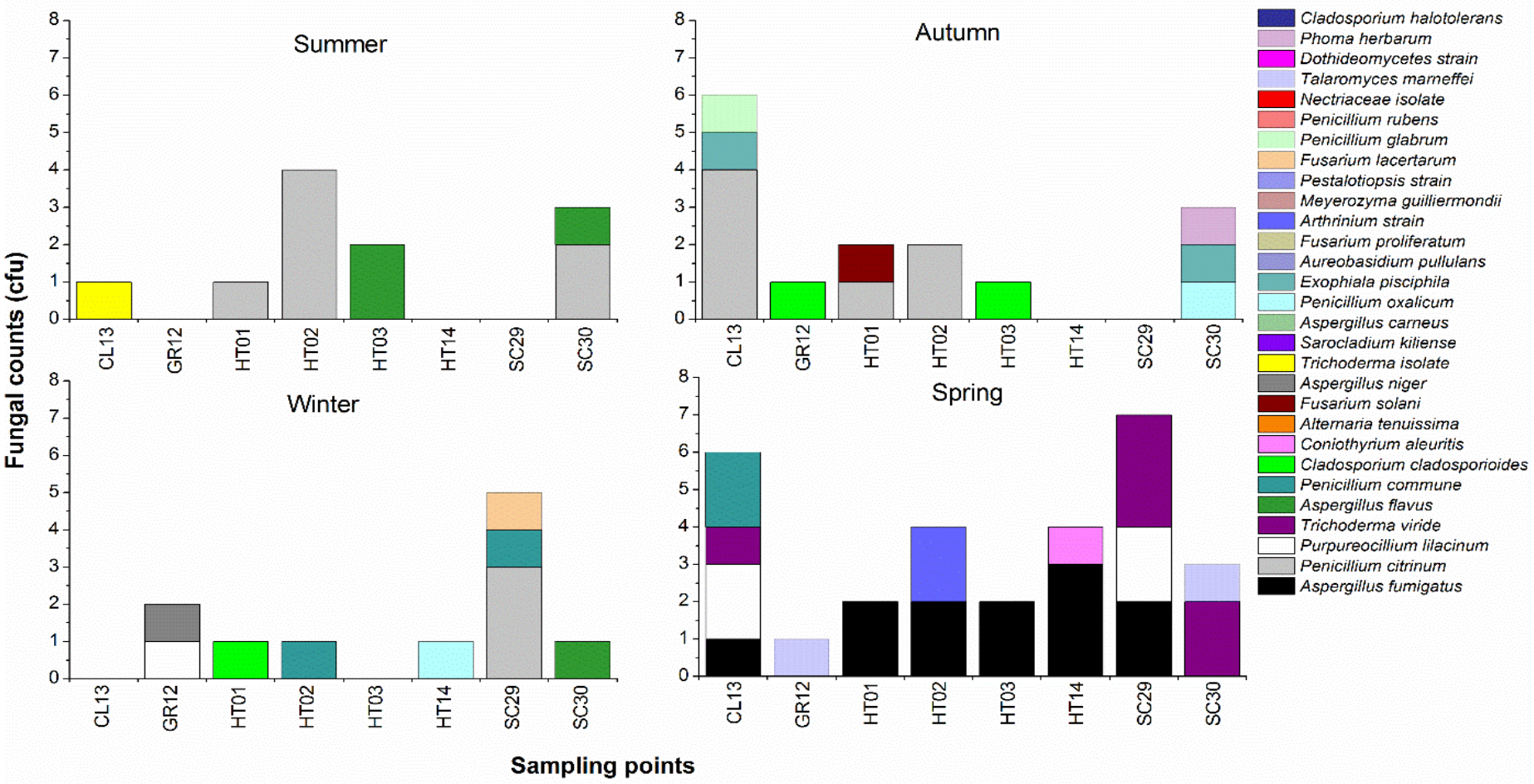

Figure 4-11: Stacked bar charts of the seasonal distribution of the fungal ITS gene clusters of all sequenced isolates.

4.5.3 Phylogenetic analysis of the fungal ITS sequences

A phylogenetic tree was constructed to show the relationships between the OTUs (31 OTUs, representing 282 sequences) and their closest neighbours (Figure 4-12). The phylogenetic affiliations of the fungal isolates per site, grouped into different OTUs as given in Table 4-3, ranging from 2 to 55. Based on the valid reference tree, all the fungal isolates grouped in the phylum *Ascomycota*, with majority of OTUs being classified as *Aspergillus*, *Penicillium* and *Trichoderma*. A total of five OTUs representing 91 sequences were classified within genus *Aspergillus*, followed by *Penicillium* (five OTUs representing 65 sequences, *Trichoderma* (two OTUs representing 31 sequences). Other less prevalent fungal isolates included *Cladosporium species*, *Alternaria species*, *Fusarium species*, *Purpureocillium species* and *Phoma species*. A study by Memon (2012) also revealed that samples of drinking water tested from the distribution system of the city of Hyderabad (Pakistan), contained these identified fungal species. Figure 4-12 shows that the most prevalent fungi found in treated drinking water distribution network supplying Johannesburg West are *Penicillium, Aspergillus, Cladosporium, Alternaria, Phoma, Epicoccum* and *Trichoderma species.* This was also confirmed through phylogenetic analysis, where the greatest number of OTUs grouped with the *Penicillium* clade (Figure 4-12). These results are consistent with findings that members of the ascomycetous filamentous fungi genera *Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium* and *Trichoderma* are the main groups detected in treated potable water using cultivation techniques. The second most cultivated group are fungi from the subphylum *Mucormycotina* (former phylum *Zygomycota*), which was not detected in the current study (Babič et al., 2017).

Most of the fungal species identified in the present study are known to be of public health concerns. *Penicillium, Aspergillus* and *Cladosporium species* have been implicated in a numerous health conditions including allergies, respiratory illness, cutaneous infection and life-threatening meningitis (Sulaiman et al., 2014). *Alternaria* species*, Cladosporium* species*, Aspergillus* species*, Penicillium* species and *Fusarium* species have also been linked to allergies and respiratory illness (Korzeniewska, 2011). Taste and odor problems in water are caused by *Aspergillus species, Acremonium species* and *Penicillium* species (Sonigo et al., 2011; Hageskal et al., 2006). In addition to their health implications, fungi such as *Rhizopus, Fusarium, Alternaria, Aspergillus* and *Penicillium* are known to produce mycotoxins that are harmful to public health as they may be carcinogenic and have the ability to impair the immune system (Bhat et al., 2010). Mycotoxins of great concern for public health include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F) and ergot alkaloids (Zain, 2011).

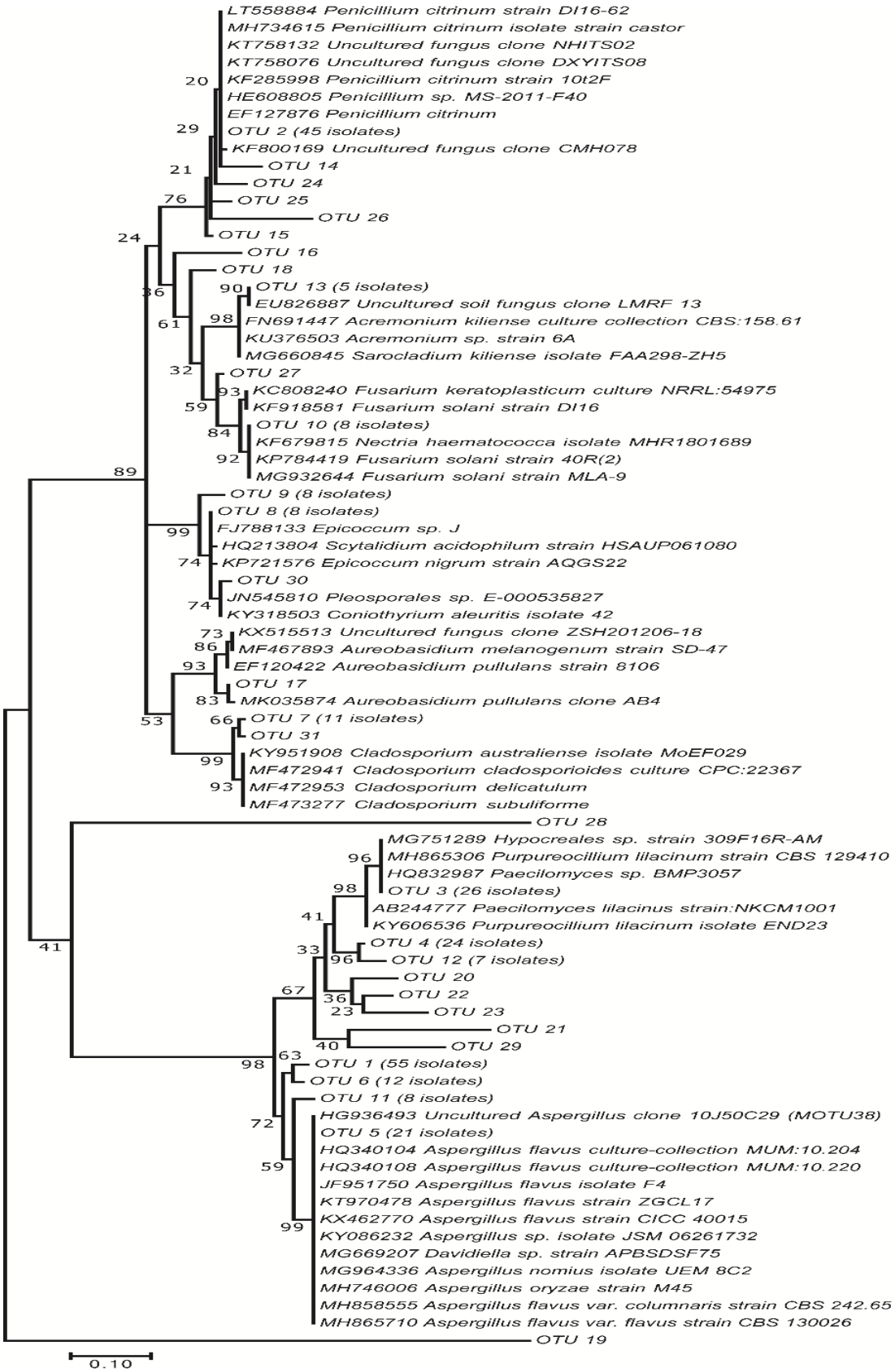

Figure 4-12: Maximum likelihood phylogenetic tree based on analysis of the representative ITS gene sequences obtained from different OTUs. The tree was constructed using the neighbour-joining method in MEGA 7. Bootstrap analysis was conducted using 1000 replicates.

