OPPORTUNISTIC FUNGAL PATHOGENS IN THE PLANKENBRUG/EERSTERIVIER SYSTEM WITHIN THE STELLENBOSCH REGION

Report to the

Water Research Commission

by

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WRC Report No. 3101/1/23
ISBN 978-0-6392-0558-8

November 2023

WATER RESEARCH COMMISSION

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This is the final report of WRC project no. C2019-2020-00137.

EXECUTIVE SUMMARY

PROJECT BACKGROUND

The AIDS pandemic is associated with a global increase in the incidence of mycosis, a disease caused by several opportunistic pathogenic fungi, which normally reside in the natural environment. Knowledge of the environmental prevalence and interactions of these opportunistic fungal pathogens is thus important to ameliorate their impact on humans, especially on the poor living in ever expanding informal settlements. The rapid expansion of these informal settlements on the outskirts of cities and towns has resulted in an increase in the volume of untreated polluted urban runoff that flows into various river systems. Although such polluted waters are known to harbour numerous opportunistic pathogenic fungi, including yeasts, relatively little is known about the occurrence and interactions of these fungi in South African urban river systems. A better understanding of the presence and environmental behaviour of these clinically relevant fungi is essential for the development of strategies to manage this threat. This study is therefore aimed at determining the presence, identity, and antifungal resistance of potentially pathogenic fungi, especially clinically relevant yeast species, in the Plankenbrug / Eersterivier system in Stellenbosch. In addition, the health risks associated with using the river water for domestic use and drinking is exemplified here through a provisional quantitative microbial risk assessment (QMRA).

AIMS

The aims for the project:

1. To provide a literature review on existing knowledge of opportunistic fungal pathogens in South African surface waters and the potential effects of pollution and rising environmental temperatures on these fungi.
2. To determine the presence and concentrations of opportunistic pathogenic fungi within different sections of the Plankenbrug / Eersterivier system as well as report on the level of water pollution by means of physicochemical and bacterial indicator data.
3. To determine the antifungal sensitivity of opportunistic pathogenic fungi originating from the different sections in the river system, which are subjected to different levels of pollution.
4. Carry out quantitative microbial risk assessment (QMRA) to determine the risk of yeast infections after exposure to the river water.
5. Provide recommendations for routine monitoring for potential pathogenic fungi to serve as an early warning to the emergence of fungal infections.

METHODOLOGY

A literature review was compiled in order to report on the presence of opportunistic and pathogenic fungi present in surface water sources, such as rivers, both globally and in South Africa. The review also aimed at addressing the effects of pollution as well as rising environmental temperatures on the emergence and / or occurrence of these microorganisms in the aquatic environment. Additionally, this literature review was compiled in preparation for the experimental work that was carried out for this study.

River water sampling was carried out in Stellenbosch, South Africa. River sections varying in pollution levels were selected in order to evaluate the potential associations between polluted environments and fungal contaminants. Membrane filtration and thermally selective incubation was employed to enumerate and isolate potentially pathogenic yeast species. Additionally, high throughput sequencing was applied on one occasion to investigate and compare the presence of pathogenic fungi in the river water using molecular and culture-based methods. Metagenomic analyses of the high throughput sequence results revealed the viable whole-community fungal composition of the river water, in which some pathogenic species were detected. Yeasts isolated from the river water were identified using 28S rDNA sequencing and phylogenetic analyses. Subsequently, the cultured yeasts identified as representatives of known pathogenic species were subjected to antifungal susceptibility testing to determine the level of antifungal resistance among the isolates against commonly used antifungal drugs, i.e. Amphotericin B (AmB) and Fluconazole (FLU). Finally, the concentrations of culturable pathogenic yeasts species in the river water were used to carry out a quantitative microbial risk assessment (QMRA) for yeasts present in the river system. This was achieved using the limited available literature detailing the pathogenic potential of a select few yeast species.

RESULTS AND DISCUSSION

The threats that opportunistic and pathogenic fungi pose due to their potential to acquire thermotolerance and proliferate in polluted environments (Figure 2.1) were discussed in the literature review. It was highlighted that a lack of knowledge exists on the occurrence and biology of fungi in polluted surface waters, especially in developing countries burdened with many immunocompromised individuals, who are known to be highly susceptible to fungal infections.

Worryingly, using metagenomic analysis and culture-based methods, it was found that pathogenic fungal species occur in the river system that was studied. The resolution power of the culture-based methods was found to be higher for detection of pathogenic yeast species, compared to the metagenomic analysis. On the other hand, the metagenomic analysis revealed that the fungal composition between highly polluted and less polluted waters were significantly different, which was not observed when the thermotolerant culturable yeast populations were analysed. It was however observed that culturable yeast concentrations increase greatly during rainy seasons, concomitantly with the increase in predominance of specific yeast species. It was contended that natural factors, other than increased oxygen concentrations, could play a major role in fungal species predominance in these waters.

The presence of clinically relevant yeast species prompted further investigation to determine their antifungal susceptibility profiles. As such a few isolates resistant to FLU were recovered suggesting that these isolates either acquired resistance within these polluted environments or originated from patients treated with antifungal drugs. Consequently, the presence of these fungi in the water necessitated further investigations into the potential risk of infections via river water ingestion. As such, a provisional QMRA study was carried out providing the first quantified risk of yeast infection from drinking river water. Overall, this study gave insight into the potential health risks associated with exposure to polluted

surface water, especially focussing on the threat that fungal species have on immunocompromised individuals.

GENERAL

All of the aims of this study were achieved by reporting the presence of opportunistic and pathogenic fungi in river water; evaluating the level of pollution in these waters and their possible associations with fungi; investigating the antifungal susceptibility profiles of the pathogenic yeasts detected in the river water; and finally highlighting the health risks associated with river water exposure through a provisional QMRA for yeast infections. Moreover, recommendations for future research highlighted methods that could be employed to strengthen the quantification and detection of pathogenic fungal species as well as improve on the QMRA of yeast infections.

CONCLUSIONS

Combinatorial effects of global warming and increasing pollution in river water might promote the emergence of mycoses cases in developing countries such as South Africa. This is especially concerning due to the large population of immunocompromised people residing in these countries. Moreover, many of these individuals form part of communities that live in rural areas which primary water sources are nearby rivers. These environments, due to pollution, have been burdened with microbial contaminants. This study revealed that various opportunistic and pathogenic fungi are present in these environments. Metagenomic analyses indicated that fungal composition differs between highly and less polluted waters. Furthermore, the isolation of yeast species from these waters have indicated that natural factors such as higher dissolved oxygen levels might affect the growth and distribution of yeast species. These observations prompt further investigation into the physiology and interactions of fungal species in polluted environments. Moreover, the correlation observed between opportunistic pathogenic species, such as *C. glabrata* and *C. lusitaniae*, and pollution indicators suggests possible pollution associated recurrence. The presence of fungal pathogens in these waters also suggests that there is a risk of fungal infection; however, this risk requires quantification in order to fully determine the level of the threat. This study therefore employed a QMRA for yeast infections when river water is used for drinking. Despite the limited data available to perform such an assessment, this study calculated the risk which indicated that the risk of infection is higher when consuming more polluted water. Overall, this study highlighted the risks associated with exposure to polluted river water, focussing on the impact of opportunistic and pathogenic fungi.