Table 4-3: Phylogenetic affiliation of fungal isolates obtained from treated municipal water samples collected from different sources in Johannesburg as deduced from BLAST search of the UNITE fungal ITS database

OTU	Representative isolate	No. of similar sequences	Phylogenetic description	Closest relative (accession no.)	Identity (%)
1	SCOC17_49	55	Eurotiomycetidae	*Aspergillus fumigatus* strain SN-A4 (HQ285578)	99
2	CMJN17_91	45	Eurotiomycetidae	*Penicillium citrinum* (KM491892)	99
3	RSOSP17_18B	26	Hypocreomycetidae	*Purpureocillium lilacinum* isolate ATT161 (HQ607867)	99
4	CMSP17_25B	24	Hypocreomycetidae	*Trichoderma viride* isolate NW652 (EU520205)	99
5	HTJA17_150	21	Eurotiomycetidae	*Aspergillus flavus* isolate F4 (JF951750)	99
6	CMAP17_42	12	Eurotiomycetidae	*Penicillium commune* isolate NJP11 (HQ710540)	99
7	RSIJN17_69	11	Dothideomycetidae	*Cladosporium cladosporioides* strain LPSC1202 (KX463059)	99
8	FPDC16_49A	8	Pleosporomycetidae	*Coniothyrium aleuritis* isolate 42 (KY318503)	97
9	CMMY17_35	8	Pleosporomycetidae	*Alternaria tenuissima* strain CHR-1 (KJ082100)	98
10	CMMY17_38	8	Hypocreomycetidae	*Fusarium solani* isolate F2-3-18 (KX349467)	98
11	CMAP17_79	8	Eurotiomycetidae	*Aspergillus niger* strain NJA-1 (KJ365316)	99
12	CLDC16_130	7	Hypocreomycetidae	*Trichoderma* sp. isolate HYKH3/C1 (AY514865)	99
13	FPMA17_26	5	Hypocreomycetidae	*Sarocladium kiliense* strain kw63-15 (LN864540)	100
14	TWJN17_90	4	Eurotiomycetidae	*Aspergillus carneus* strain W2-4(HQ889708)	99
15	HTJN17_92	4	Eurotiomycetidae	*Penicillium oxalicum* isolate B3-11(2) (JQ446378)	99
16	CLFB17_167	4	Chaetothyriomycetidae	*Exophiala pisciphila* strain IHEM 3404 (KP132125)	100
17	TWDC16_57A	3	Dothideomycetidae	*Aureobasidium pullulans* strain YY20 (KR912253)	98
18	RSOAP17_31	3	Hypocreomycetidae	*Purpureocillium lilacinum* isolate A546 (KX463002)	99
19	CMAG17_02A	2	Hypocreomycetidae	*Fusarium proliferatum* isolate P53(HF936728)	83

OTU	Representative isolate	No. of similar sequences	Phylogenetic description	Closest relative (accession no.)	Identity (%)
20	HTOC17_82B	2	Xylariomycetidae	*Arthrinium sp. strain GU071007 (AB471012)*	99
21	CMJN17_79	2	Saccharomycetales	*Meyerozyma guilliermondii* strain Kw2680-2-14 (LN626314)	99
22	CMJN17_84A	2	Xylariomycetidae	*Pestalotiopsis* sp. strain 39 (KX271321)	97
23	SCJN17_74	2	Hypocreomycetidae	*Fusarium lacertarum* strain NRRL 52753 (JF740923)	98
24	TWJN17_93	2	Eurotiomycetidae	*Penicillium glabrum* strain DI16-96 (LT558918)	99
25	CMSP17_17	2	Eurotiomycetidae	*Aspergillus fumigatus* strain AN1 (KJ820681)	99
26	RSODC16_134	2	Eurotiomycetidae	*Penicillium rubens* isolate 0911MAR15O1 (LN808905)	98
27	RSIAP17_63	2	Hypocreomycetidae	*Nectriaceae* sp. isolate E9322a (JN545766)	99
28	SCOC17_44		Eurotiomycetidae	*Talaromyces marneffei* strain F277934 (KC427058)	82
29	RSIOC17_04	2	Dothideomycetidae	*Dothideomycetes* sp. (KX908683)	96
30	RSOAP17_69	2	Pleosporomycetidae	*Phoma herbarum* strain VL168 (JF440609)	99
31	CMMA17_28	2	Dothideomycetidae	*Cladosporium halotolerans* strain UTHSC DI-13-249 (LN834373)	99

4.5.4 Metagenomics community analysis of the fungi

Phylogenetic diversity of fungi in the environment is still largely overlooked (Maza-Márquez et al., 2016), especially where drinking water is concerned. However, exploration of fungal biodiversity in aquatic habitats is gaining momentum as new molecular tools and approaches like next-generation sequencing have revealed an unexpected abundance of fungi with unidentified ecological functions and unclear phylogenetic placement (Grossart et al., 2016). Out of 15 selected samples targeted for total DNA extraction, three samples had good quality DNA for downstream metagenomics analysis. A total of 54767 quality-filtered reads were obtained from the selected three water samples after removal of PCR artifacts and chimeric sequences and used for further analysis. The complete phylogenetic taxonomy analysis assigned the fungal reads to 6 phyla, 31 classes and 92 genera in all drinking water samples (Figure 4-13). Phylum level phylogenetic fingerprint of fungal communities in this study produced a total of 6 phyla dominated by *Basidiomycota* whose relative abundance ranged from 56.12% in CM26 (communal tap) to 88.03% in CM24 (communal tap) samples followed by *Ascomycota* with a relative abundance of 9.66% in CL13 (clinic tap) to 22.85% in CM26. Similar to the present findings, previous studies on fungal diversity in terrestrial

system using amplicon metagenomic sequencing in Chile found fungal communities to be dominated by fungi of the phyla *Basidiomycota* and *Ascomycota* (Baeza et al., 2017).

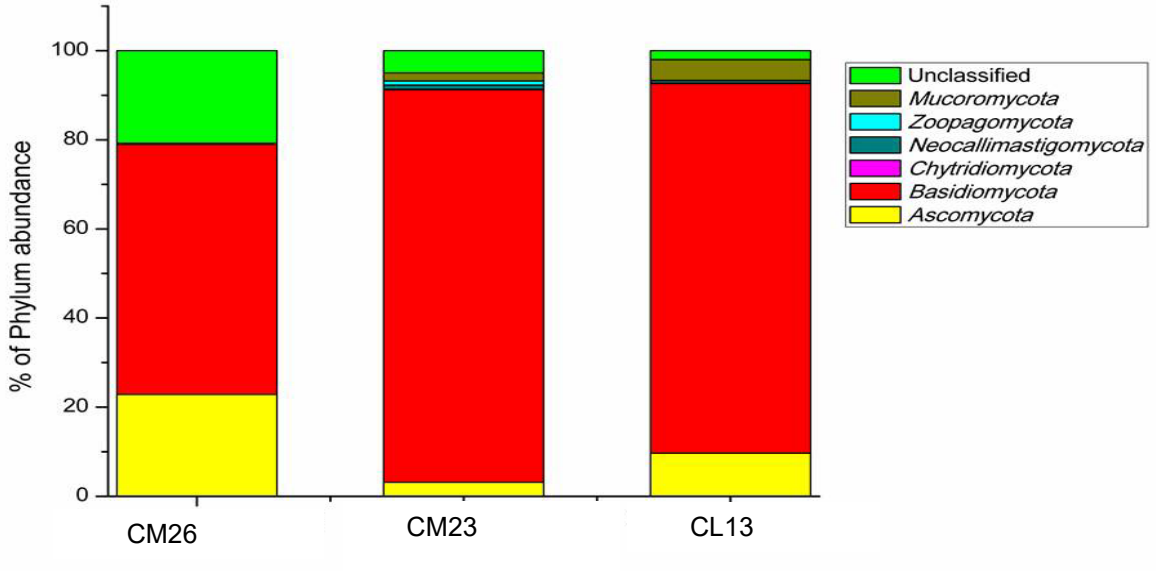

Figure 4-13: Relative abundances of fungal phyla from three different drinking water samples. Sequences that could not be classified into any known group of phyla were assigned as "unclassified" fungi.

The overwhelming abundance of fungi belonging to these two phyla may be attributed to fact that *Ascomycota and Basidiomycota* constitutes the largest phyla of fungi encompassing more than 33,000 named species and a vast number of undescribed fungi (Money, 2016; Peralta et al., 2017). Other phyla including *Chytridiomycota, Glomeromycota* and *Neocallimastigomycota*, *Zoopagomycota* and *Mucoromycota* occurred in low percentages. The occurrence of the fungal phyla *Glomeromycota* and *Neocallimastigomycota* in drinking water corroborates the findings of *previous* research (Babič et al., 2017). Unclassified fungal sequences occurred at relatively high abundance ranging from 1.99% in clinic tap water to 20.75% in the communal informal setting, hypothetically suggesting that these water samples may contain different fungal species. The detailed distribution of the fungal phyla is given in Figure 4-13. For the in-depth analysis, the top 15 OTUs of three different water samples were analysed from the metagenomic data. Samples from the communal informal setting water exhibited the following fungal members; *Agaricus bisporus, Naematelia encephala, Pleosporineae genus, Fibulorhizoctonia sp., Penicillium sp., Aspergillus sp., Heterobasidion annosum, Wickerhamomyces anomalus, Gloniaceae sp., Basidiomycota_species, Mycosphaerellaceae_genus, Pleurotus ostreatus, Eurotiales sp. and Pleosporomycetis sp.*

While the samples collected from communal tap (CM26) exhibited following fungal members; Sistotremastrum sp., Naematelia encephala, Rhodotorula graminis, Agaricus bisporus, Aspergillus sp., Lobosporangium transversal, Pleosporineae sp., Wickerhamomyces anomalus NRRL Y-366-8, Basidiobolus meristosporus CBS, Piromyces finnis, Eurotiales, Pleosporomycetidae, Pichia and Asc0 members, samples collected from the clinical tap (CL13 demonstrated the following fungal members; Sistotremastrum niveocremeum, Lobosporangium transversal, Agaricus bisporus, Aspergillus sp., Capnodiales, Pleosporomycetidae incertae sedis, Hypocreales, Schizophyllum commune, Gelatoporia subvermispora, Metschnikowia bicuspidate, Pichia kudriavzevii, Aspergillus aculeatus, Aspergillus lentulus. Detailed analysis of top 15 genera is given in Figure 4-14.

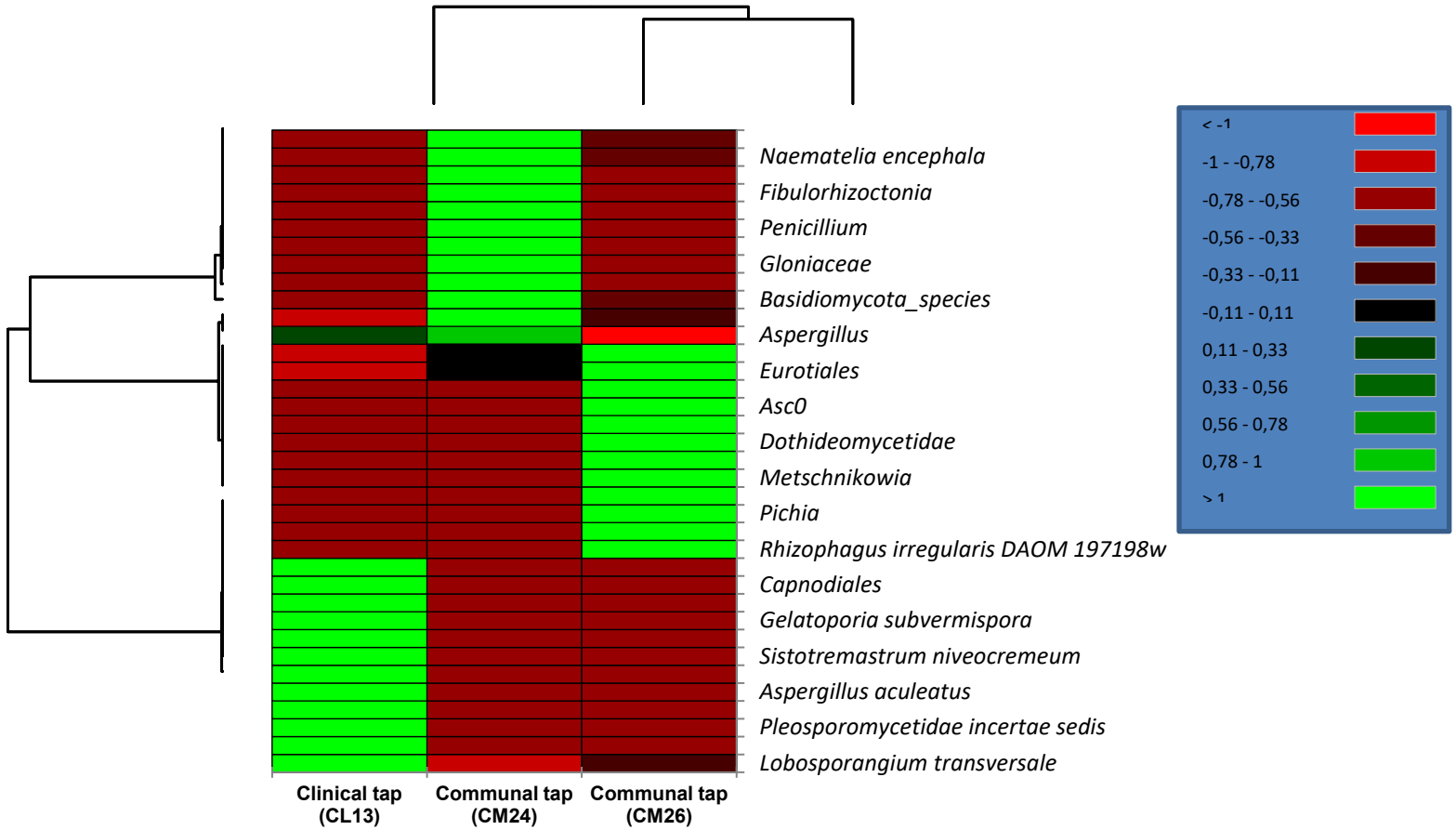

Figure 4-14: Heat map graph of hierarchy cluster for the top 15 genera. The colour intensity indicates the relative abundance of each genus within each sample.

Culture-based methods are often biased by the selection of culture media. Moreover, dead microorganisms are not culturable even though they may retain activity linked to allergenic proteins or toxic secondary metabolites (Babič et al., 2017). NGS is a growing sequencing technology that can identify the fungal genera that are not only the most frequently reported in drinking water but also often being recognised as causative agents of diseases. Apart from the fungal isolate identification, bacterial genera from the collected drinking water were also identified using metagenomics approach. Metagenomics results demonstrated various pathogenic bacterial members in collected drinking water including members such as *Acinetobacter baumannii, Arcobacter cryaerophilus, Brucella suis, Candidatus Harrisonbacteria bacterium, Corynebacterium xerosis, Cutibacterium acnes, Enterococcus casseliflavus, Enterococcus faecalis, Enterococcus faecium, Erwinia amylovora, Escherichia coli, Galdieria sulphuraria, Legionella pneumophila, Listeria monocytogenes, Microbacterium aurum, Mycobacterium abscessus subsp. Abscessus, Mycobacterium tuberculosis complex, Propionibacterium acnes HL043PA2, Propionibacterium acnes HL053PA2, Pseudomonas oleovorans/pseudoalcaligenes group, Salpingoeca rosetta, Serratia marcescens, Staphylococcus aureus, Staphylococcus sciuri, Streptococcus pneumonia, Streptomyces himastatinicus ATCC 53653, Streptomyces himastatinicus ATCC 53653, Vibrio anguillarum.* The results of this study suggest that the ecology and pathogenesis of fungal contaminants in water is essential to measure and understand in drinking water, particularly in environments with high numbers of immunocompromised people.

4.6 MYCOTOXINS IN WATER

4.6.1 Concentrations of mycotoxins detected in water

There are hundreds of different types of mycotoxins of which only aflatoxins, patulin, ochratoxin A, fumonisins, zearalenone, sterigmatocystin, nivalenol and deoxynivalenol present a concern to both human and animal health (Zain, 2011). Of all mycotoxins, aflatoxins are the most potent mutagenic and carcinogenic compounds to animals and humans (Chen, 2017; Kamika et al., 2016). It should be noted that mycotoxins are commonly present in a variety of food-crops, foodstuffs and beverages (Babič et al., 2017). However, very few studies have reported their presence in water destined for drinking (Sonigo et al., 2011; Mata et al., 2015). Figures 4-15 and 4-16 show the LC-MS/MS chromatographic profiles of the different mycotoxins. All analysed samples contained the trichothecenes deoxynivalenol (8.405 to 96.139 ng/Kg), 15-acetyldeoxynivalenol (15.154 to 71.606 ng/Kg), 3-acetyldeoxynivalenol (18.737 to 145.689 ng/Kg). Trichothecenes are a group of mycotoxins that are produced mainly by the *Fusarium* genus and Deoxynivalenol (DON) has shown to be one of the most abundant trichothecenes in food and animal feed frequently occurring in toxicological conditions. Complete avoidance of these toxins by organisms is a bit difficult as they are dependent on environmental conditions such as humidity and temperature. Continuous exposure to these toxins can be a permanent health risk for human beings (Awad, 2010).