RECOMMENDATIONS AND FUTURE RESEARCH

The final objective of our study was to provide recommendations for the monitoring of opportunistic and pathogenic fungi in river water to evaluate any signs of health risks that should be considered. Based on the results reported in the experimental chapter, it became evident that a standard procedure for fungal pathogen detection is direly needed. We recommend that future studies focus on quantifying fungi on a species-specific level. With current available technologies, the most feasible approach is to employ molecular techniques. However, we also recommend conducting more studies similar to ours which employed both molecular and culture-based techniques in order to be able to study the

physiology of the opportunistic or pathogenic fungi occurring in the polluted water. This would lay the foundations for an improved QMRA focussing on clinically relevant fungi in future. These research opportunities would greatly contribute to future monitoring efforts for mitigating the threat that fungi pose in polluted river water.

ACKNOWLEDGEMENTS

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

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ACRONYMS and ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
AmB	Amphotericin B
BLAST	Basic Local Alignment Search Tool
CCS	Circular Consensus Sequences
COD	Chemical Oxygen Demand
COVID-19	Corona Virus Disease
CWI	Cell Wall Integrity
DER	Downstream Eerste River
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
EMA	Ethidium monoazide
ERC	Eerste River Catchment
ESR	Environmental Stress Response
FLU	Fluconazole
HIV	Human Immunodeficiency Virus
HP	Highly Polluted
HSP	Heat Shock Protein
HSR	Heat Shock Response
Htg^+	High temperature growth phenotype
ITS	Internal Transcribed Spacer
KR	Krom River
LP	Less Polluted
LSU	Large Subunit
MBA	Molybdate Agar
MIC	Minimum Inhibitory Concentration
MLGA	Membrane Lactose Glucuronide Agar
NCBI	National Centre for Biotechnology Information
OTU	Operational Taxonomic Unit
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PMA	Phosphomolybdic acid
PP	Pathogenic Potential
PR	Plankenbrug River
QMRA	Quantitative Microbial Risk Assessment
RNA	Ribonucleic acid
SANS	South African National Standards
SDA	Sabouraud Dextrose Agar
SEM	Standard Error of Means
T_{max}	Maximum Temperature of Growth
UER	Upstream Eerste River
UNAIDS	United Nations Programme on HIV / AIDS
WWTP	Wastewater Treatment Plant
YMA	Yeast Malt extract Agar

GLOSSARY

Allochthonous – denoting a microorganism that originated at a different environmental location.

Anthropogenic environments – environments that were created by humans, such as buildings.

Antifungal resistance – an acquired or intrinsic trait of a fungus that allows growth in the presence of an antifungal agent

Chimeric sequences – artifacts in sequences that results due to faulty PCR

Climate Change – change in global climate patterns such as unusual seasonal changes, natural disasters, etc.

Dose response model – examines the response of an agent (microbial) within a host

Exposure assessment – a process during which the route, frequency, and magnitude a specific agent is exposed host is evaluated

Global Warming – increase in atmospheric temperatures induced by anthropogenic activities including industrialisation, urbanisation, and farming, all of which increase the release of greenhouse gasses into the atmosphere.

High-throughput sequencing – parallel sequencing in order to investigate whole-community data sets

Immunocompromised – lacking proper immune response to eliminate infectious propagules.

Invasive infection – infection that enters blood stream and can disseminate to the rest of the body.

Law of Parsimony – also known as Occam's Razor, here interpreted to mean that investigating a less complex system would reduce the number of assumptions made.

Mass-specific metabolic rate – the rate at which an organism uses energy per gram body weight.

Metagenomics – study of genetic material that is of environmental origin

Microevolution – changes in organisms over short period of time (decades instead of millennia).

Multivariate correlations – linear correlations between a variable and a set of other variables

Mycoses (singular, mycosis) – diseases caused by fungal species.

Opportunistic (fungi) – ability of a microorganism generally regarded as safe to cause infection in immunocompromised individuals.

Polyphyletic – a group of organisms with similar traits without common ancestor.

Relative abundance – the abundance of a particular species / genus / phylum relative to the total number of the respective group

Species richness – denotes the number of species identified in a sample / region.

Subcutaneous infection – infection that breaches skin barrier but does not enter blood stream.

Superficial infection – infection that primarily occurs on surface of skin.

Thermal pollution (water) – increase in ambient temperatures of water sources caused by anthropogenic activities.

Thermal restriction zone – a temperature threshold that prevents many fungal species from surviving within mammalian species.

Thermotolerant (fungi) – ability of a fungus to grow at temperatures higher than or equal to mammalian body temperatures.

CHAPTER 1: BACKGROUND

1.1. PROBLEM STATEMENT

The state of rivers in South Africa can have a negative impact on the health of many individuals who are dependent on river water for domestic purposes. Generally, health risks associated with polluted surface waters are determined by analysing the bacterial or viral load within these waters; however, very little focus is given to other microorganisms, such as opportunistic and pathogenic fungi. The information regarding clinically relevant fungi present in aquatic environments is therefore limited. Moreover, little is understood regarding the association between fungi and polluted water sources. Although a few studies have investigated the above-mentioned, more surveys are needed to draw any definitive conclusions about the association between fungal populations and pollution. In addition, research on the thermotolerant nature of fungal species identified in rivers and many other natural environments can provide insight on the potential for harmless fungi to emerge as human pathogens in the future. This entails studies on the effects of global warming on microevolution of fungi to gain thermotolerance and thus increase the chances for infection in mammalian species. Considering that higher temperatures are one of the major obstacles preventing fungi from causing infections, determining the potential of fungi to become thermotolerant is crucial. It is therefore important that the role of opportunistic pathogenic fungi in resource environments such as rivers be investigated in order to emphasise the potential for these microorganisms to exacerbate fungal disease rates in developing countries such as South Africa.

1.2. AIMS

The aims for the project:

1. To provide a literature review on existing knowledge of opportunistic fungal pathogens in South African surface waters and the potential effects of pollution and rising environmental temperatures on these fungi.
2. To determine the presence and concentrations of opportunistic pathogenic fungi within different sections of the Plankenbrug / Eersterivier system as well as report on the level of water pollution by means of physicochemical and bacterial indicator data.
3. To determine the antifungal sensitivity of opportunistic pathogenic fungi originating from the different sections in the river system, which are subjected to different levels of pollution.
4. Carry out quantitative microbial risk assessment (QMRA) to determine the risk of yeast infections after exposure (ingestion) to the river water.
5. Provide recommendations for routine monitoring for potential pathogenic fungi to serve as an early warning to the emergence of fungal infections.