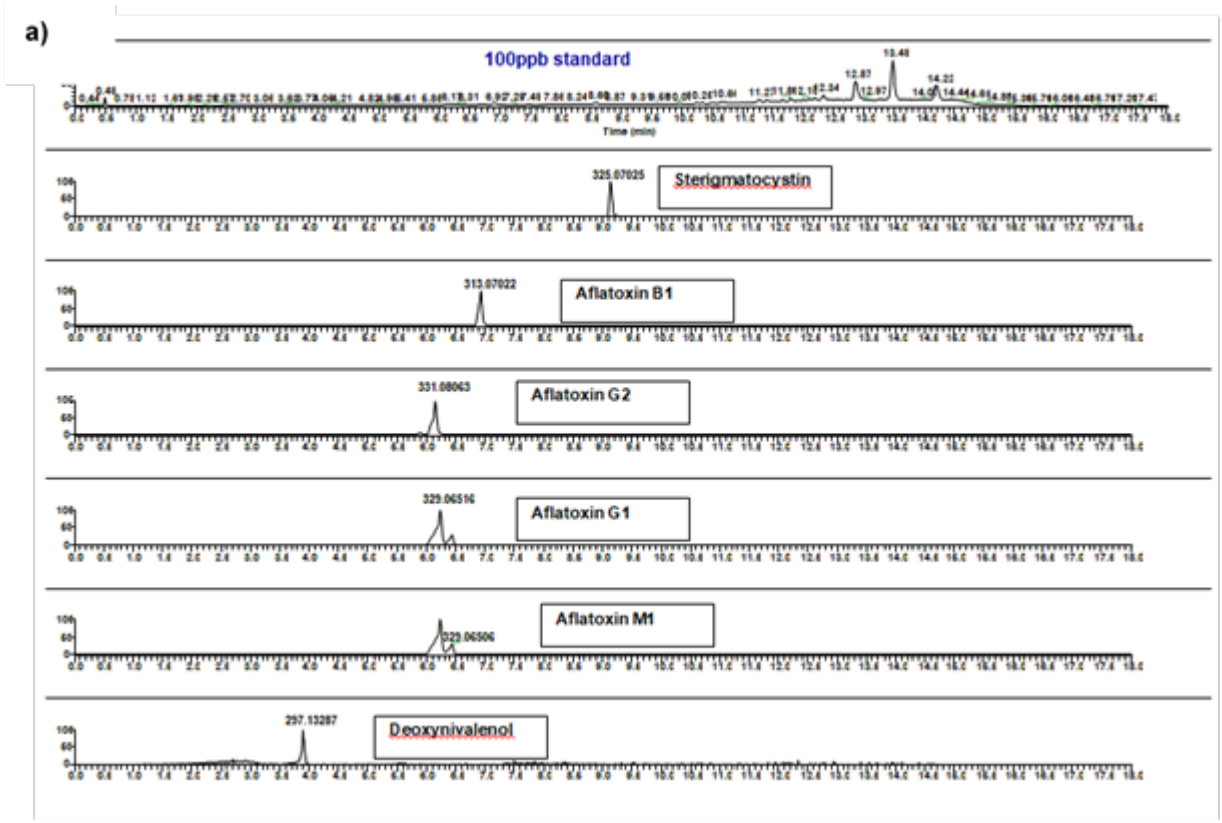

Figure 4-15: LC-MS/MS chromatographic profiles of the different mycotoxins showing peak detection of different mycotoxins (1000ppb).

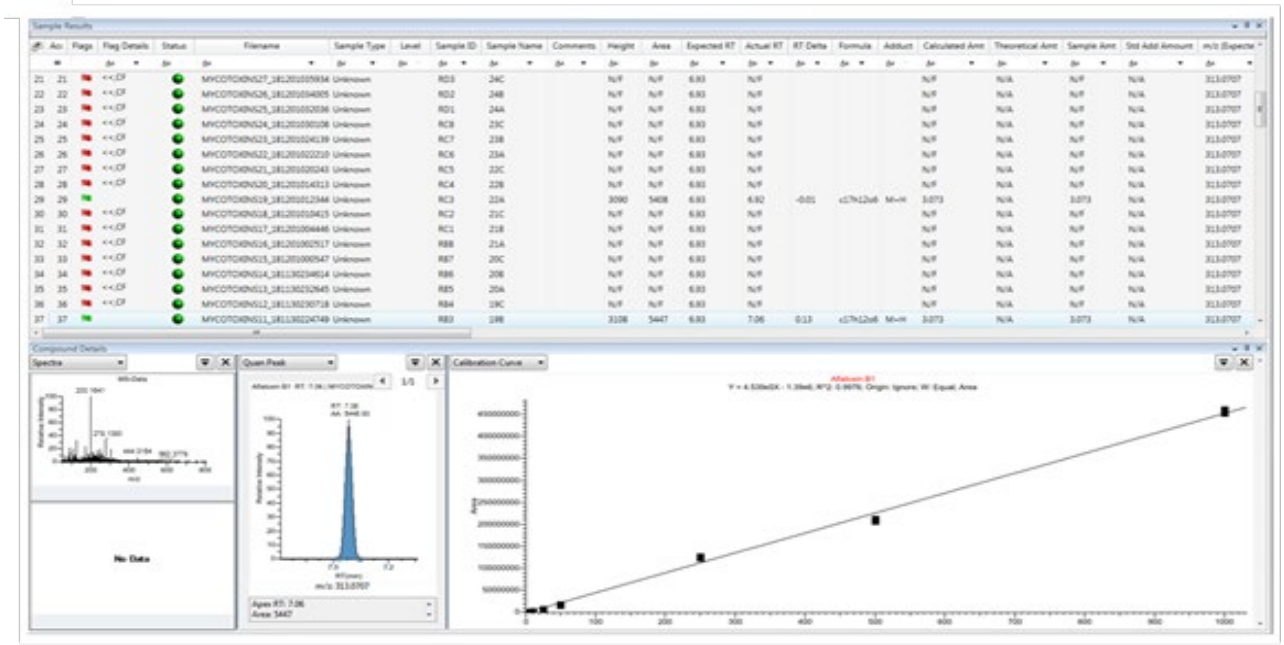

Figure 4-16: LC-MS/MS chromatographic profiles of the different mycotoxins showing peak detection of Aflatoxin B1 from sample view in tracefinder.

In the present study, 15-acetyldeoxynivalenol, nivalenol, tenuazonic acid, deoxynivalenol, 3-acetyldeoxynivalenol, aflatoxin G2, aflatoxin G1, aflatoxin M1, aflatoxin B1, sterigmatocystin and patulin were detected from the collected drinking water samples (Table 4-4). Thus, despite the low levels detected in this study, long term intake from water among other sources can be a risk factor in human health. In this study the results of 15-acetyl deoxynivalenol, deoxynivalenol and 3-acetyl deoxynivalenol were below the maximum standard limits in food and animal feed (Table 4-4 and 4-5). It is assumed therefore that they were secreted in low quantities in addition to possible microbiological degradation and detoxifying that can occur in biofilms in water distribution pipelines. Aflatoxins are furanocoumarins produced by Aspergillus species. Aflatoxins are carcinogenic to humans. In this study aflatoxins, especially aflatoxin B1 was noted in 37% of all analysed samples at concentration range 3.069 to 3.083 ng/Kg.

Table 4-4: Mycotoxin contents of drinking water (ng/Kg), collected from Johannesburg West.

Sample	DON	15 acetyl DON	3 acetyl DON	Aflatoxin G2	Aflatoxin G1	Aflatoxin M1	Aflatoxin B1	Sterigmatocystin
FP27	15.361	15.154	20.891	3.049	0	0	0	0.223
SC30	12.593	20.308	28.059	3.06	0	0	0	0
CL13	17.733	69.106	60.472	3.066	0.008	0.044	0	0
RSO06	19.270	30.572	24.417	3.048	0	0	3.069	0
HT14	26.355	27.006	33.521	0	0	0	3.066	0
FP28	75.130	30.917	57.915	0	0	0	3.073	0
TW15	43.551	30.699	26.589	3.068	0	0	0	0
RSO08	17.535	29.226	29.825	0	0	0	0	0
RSO06	8.405	37.173	38.645	0	0	0	0	0
HT14	24.289	20.361	18.737	3.085	0	0.099	3.079	0
RSI07	21.062	28.293	32.912	0	0	0	3.071	0
CL13	44.142	32.159	36.575	0	0	0	3.074	0
TW15	12.787	32.603	40.919	0	0	0	0	0
CM26	44.667	47.456	47.221	0	0	0	3.073	0
SC30	21.526	25.913	32.202	3.053	0	0	0	0
HT03	44.846	51.022	46.921	3.047	0	0	0	0
CM18	44.667	47.456	47.221				3.073	
FP28	52.298	28.518	26.237	0	0	0	0	0
CM19	96.139	31.086	23.574	3.06	0.013	0	0	0
CM17	11.884	57.472	19.923	0	0	0	3.076	0
FP27	87.827	45.940	46.705	0	0	0	3.071	0
CM24	53.500	71.606	145.689	3.183	0	0	3.083	0

From the analysed samples, Sterigmatocystin (0.223 ng/Kg) could only be detected in one sample (FP27) collected in May 2017. The aflatoxin concentrations were below the maximum standard limits in food and animal feed (Table 4-4 and 4-5). Sterigmatocystin was also below the maximum standard limits in food and animal feed (Table 4-4 and 4-5). Apart from the deoxynivalenol and its conjugates, four samples collected from September (RSO06, RSO08, TW15 and FP28) revealed no other detectable mycotoxins. RSO06 had high Fusarium which is known to produce deoxynivalenol.

Aflatoxins and Sterigmatocystin may have also been reduced by microbiological degradation and detoxification in biofilms in water distribution pipelines. Since no mycotoxin legal limits have been set for drinking water, maximum acceptable concentrations of targeted mycotoxins from food-crops, foodstuffs as well as beverages set by South Africa, UN-FAO, European Union and the United States of America were used (Mazumder and Sasmal, 2001). When comparing the current results to maximum legal concentration set by South Africa for aflatoxins (aflatoxin B1: 5ug/Kg; total aflatoxin: 10 µg/Kg), patulin (50 µg/Kg) and fumonisin (100-200 µg/Kg), and by those set by the United States of America for deoxynivalenol and its conjugates (1000 µg/Kg), it was noted that none of the mycotoxins exceeded the set legal limits (Mazumder and Sasmal, 2001; Kamika et al., 2016). However, since the set acceptable limits were not for drinking water, it is recommended to establish new maximum limits for this matrix. These findings suggest that the contamination of the collected drinking water is not of toxicological concern but can still contribute to the overall intake of mycotoxins and lead to exceeding the maximum acceptable concentration for the detected mycotoxins.

4.6.2 Mycotoxin average daily dose for human health

Human health problems from mycotoxin exposure are linked to cancer induction, kidney toxicity and immune suppression among other effects. The exposure to mycotoxins is chronic due to low-dose contact over long periods of time (Bennett and Klich, 2003). The toxicity of mycotoxins may depend on the type of mycotoxin, amount consumed, duration of consumption and the health status of the exposed individual (Mengelers & van Eijkeren, 2015). Codex has set the maximum levels for aflatoxins in grains, dried nuts, figs and milk at the range of 0.5 to 15 µg/kg. The Codex maximum limit for patulin in apple juice is 50 µg/L (Welke et al., 2009). Food safety authorities have come up with measures to ensure that human exposure is below health-based guidance values, such as tolerable daily or weekly intake or reference dose (Welke et al., 2009). In this study the average daily dose (ADD) was calculated on daily consumption of 1.5 litres of drinking water and average adult weight of 70kg (Mata et al., 2015). Estimated ADD were below tolerable daily intake, suggesting that analysed drinking water do not pose toxicological risk (Table 4-5).

Table 4-5: Mycotoxin detection and calculated daily exposure level from drinking water

Mycotoxin	Detected levels (ng/kg)	Standard maximum limit in food (mg/kg)	Calculated daily intake[1] (ng/kg bw/day)	Tolerable daily limit (TDI)
Aflatoxin B1	**3.07-3.08**	4-10 (EU, 2006)	**1.432**	1.5 ng/kg bw (Mata et al., 2015
Aflatoxin M1	**0.099**		**0.002**	-
Aflatoxin G1	**0.008**	4-10 (EU, 2006)	**0.0001**	-
Aflatoxin G2	**3.18**	4-10 (EU, 2006)	**0.064**	-
Sterigmatocystin	**0.22**		**0.0045**	
15-acetyl deoxynivalenol	**15.15-71.61**		**1.432**	1 mg/kg bw/day (EFSA, 2013)
deoxynivalenol	**8.41-96.14**		**1.923**	
3-acetyl deoxynivalenol	**18.74-145.69**	300-2000 (FAO, 2003)	**2.914**	1 mg/kg (WHO, 2011)

[1]Average daily dose (ADD) was calculated on daily consumption of 1.5 litres of drinking water and average adult weight of 70 kg.

4.7 SUMMARY OF FINDINGS

The work so far shows that a wide range of potentially pathogenic and mycotoxigenic fungi contaminate treated drinking water from Johannesburg West distribution system. The most prevalent fungi detected at most sites and sampling intervals using culture method were *Aspergillus* (32% of isolates), *Penicillium* (23% of isolates), *Trichoderma* (11% of isolates), and *Purpureocillium* (10% of isolates) belonging to the Ascomycota phylum. Other genera isolated included *Fusarium, Alternaria, Coniothyrium, Cladosporium, Sarocladium, Exophiala, Auerobasidium, Arthrinium, Meyerozyma, Phoma, Talaromyces,* and unclassified *Nectriaceae* and *Dothidomycetes*. In Contrast, metagenomics study showed that fungal community was populated by six phyla with Basidiomycota as the most predominant. These results indicate that the use of both culture method and metagenomics should be used in order to elucidate the complete picture of the prevalence of fungi in drinking water. The study further established a moderate correlation between total coliform and fungal occurrence in the drinking water system and this suggest that total coliform as well as faecal coliform cannot be a good indicator for fungal contamination. Despite the stipulated chlorine level in the collected drinking water, fungal contamination was prevalent hence a weak correlation was noted (r= 0.4266). Furthermore, LC-MS results showed that the collected drinking water samples were contaminated with mycotoxins such as 15-acetyldeoxynivalenol, nivalenol, tenuazonic acid, deoxynivalenol, 3-acetyldeoxynivalenol, aflatoxin G2, aflatoxin G1, aflatoxin M1, aflatoxin B1 and Sterigmatocystin at concentration below the acceptable maximum limits for food, foodstuffs and beverages in South Africa.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

Findings from this study suggest that contamination of the collected drinking water with fungi is prevalent but it is not of toxicological concern but can still contribute to the overall intake of mycotoxins and lead to exceeding the maximum acceptable concentration for the detected mycotoxins. There was detection of some potentially pathogenic species and these can possibly lead to other health risks such as allergies and fungal infection diseases, thus further studies on fungal pathological risks need to be done. Coliforms were found not to be a good indicator for fungi, thus there is need to establish water quality standards that are specific to fungi. Also residual disinfect which was always within acceptable set limits was not effective in excluding the occurrence of fungi in the treated water. Despite the low values in mycotoxin concentrations, long-term studies need to be done to address the exposure effects of detected mycotoxins to human health. This study also suggests that there is a need for further study to establish appropriate monitoring systems and standards in South Africa that are specific for fungi and mycotoxins in drinking water.

5.2 RECOMMENDATIONS

i. There is a need to establish a direct link between mycotoxins and mycotoxigenic fungal occurrence and environmental conditions promoting the production of the toxins.
ii. Origin of fungi and mycotoxins in drinking water should be investigated including analysis of the raw water sources.
iii. More studies towards the establishment of monitoring standards for fungal contamination in drinking water are needed
iv. A more comprehensive monitoring scheme, based on the water safety planning process, is required to ensure surveillance of the distribution system is done on a wider area and even during and after maintenance of the distribution system. This will help to properly ascertain the public health implications of fungal occurrence in the distribution systems and whether there is a valid motivation for the inclusion of fungi in the water regulation standards.
v. Noting that no biofilm studies were done due to consent restrictions, there is need that studies that involve microbial biofilm studies and fungal occurrence be also done if the prevalence of fungi in water system is to be well characterised.

REFERENCES

1. Abbott, S.P. (2002). Mycotoxins and Indoor Molds. *Indoor Envronment Connections*, 3(4), pp.14-24.

2. Adam, K., Heath, R.G.M. & Steynberg, M.C (1998). Invertebrates as biomonitors of sand-filter efficiency. *Water SA*, 24(1), pp.43-48.

3. Al-berfkani, M.I., Zubair, A.I. & Bayazed, H. (2014). Assessment of chlorine resistant bacteria and their susceptibility to antibiotic from water distribution system in Duhok province. *Journal of Applied Biology and Biotechnology*, 2(06), pp.10-13. Available at: http://jabonline.in/counter.php?aid=52.

4. Al-gabr, H.M., Zheng, T. & Yu, X. (2014). Occurrence and quantification of fungi and detection of mycotoxigenic fungi in drinking water in Xiamen City, China. *The Science of the total environment*, 466-467, pp.1103-11.

5. Al-mamun, A., Alam, Z. & Raus, R.A. (2016). Fungal Coagulant for Reduction of Water Turbidity. 3rd International Conference on Civil, Biological and Environmental Engineering (CBEE-2016) Feb. 4-5, 2016 Bali (Indonesia), pp.10-12.

6. Ali, E.A., Abdel-Rahman, T.M., Sayed, M.A.E., & Al Khalek, S.H.A. (2017). Occurrence of Fungi in Drinking Water Sources and Their Treatment by Chlorination and UV-Irradiation. Egyptian Journal of Botany, 57(3), pp.621-632.

7. Araj, G.F., Asmar, R.G. & Avedissian, A.Z. (201)5. Original Article Candida profiles and antifungal resistance evolution over a decade in Lebanon. *Journal of Infection in Developing Countries*, 9(9), pp.997-1003.

8. Arroyo-Manzanares, N., Huertas-Pérez, J.F., Gámiz-Gracia, L. & García-Campaña, A.M. (2015). Simple and efficient methodology to determine mycotoxins in cereal syrups. *Food Chemistry*, *177*, 274-279.

9. Arvanitidou, M., Kanellou, K., Constantinides, T.C. & Katsouyannopoulos, V. (1999). The occurrence of fungi in hospital and community potable waters. *Letters in Applied Microbiology*, *29*(2), 81-84.

10. Arvanitidou, M., Kanellou, K., Katsouyannopoulos, V. & Tsakris, A. (2002). Occurrence and densities of fungi from northern Greek coastal bathing waters and their relation with faecal pollution indicators. *Water Research*, *36*(20), 5127-5131.

11. Ashbolt, N., Grabow, W. & Snozzi, M (2001). Indicators of microbial water quality. *Water Quality: Guidelines, Standards and Health*, (Grabow 1996), pp.289-316.

12. Auwal, H. & Taura, D. (2013). Prevalence of Moulds in Households Drinking Water of Some Local Government Areas of Kano, Nigeria. *Greener Journal of Biological Sciences*, 3(5), pp.179-186.

13. Awad, W.A., Ghareeb, K., Böhm, J. & Zentek, J. (2010). Decontamination and detoxification strategies for the Fusarium mycotoxin deoxynivalenol in animal feed and the effectiveness of microbial biodegradation. *Food Additives and Contaminants*, *27*(4), 510-520.

14. Awopetu, M.S., Coker, A.O., Aribisala, J.O. & Awopetu, S.O. (2013). Water quality in a pipe distribution network: a case study of a communal water distribution network in Ibadan, Nigeria. *WIT Transactions on Ecology and the Environment*, *171*, 175-186.

15. Ayanshola, A.M., Mandal, K., Bilewu, S.O. & Salami, A.W. (2015). Pragmatic approach to the combination and selection of tanks for water distribution pipe network based on pressure simulation. *Ethiopian Journal of Environmental Studies and Management*, *8*(2), 130-140.

16. Babič, M.N., Gunde-Cimerman, N., Vargha, M., Tischner, Z., Magyar, D., Veríssimo, C., ... & Brandão, J. (2017). Fungal contaminants in drinking water regulation? a tale of ecology, exposure, purification and clinical relevance. *International Journal of Environmental Research and Public Health*, *14*(6), 636.

17. Babič, M.N., Zalar, P., Ženko, B., Džeroski, S. & Gunde-Cimerman, N. (2016). Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances. *Fungal Ecology*, *20*, 30-39.