1.3. SCOPE OF STUDY

1.3.1. Literature review – Chapter 2

Reporting the presence of the clinically relevant fungi that have been identified in various water sources across the globe required extensive research. Two major points were considered while listing these fungi: specific water source from which the fungus was isolated (in this review focus was given to natural surface waters) and determining clinical relevance (based on clinical cases and literature). Additionally, the level of pollution of the studied water sources was noted to emphasise the current understanding concerning the association between pollution and fungal numbers. Regarding the impact on rising environmental temperatures, a holistic approach was applied to address various points of discussion such as: activities leading to increase in global temperatures, rising air temperatures linked to increased river water temperature, natural origin of thermally tolerant fungi and molecular processes within fungi allowing adaptation to increased temperatures. Lastly, the combinatorial effects of both global warming and polluted surface waters were interpreted and discussed.

1.3.2. Experimental work – Chapter 3

The large population of immunocompromised individuals in South Africa who are, susceptible to fungal infections, and dependant on river water for their livelihoods provided motivation for this study. The study therefore reported on the presence of potentially pathogenic fungi in polluted surface waters, specifically an urban river system. The sampling sites that were selected for the study included tributaries and main river sections that would provide a clear picture of the pollution levels, sources of pollution and the presence of clinically relevant fungi in the water. The latter was determined using both metagenomic analyses of the mycobiome and enumeration of culturable thermotolerant yeast strains. While results of both these methods was found to be informative, some limitations of the methodology can be highlighted. The first being that only culturable yeasts were enumerated and not culturable filamentous fungi, since the spreading growth of the latter on isolation plates renders counting of culturable filamentous fungi inaccurate. The second limitation was that compared to the culture method used, the metagenomic analysis provided quite limited resolution with regard to the number of clinically relevant species in the water. Nevertheless, our results revealed a wide diversity of fungal taxa in the waters.

In addition to determining the fungal diversity in the river system, a provisional QMRA of yeast infections was conducted for the river water by using the culturable pathogenic yeast concentrations obtained in the calculations. The QMRA framework, however, was dependent on limited literature available on the pathogenic potential of only six pathogenic yeast species. Despite this, robust probabilities of infection were determined to pave the way for future yeast QMRA studies.

CHAPTER 2: LITERATURE REVIEW

This literature review titled has been published in the journal Water SA: Steffen HC, Bosch C, Smith K, Wolfaardt GM and Botha A (2022). Rising Environmental Temperatures and Polluted Surface Waters: The Prelude to the Rise of Mycoses in South Africa. Water SA 48 (2) 199-216 / Apr 2022
https://doi.org/10.17159/wsa/2022.v48.i2.3918

2.1. ABSTRACT

South Africa's rivers are frequently used by communities lacking proper sanitation infrastructure for domestic purposes; however, these surface waters may pose a health risk to immunocompromised individuals due to the presence of opportunistic pathogenic fungi in the polluted water. Although only a few studies have focused on the presence of clinically relevant fungal species in South African rivers, many known opportunistic pathogenic species were found to be predominant in these waters. Furthermore, strong evidence exists that increased numbers of clinically relevant species may be observed in future due to fungi acquiring thermotolerance in response to the global increase in temperature. Thermotolerance is a major factor contributing to pathogenesis in fungi, due to the generally low tolerance of most fungi toward mammalian body temperatures. It is therefore contended that combinatorial effects of water pollution and rising environmental temperatures could lead to an increase in the incidence of mycoses in South Africa. This is especially concerning since a relatively large population of immunocompromised individuals, represented mostly by HIV-infected people, resides in the country.

Key words: River water; pollution; global warming; mycoses; thermotolerance

2.2. INTRODUCTION

Despite the global increase in the incidence of fungal infections, which affect 1 billion individuals and cause nearly 1.6 million deaths annually (Brown et al., 2012; Bongomin et al., 2017; Almeida et al., 2019), only a few fungal taxa have been identified as causative agents of mycoses (Robert and Casadevall, 2009; Almeida et al., 2019). This phenomenon was ascribed to the relatively low maximum thermal tolerances of most fungi, which are lower than mammalian body temperatures (Robert and Casadevall, 2009). Nevertheless, fungi that can grow at mammalian body temperatures cause infections that may vary from being superficial, to subcutaneous to systemic (Dupont et al., 2000; Garber, 2001; Bicanic and Harrison, 2014; Almeida et al., 2019). Incidents of such mycoses are scrupulously reported in developed countries; however, the opposite is true for developing countries, where the statistics on fungal infections are likely underestimated to a great degree. Furthermore, the largest populations of individuals suffering from HIV / AIDS reside in developing countries (Vearey, 2011; UNAIDS, 2017; Schwartz and Denning, 2019), which could potentially increase the incidence of fungal diseases in these regions, since such immunocompromised individuals are at greater risk of acquiring fungal infections (Dupont et al., 1994; Schwartz and Denning, 2019). This is especially relevant to South Africa, with its disproportionately large population of HIV-infected people (UNAIDS, 2021; Schwartz and Denning, 2019; Weimann and Oni, 2019).

There are various mycoses known to be associated with immunocompromised individuals (Guarner, 2017). Candidiasis, caused by ascomycetous yeasts belonging to the genus *Candida*, is the most common opportunistic fungal infection among HIV-infected patients (Garber, 2001; Moran et al. 2012; Guarner, 2017). Of the estimated 3.2 million fungal infections reported annually in South Africa, candidiasis represents nearly 2 million (Schwartz and Denning, 2019). Other important mycoses include invasive aspergillosis, cryptococcal meningitis, histoplasmosis, as well as *Pneumocystis*- and dermatophyte infections (Guarner, 2017). The causative agents of these infections are regularly isolated from natural and anthropogenic environments (Garber, 2001).

Although mycoses may be obtained from nature, hospital-acquired fungal infections are most common and represent the majority of candidiasis and other fungal disease cases reported in literature (Garber, 2001; Suleyman and Alangaden, 2016; Moazeni et al., 2018). These clinical cases often include fungal infections as a result of contaminated catheters, needles, and decreased immunity during organ transplants (Suleyman and Alangaden, 2016). Additionally, infections caused by members of the genus *Aspergillus* are acquired due to the exposure of airborne propagules associated with, among others, construction sites and ventilation systems of hospitals (Cortez et al., 2008; Benedict et al., 2017). Aspergillosis may also develop due to close interaction with certain contaminated foodstuffs and vegetation (Walsh and Dixon, 1989). Other habitats, such as decaying trees and pigeon guano, harbour notorious members of *Cryptococcus,* which can infect humans in proximity via inhalation (Restrepo et al., 2000; Garber, 2001). Additionally, representatives of pathogenic fungal species were recovered from polluted river systems (Luplertlop et al., 2016; Postma, 2016; Assress et al., 2019). Although pathogenic fungi are plentiful in nature, studies commonly focus on nosocomial mycoses (Jarvis, 1995; Guinea, 2014). The prevalence and interactions of opportunistic fungal pathogens within the natural environment, such as rivers, are rarely studied, despite indications that fungal virulence results from adaptations that have evolved for protection against adverse conditions in nature (Steenbergen et al., 2001; Bosch et al., 2020). These conditions include rising environmental temperatures and other anomalies as a result of climate change and have been linked with increases in the prevalence of fungal diseases in mammals, including humans (Garcia-Solache and Casadevall, 2010; Araújo et al., 2017). Research on the ecology of opportunistic fungi in environments conducive to their proliferation, such as river systems, is therefore expedient. Focus should be given to developing countries such as South Africa, with its large population of immunocompromised individuals (UNAIDS, 2021) and inadequate infrastructure accompanied by rapid urbanisation (Schwartz and Denning, 2019). With the ultimate goal of obtaining an indication of the potential effects of climate change and anthropogenic pollution on the ecology of opportunistic pathogenic fungi in South African rivers, the aim of this literature review was to survey existing knowledge on the occurrence of these fungi in the river systems of the region. Additionally, this review reflects on the potential effect of increased environmental temperatures, in combination with pollution, on the biology of opportunistic pathogenic fungi occurring in these rivers.