18. Baeza, M., Barahona, S., Alcaíno, J. & Cifuentes, V. (2017). Amplicon-Metagenomic Analysis of Fungi from Antarctic Terrestrial Habitats. *Frontiers in microbiology*, *8*, 2235.

19. Bennett, J. & Klich, M. (2003). Mycotoxins. *Clinical microbiology reviews*, *16*(3), 497-516.

20. Bennett, J.W. (1987). Mycotoxins, mycotoxicosis, mycotoxicology and mycopathology. *Mycopathologia*, 100(1), pp.3-5.

21. Berry, D., Xi, C. & Raskin, L. (2006). Microbial ecology of drinking water distribution systems. *Current Opinion in Biotechnology*, 17(3), pp.297-302.

22. Bhat, R., Rai, R.V. & Karim, A.A. (2010). Mycotoxins in Food and Feed: Present Status and Future Concerns. *Comprehensive Reviews in Food Science and Food Safety*, 9(1), pp.57-81.

23. Biedunkiewicz, A., Kowalska, K., Schulz, L., Stojek, K., Dynowska, M., Ejdys, E., ... & Kubiak, D. (2014). Mycological monitoring of selected aquatic ecosystems in the context of epidemiological hazards. Drinking water. *Annals of Parasitology*, *60*(3).

24. Black, K.E. & Baden, L.R. (2007). Fungal infections of the CNS: Treatment strategies for the immunocompromised patient. *CNS Drugs*, 21(4), pp.293-318.

25. Bloom, E. (2008). Mycotoxins in indoor environments. Determination using mass spectrometry. *Lund University, Faculty of Medicine Doctoral Dissertation Series*, *2008*(112).

26. Boe-Hansen, R., Martiny, A.C., Arvin, E. & Albrechtsen, H.J. (2003). Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. *Water Science and Technology*, *47*(5), 91-97.

27. Boer, W.D., Folman, L.B., Summerbell, R.C. & Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS microbiology reviews*, *29*(4), 795-811.

28. Buse, H.Y., Lu, J., Struewing, I.T. & Ashbolt, N.J. (2013). Eukaryotic diversity in premise drinking water using 18S rDNA sequencing: implications for health risks. *Environmental Science and Pollution Research*, *20*(9), 6351-6366.

29. Cabral, D. & Fernandez, P. (2002). Fungal spoilage of bottled mineral water. *International journal of food microbiology*, 72(1-2), pp.73-76.

30. Cabral, J.P.S. (2010). Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health*, 7(10), pp.3657-3703.

31. Campbell, A.W., Thrasher, J.D., Gray, M.R. & Vojdani, A. (2004). Mold and mycotoxins: effects on the neurological and immune systems in humans. *Advances in Applied Microbiology*, *55*, 375-408.

32. Castiglia, V.C. and Kuhar, F. (2015). Deterioration of expanded polystyrene caused by Aureobasidium pullulans var. melanogenum. *Revista Argentina de Microbiología*, *47*(3), pp.256-260.

33. City of Johannesburg (2013). *2012/16 Integrated Development Plan: 2013/14 Review*, Available at: http://joburg.org.za/index.php?option=com_content&task=view&id=722&Itemid=131.

34. Coelho, M.A.Z., Amaral, P.F.F., Belo, I., Yarrowia lipolytica: an industrial workhorse. In: Mendez-Vilas A, editor. Current research, technology and education topics in Applied Microbiology and Microbial Biotechnology. Badajoz: Formatex Research Center; 2010. p. 930e44.

35. Corona-Vasquez, B., Rennecker, J.L., Driedger, A.M. & Mariñas, B.J. (2002). Sequential inactivation of Cryptosporidium parvum oocysts with chlorine dioxide followed by free chlorine or monochloramine. *Water Research*, *36*(1), 178-188.

36. Cosgrove, W.J. & Loucks, D.P. Water management: Current and future challenges and research directions. *Water Resources Research*, 51(6), pp.4823-4839. Available at: https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1002/2014WR016869.

37. Dao, H.P., Mathieu, F. & Lebrihi, A. (2005). Two primer pairs to detect OTA producers by PCR method. *International Journal of Food Microbiology*, 104(1), pp.61-67.

38. Doull, J., Andelman, J.B., Buhler, D.R., Characklis, W.G., Christman, R.F., Cohen, S.D., Engelbrecht, R.S., Hayes, A.W., Hughes, J.M., Olivieri, V.P., Pike, M.C., Schnell, R.C., Street, J.C.& Tate, C.H. (1982). Drinking water and health. National Research Council (US) Safe Drinking Water Committee, Washington (DC): *National Academies Press (US),* 4, pp. 1-312

39. Douterelo, I., Boxall, J.B., Deines, P., Sekar, R., Fish, K.E. & Biggs, C.A. (2014a). Methodological approaches for studying the microbial ecology of drinking water distribution systems. *Water research*, *65*, 134-156.

40. Douterelo, I., Husband, S. & Boxall, J.B. (2014b). The bacteriological composition of biomass recovered by flushing an operational drinking water distribution system. *Water Research*, 54, pp.100-114.

41. DWAF (1996). South African Water Quality Guidelines: Domestic Water Use. *Department of Water Affairs and Forestry*, 1(2nd ed.), pp.1-197.

42. DWS (2015). Has the Blue or Green Drop status truly aided in the delivery of safe. *Department of Water and Sanitation*, (February), pp.2012-2014.

43. Earle, A., Goldin, J. & Kgomotso, P. (2005). Domestic Water Provision in the DemocraticSouth Africa – changes and challenges. *Nordic Africa Institute's Conflicting Forms of Citizenship Programme*, pp.1-40.

44. El-Chakhtoura, J. et al. (2015). Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. *Water Research*, 74(0), pp.180-190.

45. EPA (2013). Water Treatment Manual : Disinfection. *The Environmental Protection Agency-Ireland*, (11/11/1000), pp.1-200.

46. Fish, K.E., Collins, R., Green, N.H., Sharpe, R.L., Douterelo, I., Osborn, A.M. & Boxall, J.B. (2015). Characterisation of the physical composition and microbial community structure of biofilms within a model full-scale drinking water distribution system. *PLoS One*, *10*(2), e0115824.

47. Fox, A.R., Houser, K.H., Morris, W.R., & Walton, R.C. (2016). Dematiaceous fungal endophthalmitis: report of a case and review of the literature. *Journal of Ophthalmic Inflammation and Infection*, *6*(1), 43.

48. Freese, S.D. & Nozaic, D.J. (2004.) Chlorine: Is it really so bad and what are the alternatives? *Water SA*, 30(5), pp.566-572.

49. Frey-Klett, P. et al. (2011). Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol Mol Biol Rev*, 75(4), pp.583-609. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22126995.

50. Gashgari, R.M., Elhariry, H.M. & Gherbawy, Y.A (2013). Molecular Detection of Mycobiota in Drinking Water at Four Different Sampling Points of Water Distribution System of Jeddah City (Saudi Arabia). *Geomicrobiology Journal*, 30(1), pp.29-35.

51. Gonçalves, A.B., Paterson, R.R.M. & Lima, N. (2006). Survey and significance of filamentous fungi from tap water. *International Journal of Hygiene and Environmental Health*, 209(3), pp.257-264.

52. Göttlich, E., Van der Lubbe, W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., ... & de Hoog, S. (2002). Fungal flora in groundwater-derived public drinking water. *International journal of hygiene and environmental health*, *205*(4), 269-279.

53. Grabińska-Łoniewska, A., Konieczny-Kowalska, T., Wardzyńska, G. & Boryn, K. (2007). Occurrence of Fungi in Water Distribution System. *Polish Journal of Environmental Studies*, *16*(4).

54. Grossart, H.P., Wurzbacher, C., James, T.Y. & Kagami, M. (2016). Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. *Fungal Ecology*, *19*, 28-38.

55. Hageskal, G., Gaustad, P., Heier, B.T. & Skaar, I. (2007). Occurrence of moulds in drinking water. *Journal of applied microbiology*, *102*(3), 774-780.

56. Hageskal, G., Knutsen, A.K., Gaustad, P., De Hoog, G.S. & Skaar, I. (2006). Diversity and significance of mold species in Norwegian drinking water. *Applied and Environmental Microbiology*, *72*(12), 7586-7593.

57. Hageskal, G., Lima, N. & Skaar, I. (2009). The study of fungi in drinking water. *Mycological Research*, 113(2), pp.165-172.

58. Hageskal, G., Tryland, I., Liltved, H. & Skaar, I. (2012). No simple solution to waterborne fungi: various responses to water disinfection methods. *Water Science and Technology: Water Supply*, *12*(2), 220-226.

59. Housewright, R.D. et al. (1982). *Drinking Water and Health, Volume 4.*

60. Hundalani, S. & Pammi, M. (2013). Invasive fungal infections in newborns and current management strategies. *Expert Review of Anti-infective Therapy*, 11(7), pp.709-721. Available at: https://doi.org/10.1586/14787210.2013.811925.

61. Hurtado-McCormick, S., Sanchez, L., Martínez, J., Calderón, C., Calvo, D., Narvaez, D., ... & Rodriguez-Susa, M. (2016). Fungi in biofilms of a drinking water network: Occurrence, diversity and mycotoxins approach. *Water Science and Technology: Water Supply*, *16*(4), 905-914.Hussein, H.. & Brasel, J.., 2001. Toxicity, metabolism and impact of mycotoxins on human and animals. *Toxicology,*, 167, pp.101-134.

62. Jun, N.A.N., Weipeng, H.E., Xinin, S.O.N.G. & Guibai, L. I. Impact of dynamic distribution of floc particles on flocculation e ect. *Journal of Environmental Sciences*, *21*(8), 1059-1065.