2.3. POLLUTED SURFACE WATERS

Rivers may become polluted as a result of various anthropogenic activities: agricultural practices, industrial processes, expanding urban communities with poor sanitation infrastructure, as well as faulty

wastewater treatment operations (Vearey, 2011; Wang et al., 2012; Glińska-Lewczuk et al., 2016; Liyanage and Yamada, 2017; Cullis et al., 2019). These may largely contribute to the fungal load in water due to the nutrient-rich characteristics of most pollutants. For several decades, the quality of water, whether it be used for drinking, ablutions, or irrigation purposes, was evaluated by determining the microbiological load thereof (Havelaar et al., 1986). Bacterial indicators have been, and are being, extensively used to determine water quality, especially in relation to the level of faecal pollution (Havelaar et al., 1986; Haack et al., 2009). Only relatively recently were studies conducted linking fungal numbers to pollution levels in river systems (De Almeida, 2005; Brandão et al., 2010; Medeiros et al., 2012; Stone et al., 2013). Previously the only indicators of fungal contaminants used were taste, odour and the number of culturable moulds in the water (Doggett, 2000). While very few studies were aimed at investigating the link between fungal numbers and pollution, several authors have studied fungal diversity in a wide range of surface water sources – including tap water, rivers, lakes and estuaries (Kwasnieskwa, 1988; Hageskal et al., 2009; Pereira et al., 2009; Magwaza et al., 2017; Assress et al., 2019). Some of the fungi identified during these studies include known clinically relevant yeasts and filamentous fungi.

2.3.1 Clinically relevant fungi

Pathogenic and opportunistic pathogenic fungi are ubiquitous in the environment (Restrepo et al., 2000; Gostinčar et al., 2011; Babič et al., 2017). While the occurrence of some of these fungi has been studied extensively in atmospheric (Newbound et al., 2009) and terrestrial environments including animals, plants and soils (Restrepo et al., 2000; Gostinčar et al., 2011), relatively little knowledge exists on the ecology of clinically relevant fungi in aquatic environments, with only a few studies focusing on polluted water. Regardless of the lack of available information, some findings have allowed researchers to hypothesise that fungi, particularly yeasts, have the potential to be used as indicators of pollution in rivers and other water bodies (De Almeida, 2005; Brandão et al., 2010; Medeiros et al., 2012; Stone et al., 2013; Postma, 2016). However, it should also be made a priority to determine the pathogenic fungal taxa occurring in polluted rivers, since individuals utilizing these water sources are at risk of acquiring mycoses (Weimann and Oni, 2019).

2.3.1.1. Filamentous fungi

The most common water-borne filamentous fungi are members of the genera *Penicillium, Trichoderma, Acremonium, Cladosporium, Aspergillus, Fusarium* and *Mucor* (Babič et al., 2017). Representatives of the latter three genera are considered opportunistic, causing invasive fungal infections in immune-deficient individuals. Members of the remaining genera cause allergies, as well as subcutaneous and superficial infections, especially among those suffering from immunodeficiency. Despite the low health risk, compared to invasive infections, superficial infections remain concerning due to the associated negative impact on quality of life (Weimann and Oni, 2019).

To date, no substantial evidence exists on the presence of pathogenic filamentous fungi in the river systems of South Africa. This, however, does not negate the danger associated with the presence of these fungi in rivers, but rather encourages researchers to investigate neglected environments (Schwartz and Denning, 2019). Investigating such understudied environments is of particular

importance considering current evidence of disease-causing filamentous fungi detected in different European water sources – especially tap water (Novak Babič et al., 2018). It is well known that surface waters are sources for drinking and tap water (UN Water, 2006; WHO, 2017). These water sources are treated before distribution, but the treatment processes are not feasible for rivers, lakes and other surface waters that are used as primary water sources by some communities (Colvin et al., 2016; Edokpayi et al., 2018). Recent studies, however, have revealed that fungi can survive conventional treatment processes and persist in tap water, as was observed in some hospitals (Arroyo et al., 2020; Caggiano et al., 2020). Nevertheless, the fungal composition of treated surface water could be significantly different from untreated surface water and therefore in this review only fungi present in rivers and other natural water sources were evaluated.

Globally, two major fungal groups were detected in the few river systems that were studied: Mucoromycota and Ascomycota (Arvanitidou et al., 2005; Pietryczuk et al., 2018; Machido et al., 2015). Mucoralean fungi were found to be the only representatives of the phylum Mucoromycota that occurred within rivers in Greece. These included members of *Mucor*, *Absidia* and *Rhizopus* (Arvanitidou et al., 2005). Species within these genera cause mucormycosis – an invasive fungal infection which can manifest in the lungs, cutaneous and subcutaneous dermis, nose cavities, brain, intestines and bloodstream (Spellberg and Maertens, 2019). Recently, cases of mucormycosis have increasingly been reported among patients suffering from Covid-19 (Garg et al., 2021; Werthman-Ehrenreich et al., 2021). Members of *Absidia* have also caused infections in individuals suffering from burn wounds (Christiaens et al., 2005). These infections are invasive, invading organs and the bloodstream, with detrimental health outcomes (Harrison and Brouwer, 2009).

It must be noted that although immunosuppressed individuals are especially susceptible to invasive fungal infections, rare cases of rhinocerebral mucormycosis, as well as other mycoses, have been observed in immunocompetent patients (Hussain et al., 1995; Leyngold et al., 2014). In contrast, non-invasive superficial fungal infections caused by a variety of different fungi are very common among patients with immune deficiencies (Huang et al., 2004), and noticeable superficial infections on epidermal regions are considered a presenting feature of individuals diagnosed with AIDS (Lohoué Petmy et al., 2004; Benedict et al., 2017).