63. Kamili, A.N., Ganai, B.A., Saleem, S., Lone, B.A. & Nissa, H. (2012). First qualitative survey of filamentous fungi in Dal Lake, Kashmir. *Journal of Yeast and Fungal Research*, *3*(1), 7-11.

64. Kamika, I., Ngbolua, K. te N. & Tekere, M (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. *Food Control*, 69, pp.292-296. Available at: http://dx.doi.org/10.1016/j.foodcont.2016.05.014.

65. Kanzler, D., Buzina, W., Paulitsch, A., Haas, D., Platzer, S., Marth, E. & Mascher, F. (2008). Occurrence and hygienic relevance of fungi in drinking water. *Mycoses*, *51*(2), 165-169.

66. Khan, M.S.A., Ahmad, I., Aqil, F., Owais, M., Shahid, M. & Musarrat, J. (2010). Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans. In *Combating Fungal Infections* (pp. 21-45). Springer, Berlin, Heidelberg.

67. Kinsey, G., Paterson, R. & Kelley, J. (2003). Filamentous fungi in water systems. In *Handbook of Water and Wastewater Microbiology*. pp. 77-98.

68. Kinsey, G.C., Paterson, R.R. & Kelley, J. (1998). Methods for the determination of filamentous fungi in treated and untreated waters. *Journal of applied microbiology*, 85 Suppl 1, p.214S-224S.

69. Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008). Ainsworth's & Bisby's. *Dictionary of the Fungi (10th ed.). CAB International, Oxon, xi*.

70. Korzeniewska, E. (2011). Emission of bacteria and fungi in the air from wastewater treatment plants – a review Ewa. *Bioscience reports*, 1(3), pp.393-407.

71. Kuhn, D.M. & Ghannoum, M.A. (2003). Indoor Mold , Toxigenic Fungi , and Stachybotrys chartarum : Infectious Disease Perspective Fungal Organisms in Damp Buildings. , 16(1), pp.144-172.

72. Lee, J., Jun, M.J., Lee, M.H., Lee, M.H., Eom, S.W., & Zoh, K.D. (2010). Production of various disinfection byproducts in indoor swimming pool waters treated with different disinfection methods. *International Journal of Hygiene and Environmental health*, *213*(6), 465-474.

73. Lehner, S.M., Neumann, N.K.N., Sulyok, M., Lemmens, M., Krska, R. & Schuhmacher, R. (2011). Evaluation of LC-high-resolution FT-Orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food. *Food Additives & Contaminants: Part A*, *28*(10), 1457-1468.

74. Lim, M.Y., Kim, J.M. & Ko, G. (2010). Disinfection kinetics of murine norovirus using chlorine and chlorine dioxide. *Water Research*, 44(10), pp.3243-3251. Available at: http://dx.doi.org/10.1016/j.watres.2010.03.003.

75. Luu, S., Cruz-Mora, J., Setlow, B., Feeherry, F.E., Doona, C.J., & Setlow, P. (2015). The effects of heat activation on Bacillus spore germination, with nutrients or under high pressure, with or without various germination proteins. *Applied and Environmental Microbiology*, *81*(8), 2927-2938.

76. Luyt, C.D., Tandlich, R., Muller, W.J. & Wilhelmi, B.S. (2012). Microbial monitoring of surface water in South Africa: an overview. *International Journal of Environmental Research and Public Health*, *9*(8), 2669-2693

77. Magwaza, N., Nxumalo, E., Mamba, B. & Msagati, T. (2017). The occurrence and diversity of waterborne fungi in African aquatic systems: their impact on water quality and human health. *International Journal of Environmental Research and Public Health*, *14*(5), 546

78. Mains, C. (2008). Biofilm Control in Distribution Systems. *Biofilm Control in Distribution Systems*, 8(2), pp.1-4.

79. Manivanan, R. (2008). *Water Quality Modeling: Rivers, Streams, and Estuaries*, New India Publishing.

80. Marques, R.D.O., Seckler, S. & Filho, F. (2017). Flocculation kinetics of low-turbidity raw water and the irreversible floc breakup process. *Environmental Technology*, 37(7), pp.901-910.

81. Mata, A.T., Ferreira, J.P., Oliveira, B.R., Batoréu, M.C., Crespo, M.B., Pereira, V.J. & Bronze, M.R. (2015). Bottled water: analysis of mycotoxins by LC-MS/MS. *Food Chemistry*, *176*, 455-464.

82. Maza-Márquez, P., Vilchez-Vargas, R., Kerckhof, F.M., Aranda, E., González-López, J. & Rodelas, B. (2016). Community structure, population dynamics and diversity of fungi in a full-scale membrane bioreactor (MBR) for urban wastewater treatment. *Water Research*, *105*, 507-519.

83. Mazumder, P.M. & Sasmal, D. (2001). Mycotoxins – limits and regulations. *Ancient science of life*, *20*(3), 1.

84. Meirelles, G.C., Pippi, B., Hatwig, C., Barros, F., De Oliveira, L.F., Poser, G.L.V. & Fuentefria, A.M. (2017). Synergistic antifungal activity of the lipophilic fraction of Hypericum carinatum and fluconazole. *Revista Brasileira de Farmacognosia*, *27*(1), 118-123.

85. Memon, N.A. (2012). Isolation of Fungi in the Drinking Water Distribution System of Hyderabad (Pakistan). *quaid -E- AWAM University Research Journal of Engineering, Science and Technology*, 11(1), pp.6-9.

86. Mengelers, M.J.B. & Van Eijkeren, J.C.H. (2016). Estimation of the dietary intake of mycotoxins by means of measurements in human urine: The application of toxicokinetic models for the estimation of renal mycotoxin excretion. RIVM briefrapport 2015-0213.

87. Milani, J.M. (2013). Ecological conditions affecting mycotoxin production in cereals: a review. *Veterinarni Medicina*, *58*(8).

88. Mille-lindblom, C. (2005). *Interactions between Bacteria and Fungi on Aquatic Detritus – Causes and Consequences*. Uppsala University.

89. Mille-Lindblom, C., Fischer, H. & Tranvik, L.J. (2006). Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *OIKOS*, 113(2), pp.233-242.

90. Momba, M.N., Obi, C. & Thompson, P. (2008). *Improving Disinfection Efficiency in Small Drinking Water Treatment Plants*, Pretoria.

91. Money, N.P. (2016). Fungal diversity. In S. C. Watkinson, L. Boddy, & N. P. Money, eds. *The Fungi*. Academic press, pp. 1-36.

92. Mukherjee, S. (2012). Aflatoxin Effect On Health. In *USAID/East Africa*.

93. Mulamattathil, S.G., Bezuidenhout, C. & Mbewe, M. (2014). Biofilm formation in surface and drinking water distribution systems in Mafikeng, South Africa. *South African Journal of Science*, 110(11-12), pp.1-9.

94. Murphy, H.M., Payne, S.J. & Gagnon, G.A. (2008). Sequential UV- and chlorine-based disinfection to mitigate Escherichia coli in drinking water biofilms. *Water Research*, 42(8-9), pp.2083-2092.

95. Nasser, L.A. (2003). Distribution of Zoosporic and Terrestrial Fungi in Accumulated Rainfal Water in ABHA, South Western Region, Saudi Arbia. *Journal of Biological Sciences*, 3(9), pp.843-853.

96. Nazim, S., Dawar, S.H.A.H.N.A.Z., Tariq, M.A.R.I.U.M. & Zaki, M.J. (2008). Quantitative estimation of mycoflora in drinking water and fruit juices of Karachi. *Pakistan Journal Botany*, *40*(3), 1263-1268.

97. Obi, C.L., Igumbor, J.O., Momba, M.N.B. & Samie, A. (2008). Interplay of factors involving chlorine dose, turbidity flow capacity and pH on microbial quality of drinking water in small water treatment plants. *Water SA*, *34*(5), 565-572.

98. O'Connor, J.T. and O'Connor, T.L. (2001). Removal of Microorganisms by Rapid Sand Filtration. *H2O'C Engineering, Columbia, Mo*. pp.1-19

99. Okpako, E. C., Osuagwu, A. N., Duke, A. E., & Ntui, V. O. (2009). Prevalence and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria. *African Journal of Microbiology Research*, *3*(2), 56-61.

100. Oliveira, B.R., Crespo, M.B., San Romão, M.V., Benoliel, M.J., Samson, R.A. & Pereira, V.J. (2013). New insights concerning the occurrence of fungi in water sources and their potential pathogenicity. *Water Research*, *47*(16), 6338-6347.

101. Oliveira, H., Santos, C., Paterson, R.R.M., Gusmão, N.B. & Lima, N. (2016). Fungi from a groundwater-fed drinking water supply system in Brazil. *International Journal of Environmental Research and Public Health*, *13*(3), 304.

102. Organization, World Health. (2004). *Guidelines for Drinking-water Quality: Recommendations, Volume 1*, World Health Organization. Available at: https://books.google.com/books?id=SJ76COTm-nQC&pgis=1 [Accessed January 20, 2016].

103. Oyegbile, B., Ay, P. & Satyanarayana, N. (2016). Flocculation kinetics and hydrodynamic interactions in natural and engineered flow systems : A review. *Environmental Engineering Research*, 49, pp.1-48.

104. Page, M.A., Shisler, J.L. & Mariñas, B.J. (2009). Kinetics of adenovirus type 2 inactivation with free chlorine. *Water Research*, 43(11), pp.2916-2926. Available at: http://dx.doi.org/10.1016/j.watres.2009.03.047.

105. Parks, T.M. (2016). City of Johannesburg State of the City Address 2016 , Delivered By the Executive. *City of Johannesburg State*, (May), pp.1-23.

106. Partida-Martinez, L.P. et al. (2007). Rhizonin, the first mycotoxin isolated from the zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. *Applied and Environmental Microbiology*, 73(3), pp.793-797.

107. Paterson, R.R., Kelley, J. & Gallagher, M. (1997). Natural occurrence of aflatoxins and Aspergillus flavus (Link) in water. *Letters in Applied Microbiology*, 25(6), pp.435-436.

108. Paterson, R.R.M. & Lima, N. (2015). *Molecular Biology of Food and Water Borne Mycotoxigenic and Mycotic Fungi*, CRC Press.

109. Pellon, A., Ramirez-Garcia, A., Buldain, I., Antoran, A., Martin-Souto, L., Rementeria, A. & Hernando, F.L. (2018). Pathobiology of Lomentospora prolificans: could this species serve as a model of primary antifungal resistance?. *International Journal of Antimicrobial Agents*, *51*(1), 10-15.

110. Peralta, R.M. et al (2017). Enzymes from basidiomycetes: peculiar and efficient tools for biotechnology. In G. Brahmachari, A. L. Demain, & J. L. Adrio, eds. *Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications*. Academic Press, London, pp. 119-149.

111. Peralta, R.M., Da Silva, B.P., Côrrea, R.C.G., Kato, C.G., Seixas, F.A.V. & Bracht, A. (2017). Enzymes from Basidiomycetes—Peculiar and Efficient Tools for Biotechnology. In *Biotechnology of Microbial Enzymes* (pp. 119-149).

112. Pereira, V.J., Basílio, M.C., Fernandes, D., Domingues, M., Paiva, J.M., Benoliel, M. J., ... & San Romão, M.V. (2009). Occurrence of filamentous fungi and yeasts in three different drinking water sources. *Water Research*, *43*(15), 3813-3819.

113. Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J. & Crespo, M.B. (2013). Free chlorine inactivation of fungi in drinking water sources. *Water Research*, *47*(2), 517-523.

114. Pfaller, M.A. & Diekema, D.J. (2004). Rare and Emerging Opportunistic Fungal Pathogens : Concern for Resistance beyond Candida albicans and Aspergillus MINIREVIEW Rare and Emerging Opportunistic Fungal Pathogens : Concern for Resistance beyond Candida albicans and Aspergillus fumigatus. *JouRnal of Clinical Microbiology*, 42(10), pp.4419-31.

115. Pitt, J.I., Basilico, J.C., Abarca, M.L. & Lopez, C. (2000a). Mycotoxins and toxigenic fungi. *Medical Mycology*, *38*(sup1), 41-46.

116. Pitt, J.I. (2000b). Toxigenic fungi and mycotoxins. *British medical bulletin*, *56*(1), 184-192.

117. Plummer, J.D., Long, S.C., Charest, A.J. & Roop, D.O. (2014). Bacterial and viral indicators of fecal contamination in drinking water. *Journal-American Water Works Association*, *106*(4), E200-E211.

118. Puel, O., Galtier, P. & Oswald, I.P. (2010). Biosynthesis and toxicological effects of patulin. *Toxins*, 2(4), pp.613-631.

119. Rand Water (2008). *Sustainability Report.*

120. Rasteiro, M.G., Garcia, F.A., Hunkeler, D. & Pinheiro, I. (2016). Evaluation of the performance of dual polyelectrolyte systems on the re-flocculation ability of calcium carbonate aggregates in turbulent environment. *Polymers*, *8*(5), 174.

121. Rivett, U., Champanis, M. & Wilson-jones, T. (2012). Monitoring drinking water quality in South Africa : Designing information systems for local needs. , 39(3), pp.409-414.

122. Rojas-Valencia, M.N. (2011). Research on ozone application as disinfectant and action mechanisms on wastewater microorganisms. *Science against microbial pathogens: Communicating Current Research and Technological Advances*, pp.263-271.

123. Sammon, N.B., Harrower, K.M., Fabbro, L.D. & Reed, R.H. (2010). Incidence and distribution of microfungi in a treated municipal water supply system in sub-tropical Australia. *International Journal of Environmental Research and Public Health*, *7*(4), 1597-1611.

124. Sammon, N.B., Harrower, K.M., Fabbro, L.D. & Reed, R.H. (2011). Three potential sources of microfungi in a treated municipal water supply system in sub-tropical Australia. *International Journal of Environmental Research and Public Health*, *8*(3), 713-732.

125. SANS (2015). SANS 241-1 : 2015 South African National Standard – Drinking water Part 1. *SABS Standards Division*, (2), pp.1-5.

126. Šegvić, K.M., Rašić, D. & Peraica, M. (2013). Deleterious effects of mycotoxin combinations involving Ochratoxin A. *Toxins*, 5(11), pp.1965-1987.

127. Shadanaika, M. (2005). *Mycotoxigenic Fungi in Spices: Molecular Methods of Detection and Control* (Doctoral dissertation, University of Mysore).

128. Silva, S. & Henriques, M. (2017). Candida glabrata biofilms: how far have we come?. *Journal of fungi*, *3*(1), 11.

129. Siqueira, V., Oliveira, H., Santos, C., Paterson, R.R., Gusmão, N. & Lima, N. (2011a). Filamentous fungi in drinking water, particularly in relation to biofilm formation. *International Journal of Environmental Research and Public Health*, *8*(2), 456-469.

130. Siqueira, V.M. (2011). Characterising filamentous fungal biofilm in drinking water distribution systems using microscopic and molecular techniques. Universidade do Minho Escola de Engenharia Virgínia Medeiros de Siqueira.

131. Sisti, M., Brandi, G., De Santi, M., Rinaldi, L. and Schiavano G.F. (2012). Disinfection efficacy of chlorine and peracetic acid alone or in combination against Aspergillus spp. and Candida albicans in drinking water. Journal of Water and Health, 10(1), pp.11-19.

132. Sonigo, P., De Toni, A. & Reilly, K. (2011). A Review of Fungi in Drinking Water and the Implications for Human Health. *DEFRA*, 33(0), pp.1-107.

133. South Africa Water Act (1998) No. 36 of' 1998: Government Gazette.

134. Speight, V. (2002). Distribution Systems : The Next Frontier. *Malcolm Pirnie, Inc.*, pp.1-10.

135. Spellman, F.R. (2014). *Handbook of Water and Wastewater Treatment Plant Operations* 3rd ed., Taylor & Francis Group.

136. Sulaiman, I.M., Jacobs, E., Simpson, S. & Kerdahi, K. (2014). Molecular identification of isolated fungi from unopened containers of Greek yogurt by DNA sequencing of internal transcribed spacer region. *Pathogens*, *3*(3), 499-509.

137. Tekere, M., Lötter, A., Olivier, J., Jonker, N. & Venter, S. (2011). Metagenomic analysis of bacterial diversity of Siloam hot water spring, Limpopo, South Africa. *African Journal of Biotechnology*, *10*(78), 18005-18012.

138. Thliza, I.A., Khan, A.U. & Dangora, D.B. (2015). Fungi contamination of some selected brands of sachet water marketed in Ahmadu Bello University, Zaria, Nigeria. *Journal of Microbiology Research*, *5*(1), 23-30.

139. Thupaki, P., Phanikumar, M.S., Schwab, D.J., Nevers, M.B. & Whitman, R.L. (2013). Evaluating the role of sediment-bacteria interactions on Escherichia coli concentrations at beaches in southern Lake Michigan. *Journal of Geophysical Research: Oceans*, *118*(12), 7049-7065.

140. Tinker, S.C., Moe, C.L., Klein, M., Flanders, W.D., Uber, J., Amirtharajah, A., ... & Tolbert, P.E. (2009). Drinking water residence time in distribution networks and emergency department visits for gastrointestinal illness in Metro Atlanta, Georgia. *Journal of Water and Health*, *7*(2), 332-343.

141. Van Zyl, J.E (2014). *Introduction to operation and maintenance of water distribution systems*. Water Research Commission, South Africa

142. Volk, T.J. (2013). Fungi. *Encyclopedia of Biodiversity*, 3, pp.624-640. Available at: http://linkinghub.elsevier.com/retrieve/pii/B9780123847195000629.

143. Warris, A., Voss, A., Abrahamsen, T.G., & Verweij, P.E. (2002). Contamination of hospital water with *Aspergillus fumigatus* and other molds. *Clinical Infectious Diseases*, *34*(8), 1059-1060.

144. Welke, J.E., Hoeltz, M., Dottori, H.A. & Noll, I.B. (2009). Effect of processing stages of apple juice concentrate on patulin levels. *Food Control*, *20*(1), 48-52.

145. WHO (2011a). Mycotoxins. *Children's Health and the Environment: WHO Training Package for the Health Sector World Health Organization*, pp.1-42. Available at: www.who.int/ceh.

146. WHO (2011b). WHO guidelines for drinking-water quality. *World Health Organization*, 4th ed, pp.104-108.

147. WHO (2017). Guidelines for Drinking-water Quality. *World Health Organization*, (4th edition incorporating the 1st addendum), pp.1-631.

148. WRC (2002(. Quality of Domestic Water Supplies: Treatment Guide. *Water Research Commission No: TT 181/02*, 4(1), pp.1-99.

149. Yamaguchi, M.U., Rampazzo, R.D.C.P., Yamada-Ogatta, S.F., Nakamura, C.V., Ueda-Nakamura, T. & Dias Filho, B.P. (2007). Yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies. *Brazilian Archives of Biology and Technology*, *50*(1), pp.1-9.

150. Zain, M.E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), pp.129-144.14.

APPENDIX A: PUBLISHED REVIEW PAPER

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 need for the inclusion in treated drinking water quality regulations.

Keywords: fungi, mycotoxins, mycotoxigenic fungi, public health, treated drinking water

Mhlongo, N.T., Tekere, M. and Sibanda, T. **(2018)** Prevalence and public health implications of mycotoxigenic fungi in treated drinking water systems. **Journal of Water and Health** (Accepted).