Some fungi that are known to occur in rivers are capable of causing both invasive and superficial infections (Arvanitidou et al., 2005). These include members of the genera *Aspergillus* and *Penicillium* belonging to the orders Eurotiales; *Alternaria* and *Curvularia* of the order Pleosporales; as well as *Fusarium* and *Verticillium* belonging to Hypocreales. Representatives of the genera *Aspergillus* and *Penicillium* spp. were among the most frequently isolated filamentous fungi found during a survey of rivers in Greece. Although many beneficial strains representing *Penicillium* exist that are used in the food and pharmaceutical industries (Sousa et al., 2001; Elander, 2003), it is known that some members of this genus can cause keratitis, pneumonia, endocarditis, necrotising esophagitis, endophthalmitis, urinary tract infections and peritonises (Hu et al., 2013). *Aspergillus fumigatus* and *Aspergillus niger* that were found in Polish rivers (Pietryczuk et al., 2018) are both associated with aspergillosis, which is

one of the leading causes of death among AIDS individuals (GAFFI, 2017). Recently, cases of Covid-19 associated pulmonary chronic aspergillosis have also been reported (Arastehfar et al., 2020).

Verticillium, another genus harbouring waterborne fungi (Schiavano et al., 2014; Novak Babič et al., 2018), is represented by species associated with peritonises, as well as subcutaneous infections. The latter ensues when a microorganism breaches the epidermal barrier and migrates into the innermost layer of skin where blood vessels and nerves are located (Kujath and Kujath, 2010). While it is known that some bacteria produce proteins that enable infiltration of blood vessels, little information is available for fungi presenting the same mechanisms (Lipke, 2018). Nonetheless, in the probable event that some pathogenic fungi harbour these proteins, the proximity between the microorganisms and blood vessels in subcutaneous tissue could lead to systemic infections where the infectious agents are disseminated to other parts of the body. The subcutaneous tissue is also found directly on top of the musculoskeletal tissue, an area that some representatives of *Fusarium* are known to infect (Koehler et al., 2016). Both *Fusarium* and *Verticillium* spp. have been associated with superficial and invasive infections (Kujath and Kujath, 2010; Koehler et al., 2016), while other members of the Hypocreales from rivers, such as species of *Acremonium* and *Cylindrocarpon,* are more commonly known to cause superficial infections, such as mycetoma (Welsh et al., 2007; Arvanitidou et al., 2005; Pietryczuk et al., 2018).

Of the pleosporalean fungi that were found in Greek rivers, members of *Curvularia* and *Pyrenochaeta* were rarely encountered, whereas representatives of *Alternaria* were frequently isolated (Arvanitidou et al., 2005). *Curvularia* and *Pyrenochaeta* harbour species associated with cutaneous infections and invasive fungemia. *Alternaria* species, however, are involved in other less invasive infections such as rhinosinusitis, onychomycosis and oculomycosis (Pastor and Guarro, 2008). Additionally, in a recent study done by Pietryczuk et al. (2018), *Alternaria alternata* was identified in 4 out of 5 rivers surveyed in Poland.

Onygenalean fungi, which represent members that cause tineas, were also detected in natural surface waters (Arvanitidou et al., 2005; Machido et al., 2015; Novak Babič et al. 2018). Two genera, *Emmonsia* and *Chrysosporium*, were frequently encountered in Greek rivers (Arvanitidou et al., 2005) and represent fungi typically associated with disseminated- or superficial infections, respectively (Kenyon et al., 2013; Mijiti et al., 2017). Other members of the Onygenales which were isolated from river systems in Europe and Africa were representatives of *Microsporum* and *Trichophyton* (Arvanitidou et al., 2005; Machido et al., 2015), genera that include keratolytic fungi and are frequently associated with tineas (Schwinn et al., 1995; Brito-Santos et al., 2017). For example, *Microsporum canis,* which has been isolated from surface waters, as well as *Trichophyton tonsurans* and *Trichophyton violaceum* that have been isolated from rivers (Machido et al., 2015; Pietryczuk et al., 2018; Novak Babič et al., 2018), are all known to cause tinea capitis (Sombatmaithai et al., 2015; Pasquetti et al., 2017; Morales et al., 2019).

Other ascomycetous filamentous fungi occurring in surface waters that have mostly been associated with cutaneous infections are members of the order Chaetothyriales (Novak Babič et al., 2018; Pietryczuk et al., 2018). A chaetothyrialean fungus, *Exophalia dermatitidis*, associated with both cutaneous infections, as well as with respiratory infections in cystic fibrosis patients, was isolated from Polish rivers (Pietryczuk et al., 2018). Also isolated from these rivers were microascalean fungi

belonging to the genus *Scopulariopsis.* Representatives of this genus are known to be the causative agents for different mycoses, ranging from superficial, to pulmonary and systemic infections (Iwen et al., 2012; Sandoval-Denis et al., 2013). It is important to mention that Microascales also harbours emerging pathogens such as members of the genus *Scedosporium,* which are commonly associated with wastewater sources (Skiada et al., 2017). Considering that wastewater is a major component of the pollution in rivers, further investigation into the diversity of *Scedosporium* spp. and other opportunistic filamentous fungi in natural surface waters is imminent.

2.3.1.2. Yeasts

The majority of fungal infections are caused by yeasts (Miceli et al., 2011; Bongomin et al., 2017; Lamoth et al., 2018; Ocansey et al., 2019; Schwartz and Denning, 2019). These opportunistic unicellular fungi usually belong to genera such as *Candida* and *Rhodotorula*, which were among the prevalent yeasts occurring in polluted surface waters (Sláviková and Vadkertiová, 1997; De Almeida et al., 2005; Gadanho et al., 2006; Coelho et al., 2010; Medeiros et al., 2012; Pietryczuk et al., 2014; Monapathi et al., 2020). The invasive infections caused by these yeasts often have devastating effects on human organs, including the liver, heart, lungs, and brain. These infections, however, are rare among immunocompetent individuals and occur mostly among immunocompromised patients, such as those suffering from HIV / AIDS (Low and Rotstein, 2011).

Compared to filamentous fungi, more literature is available on the prevalence and presence of unicellular fungi in surface waters (Buzzini et al., 2017). This can be attributed to the non-spreading nature of yeast colonies on microbiological media, enabling researchers to more readily enumerate yeasts and link their numbers to environmental factors (Sláviková and Vadkertiová, 1997; De Almeida, 2005; Gadanho et al., 2006; Coelho et al., 2010; Medeiros et al., 2012; Stone et al., 2012; Van Wyk et al., 2012). Additionally, more yeast-related surveys could have been conducted in water sources than what were carried out for filamentous fungi, due to the disproportional disease burden associated with yeasts and the implicated higher health risk (Miceli et al., 2011; Bongomin et al., 2017; Lamoth et al., 2018; Ocansey et al., 2019; Schwartz and Denning, 2019).

A number of surveys on yeasts occurring in rivers was conducted across the globe (Table 1), yet there is still a paucity of published information available on yeasts associated with river systems in South Africa (Table 2). Limited data originating from only two provinces have been published thus far: Western Cape and North-Western Province (Stone et al., 2012; Van Wyk et al., 2013; Monapathi et al., 2017; Monapathi et al., 2020).

Table 2.1: List of clinically relevant yeast and yeast-like fungi found in surface waters of countries other than South Africa.

Yeast Species (Synonym)	**Infections**	**Water Source Reference**
Ascomycetes		
Aureobasidium pullulans	Fungemia, subcutaneous infections, disseminated infections [1, 2, 3]	Sláviková & Vadkertiová (1997)
Candida albicans	Candidal vulvovaginitis, candidemia, candidiasis	Buck & Bubucis (1978); Arvanitidou et al. (2005); Yamaguchi et al. (2007); Pietryczuk et al. (2018)
Candida blankii	Fungemia [4]	Medeiros et al. (2012)
Candida catenulata	Superficial- and invasive infections [5, 6]	de Almeida (2005); Medeiros et al. (2012)
Candida glabrata	Candidiasis (urogenital tracts), candidemia [7, 8, 9]	Yamaguchi et al. (2007); Coelho et al. (2010); Medeiros et al. (2012)
Candida inconspicua	Candidemia [10]	Sláviková & Vadkertiová (1997)
Candida intermedia	Fungemia [11]	Sláviková & Vadkertiová (1997); de Almeida (2005)
Candida lambica	Fungemia, polyarthritis [12, 13]	Sláviková & Vadkertiová (1997)
Candida palmioleophila	Endophthalmitis, Fungemia [14, 15]	Coelho et al. (2010); Medeiros et al. (2012)
Candida parapsilosis	Sepsis, wound and tissue infections (subcutaneous and cutaneous) [9]	Sláviková & Vadkertiová (1997); de Almeida (2005); Yamaguchi et al. (2007); Coelho et al. (2010); Medeiros et al. (2012)
Candida pararugosa	Fungemia [16]	Medeiros et al. (2012)
Candida rugosa	Candidemia (burn patients) [17]	Medeiros et al. (2012)
Candida tropicalis	Candidemia [9]	Sláviková & Vadkertiová (1997)
Candida zeylanoides	Fungemia, endocarditis [18, 19]	Arvanitidou et al. (2005); Coelho et al. (2010)
Clavispora lusitaniae (*Candida lusitaniae*)	Intra-abdominal candidiasis [20]	de Almeida (2005); Coelho et al. (2010)
Debaryomyces hansenii (*Candida famata*)	Invasive fungemia, central nervous system infections, fungemia [21, 22, 23, 24, 25]	Sláviková & Vadkertiová (1997); de Almeida (2005); Gadanho et al. (2006); Coelho et al. (2010); Medeiros et al. (2012)
Geotrichum candidum	Disseminated infections, superficial infections [26, 27]	Sláviková & Vadkertiová (1997)
Kluyveromyces marxianus (*Candida kefyr*)	Candidemia [28]	Coelho et al. (2010); Medeiros et al. (2012)
Metschnikowia pulcherrima (*Candida pulcherrima*)	Fungemia [29]	Sláviková & Vadkertiová (1997)
Meyerozyma guilliermondii (*Candida guilliermondii, Pichia guilliermondii*)	Onychomycosis (tinea), invasive infections (rare), Fungemia [30]	Sláviková & Vadkertiová (1997); de Almeida (2005); Coelho et al. (2010); Medeiros et al. (2012)
Meyerozyma caribbica (*Candida fermentati*)	Fungemia [31]	Coelho et al. (2010)
Pichia anomala (*Candida pelliculosa, Hansenula anomala*)	Fungemia (paediatric), urinary tract infection [32, 33, 34]	Sláviková & Vadkertiová (1997); Coelho et al. (2010)

Table 2.1 (continued): List of clinically relevant yeast and yeast-like fungi found in surface waters of countries other than South Africa.

Yeast Species (Synonym)	**Infections**	**Water Source Reference**
Ascomycetes		
Pichia kudriavzevii (*Candida krusei, Issatchenkia orientalis*)	Fungemia [35]	Sláviková & Vadkertiová (1997); Coelho et al. (2010); Medeiros et al. (2012)
Saccharomyces cerevisiae	Fungemia [36]	Sláviková & Vadkertiová (1997); Coelho et al. (2010)
Yarrowia lipolytica (*Candida lipolytica*)	Fungemia, superficial infections [37]	Coelho et al. (2010); Medeiros et al. (2012)
Basidiomycetes		
Cryptococcus albidus (*Naganishia albida*)	Cryptococcaemia, superficial infections [38, 39]	Sláviková & Vadkertiová (1997); Pereira et l. (2009); Coelho et al. (2010); Medeiros et al. (2012)
Cryptococcus laurentii (*Papiliotrema laurentii*)	Superficial infections, lung abscesses, fungemia, endophthalmitis [39]	Sláviková & Vadkertiová (1997); Coelho et al. (2010); Medeiros et al. (2012)
Cryptococcus luteolus	Diffuse infiltration of lungs, tenosynovitis [40]	Medeiros et al. (2012)
Cryptococcus magnus	Vulvovaginitis, cryptococcosis in cats [41, 42]	Medeiros et al. (2012)
Filobasidium uniguttulatum (*Cryptococcus uniguttulatum*)	Meningitis [43]	Coelho et al. (2010)
Rhodotorula glutinis	Fungemia, meningitis, onychomycosis (tinea) [44, 45, 46]	Sláviková & Vadkertiová (1997); Coelho et al. (2010); Medeiros et al. (2012)
Rhodotorula minuta	Endophthalmitis, onychomycosis (tinea), fungemia [47, 48, 49]	Sláviková & Vadkertiová (1997)
Rhodotorula mucilaginosa (*Rhodotorula rubra*)	Fungemia, meningitis, endocarditis, peritonises, endophthalmitis, onychomycosis (tinea) [45, 50, 51, 52, 53]	Sláviková & Vadkertiová (1997); de Almeida (2005); Gadanho et al. (2006); Coelho et al. (2010); Medeiros et al. (2012); Pietryczuk et al. (2018)
Sporobolomyces salmonicolor	Pseudomeningitis, dermatitis, endogenous endophthalmitis, fungemia [54, 55, 56, 57]	Sláviková & Vadkertiová (1997)
Trichosporon cutaneum	Disseminated infections, endocarditis, superficial infections [58, 59, 60]	Sláviková & Vadkertiová (1997)
Trichosporon mucoides	Onychomycosis, fungemia [61, 62]	Pietryczuk et al. (2018)

References to yeast epidemiology: [1] Kaczmarski et al. (1986); [2] Joshi et al. (2010); [3] Bolignano et al. (2003); [4] Nobrega de Almeida et al. (2018); [5] Radosavljevic et al. (1999); [6] Ha et al. (2018); [7] Leaw et al. (2007); [8] Jarvis et al. (1995); [9] Silva et al. (2012); [10] Guitard et al. (2013); [11] Ruan et al. (2010); [12] Trowbridge et al. (1999); [13] Vervaeke et al. (2008); [14] Datta et al. (2015); [15] Sugita et al. (1999); [16] El Helou & Palavecino (2017); [17] Pfaller et al. (2006); [18] Levenson et al. (1991); [19] Whitby et al. (1996); [20] Vergidis et al. (2016); [21] Carrega et al. (1997); [22] Prinsloo et al. (2008); [23] Beyda et al. (2013); [24] Wong et al. (1982); [25] Wagner et al. (2005); [26] Kassamali et al. (1987); [27] Sfakianakis et al. (2007); [28] Taj-Aldeen et al. (2014); [29] Bereczki et al. (2012); [30] GŘler et al. (2017); [31] Lockhart et al. (2009); [32] Baron et al. (1988); [33] Qadri et al. (1988); [34] Chakrabarti et al. (2001); [35] Scorzoni et al. (2013); [36] Muños et al., (2005); [37] Boyd et al. (2017); [38] Hoang & Burruss et al. (2007); [39] Kordossis et al. (1998); [40] Hunter-Ellul et al. (2014); [41] Ghajari et al. (2018); [42] Poth et al. (2010); [43] Pan et al. (2012); [44] Shinde et al. (2008); [45] Hsueh et al. (2003); [46] Wirth & Goldani (2012); [47] Goldani et al. (1995); [48] Pinna et al. (2001); [49] Zhou et al. (2014); [50] Lo Re et al. (2003); [51] Gyaurgieva et al. (1996); [52] Eisenberg et al. (1983); [53] Merkur et al. (2002); [54] Sharma et al. (2006); [55] Bross et al. (1986); [56] Bergman & Kauffman (1984); [57] Tang et al. (2015); [58] Gold et al. (1981); [59] Marier et al. (1978); [60] Madhavan et al. (1976); [61] Rizzitelli et al. (2016); [62] Colombo et al. (2011)

The most dominant of the opportunistic pathogenic yeasts identified in rivers of South Africa (Table 2), as well as other countries (Table 1), were found to be ascomycetous yeasts, especially members of the genus *Candida* (De Almeida, 2005; Coelho et al., 2010; Medeiros et al., 2012; Van Wyk et al., 2012; Monapathi et al., 2017). Whether the predominance of these clinically relevant ascomycetes is associated with certain environmental parameters is still unclear. Considering the positive correlation observed between pollution indicators and yeast numbers, as well as the prevalence of ascomycetes in some studies, research on the link between yeasts and physicochemical parameters is imminent (Sláviková and Vadkertiová, 1997; Gadanho et al., 2006; Coelho et al., 2010; Medeiros et al., 2012; Stone et al., 2012; Van Wyk et al., 2012). Thus far, when researchers analysed the pollution levels of river water, results varied: some authors reported higher than normal concentrations of nitrogen and phosphate (Sláviková and Vadkertiová, 1997; Medeiros et al., 2012), others reported that yeast numbers correlate with temperature and pH (Gadanho et al., 2006; Van Wyk et al., 2012) or that yeast prevalence is related to faecal coliform numbers (Coelho et al., 2010; Stone et al., 2012).

The South African rivers that were investigated were considered to be diffusely polluted, contaminated with exceedingly high levels of dissolved solids, nitrates and phosphates (Van Wyk et al., 2012; Monapathi et al., 2017), as well as faecal matter (Stone et al., 2012). The latter is of considerable concern since members of clinically relevant species including *Pichia kudriavzevii* (syn. *Candida krusei*), *Candida tropicalis, Candia parapsilosis, Candida rugosa, Papiliotrema laurentii* (syn. *Cryptococcus laurentii*), *Cyberlindnera jadinii* (syn. *Candida utilis*), *Meyerozyma guilliermondii* (syn. *Candida guilliermondii*), *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* (syn. *Rhodotorula rubra*) have previously been associated with sewage and sewage-polluted water (Cooke et al., 1960). In South Africa, many municipalities have neglected the maintenance of sewage treatment infrastructure, causing the release of raw and partially treated sewage into river systems (Herbig, 2019). The heavy load of excrement and other pollutants in sewage is well known to increase the quantity of organic carbon, including chemicals and other substrates that sustain growth of potentially harmful microorganisms (Liu et al., 2015). In tandem, anthropogenic waste depository from individuals residing near riverbanks extends the list of pollutants entering surface waters. Among the anthropogenic pollution entering river systems are chemicals such as antibiotics and even antifungals (Chitescu et al., 2015). While the above-mentioned aberrations with regard to physicochemical parameters are known to indicate pollution in river systems, no attempt was made thus far to correlate these parameters with the numbers of pathogenic yeast species in these polluted waters.

Table 2.2: List of clinically relevant yeast and yeast-like species found in surface waters of South Africa.

Yeast Species (Synonym)	**Infections**	**Water Source Reference**
Ascomycetes		
Candida albicans	Candidal vulvovaginitis, candidemia, candidiasis	Stone et al. (2012); Monapathi et al. (2017); Monapathi et al. (2020b)
Candida bracarensis	Candidemia [1]	Monapathi et al. (2017); Monapathi et al. (2020b)
Candida catenulata	Superficial- and invasive infections [2, 3]	Van Wyk et al. (2012)
Candida glabrata	Candidiasis (urogenital tracts), candidemia [4, 5, 6]	Belford (2013); Postma (2016); Monapathi et al. (2017); Monapathi et al. (2020b)
Candida globosa	Candidiasis [4, 5]	Van Wyk et al. (2012)
Candida haemulonii	Candidiasis [7]	Monapathi et al. (2020b)
Candida parapsilosis	Sepsis, wound and tissue infections (subcutaneous and cutaneous) [6]	Monapathi et al. (2017); Monapathi et al. (2020b)
Candida rugosa	Candidemia (burn patients) [9]	Van Wyk et al. (2012)
Candida sake	Endocarditis, peritonises, candidemia [10]	Van Wyk et al. (2012); Monapathi et al. (2020b)
Candida tropicalis	Candidemia [6, 11]	Van Wyk et al. (2012); Belford (2013); Bezuidenhout (2013); Postma (2016); Monapathi et al. (2017); Monapathi et al. (2020b)
Clavispora lusitaniae (*Candida lusitaniae*)	Intra-abdominal candidiasis [12]	Van Wyk et al. (2012); Bezuidenhout (2013); Postma (2016); Monapathi et al. (2017); Monapathi et al. (2020b)
Cyberlindnera fabianii	Pneumonia, fungemia, endocarditis, prostatitis [13]	Monapathi et al. (2017); Monapathi et al. (2020b)
Cyberlindnera jadinii (*Candida utilis*)	Candidemia [14, 27]	Belford (2013); Postma (2016); Monapathi et al. (2020b)
Kluyveromyces marxianus (*Candida kefyr*)	Bloodstream infection [15]	Monapathi et al. (2020b)
Meyerozyma guilliermondii (*Candida guilliermondii, Pichia guilliermondii*)	Fungemia [16]	Van Wyk et al. (2012); Bezuidenhout (2013); Monapathi et al. (2017); Monapathi et al. (2020b)
Meyerozyma caribbica (*Candida fermentati*)	Fungemia [16]	Monapathi et al. (2020b)
Pichia anomala (*Candida pelliculosa, Hansenula anomala*)	Candidemia [8]	Van Wyk et al. (2012)
Pichia kudriavzevii (*Candida krusei, Pichia guilliermondii*)	Fungemia [17, 18]	Belford (2013); Bezuidenhout (2013); Postma (2016); Monapathi et al. (2017); Monapathi et al. (2020b)
Saccharomyces cerevisiae	Fungemia [19]	Belford (2013); Postma (2016) Monapathi et al. (2020b)
Wickerhamomyces anomalus	Fungemia (neonates) [20]	Bezuidenhout (2013); Monapathi et al. (2017); Monapathi et al. (2020b)
Yarrowia lipolytica (*Candida lipolytica*)	Fungemia, superficial infections [21]	Monapathi et al. (2020b)

Table 2.2 (continued): List of clinically relevant yeast and yeast-like species found in surface waters of South Africa.

Yeast Species (Synonym)	**Infections**	**Water Source Reference**
Basidiomycetes		
Cryptococcus laurentii (*Papiliotrema laurentii*)	Superficial infections, lung abscesses, fungemia, endophthalmitis [22]	Van Wyk et al. (2012)
Rhodotorula glutinis	Fungemia, meningitis, onychomycosis (tinea) [23, 24, 25]	Van Wyk et al. (2012); Bezuidenhout (2013)
Rhodotorula mucilaginosa (*Rhodotorula rubra*)	Fungemia, meningitis, endocarditis, peritonises, endophthalmitis, onychomycosis (tinea) [25]	Van Wyk et al. (2012); Bezuidenhout (2013)
Trichosporon ovoides	White Piedra (tinea) [26]	Postma (2016); Monapathi et al. (2020b)

References to yeast epidemiology: [1] Warren et al. (2010); [2] Radosavljevic et al. (1999); [3] Ha et al. (2018); [4] Leaw et al. (2007); [5] Jarvis et al. (1995); [6] Silva et al. (2012); [7] Coles et al. (2020); [8] Jung et al. (2018a); [9] Pfaller et al. (2006); [10] Juneja et al. (2011); [11] Zuza-alves et al. (2017); [12] Vergidis et al. (2016); [13] Park et al. (2019); [14] Scoppettuolo et al. (2014); [15] Seth-Smith et al. (2020); [16] GŘler et al. (2017); [17] Scorzoni et al. (2013); [18] Nagarathnamma et al. (2017); [19] Muñoz et al. (2005); [20] Yılmaz-Semerci et al. (2017); [21] Boyd et al. (2017); [22] Molina-Leyva et al. (2013); [23] Shinde et al. (2008); [24] Hsueh et al. (2003); [25] Wirth & Goldani (2012); [26] Colombo et al. (2011); [27] Treguier et al. (2018)

To enumerate and isolate clinically relevant fungi, including yeasts, from surface waters, an incubation temperature of 37˚C was employed by some authors, (Buck and Bubucis, 1978; De Almeida, 2005; Yamaguchi et al., 2007; Coelho et al., 2010). Others incubated their isolation plates at room temperature to determine overall yeast numbers in surface waters. Interestingly, Coelho et al. (2010) identified yeasts incubated at both room temperature and 37˚C and found that the numbers of the predominant species cultivated at the latter temperature correlated with *E. coli* numbers and therefore with the level of faecal contamination. Yeast numbers occurring on isolation plates that were incubated at room temperature, however, showed no significant correlation with faecal coliform numbers.

The positive correlation between faecal coliform numbers and yeast counts obtained after incubation at 37°C, further suggests that these unicellular fungi are allochthonous – originating from other sources and subsequently introduced to the river water (Weimann and Oni, 2019; Arvanitidou et al., 2002). A potential source of these yeasts can be anthropogenic pollution (Dynowska, 1997; Coelho et al., 2010; Herbig and Meissner, 2019; Weimann and Oni, 2019). While the inhabitants of rural communities may be one of the sources of river pollution, they also tend to be at risk, especially individuals predisposed to infection because of immune deficiencies (Vearey, 2011).

It is disconcerting that some of the most clinically relevant species of *Candida* were isolated from South African rivers (Table 2): *C. albicans, C. tropicalis, C. glabrata* and *C. parapsilosis* (Miceli et al., 2011). The same representatives were isolated from surface waters in other countries such as Portugal, Greece, Poland and Brazil (Arvanitidou et al., 2005; De Almeida, 2005; Coelho et al., 2010; Medeiros et al., 2012, Pietryczuk et al., 2018). The occurrence of renowned clinically relevant yeasts in different geographical regions of the world emphasises the need for more ecological surveys for the presence of these unicellular fungi in ecosystems. To understand why these yeasts, persist and grow within ecosystems, such surveys should be accompanied by analyses of anthropological activity including

assessment of pollution indicators and measurement of physicochemical parameters. The latter includes parameters such as the nutrient levels, pH, as well as the temperature of surface waters.

2.4. RISING ENVIRONMENTAL TEMPERATURES

Life on Earth is sustained by the natural greenhouse gases found in the atmosphere; however, industrialisation and other anthropogenic activities involving fossil fuel combustion have impinged on the natural environment and largely contributed to climate change (IPCC, 2018; IPCC, 2021). Since the industrial revolution that started in 1760, the increased emissions of greenhouse- and other deleterious gases have contributed to a rise in global temperature. In addition, the exponential increase in the population caused agriculture to expand in order to meet the high food demand. Livestock farming, for instance, is now recognised as another contributor to global warming due to deforestation (land to farm) as well as an increase in released methane (Ilea, 2009). This has led to the total increase of an estimated 1°C in global temperatures and a further increase of up to 2°C has been hypothesised for future decades (IPCC, 2018; IPCC, 2021). In accordance with global predictions, South Africa has seen a 2°C increase since the industrial revolution (1760) and it is projected that a 0.12-0.5°C/decade rise will be observed in future (USAID, 2015).

This world-wide rise in temperatures has been collectively referred to as global warming (Lineman et al., 2015). Disastrous consequences of this anthropogenically influenced phenomenon include the loss of ice in Antarctica and Greenland, accompanied by a rise in sea levels (Hansen et al., 2016; IPCC, 2021). Thus far, global warming has had detrimental effects on ecosystems and biomes across the globe. Increased temperatures were found to heighten the threat of species extinction within many habitats, such as montane forests and other terrestrial environments (Malcolm et al., 2006). Recent studies have shown that climate change also increases the temperature of freshwater aquatic environments (Morrison et al., 2002; Van Vliet et al., 2011; Chen et al., 2016; Nusslé et al., 2015; Pohle et al., 2019; Kedra, 2020; Liu et al., 2020). The rising river water temperatures in some of these studies were found to be congruent with the rise in air temperatures, suggesting a direct link between thermally altered waters and global warming (Van Vliet et al., 2011; Chen et al., 2016; Pohle et al., 2018; Kedra, 2020; Liu et al., 2020). As such, excess heat because of anthropogenic activities – also referred to as thermal pollution – may therefore be one of the main causes for increased water temperatures (Nordell et al., 2003; Verones et al., 2010; Liu et al., 2020).

The effects on the microorganisms present in thermally altered waters have not been fully described, despite their crucial roles in biogeochemical cycling and other processes essential for a fully functional and diverse ecosystem (Sigee, 2005). Fungi, in particular, are largely understudied in aquatic ecosystems, with little to no knowledge available on how thermal pollution in water affects these microbes (Grossart et al., 2019). However, potential links between the emergence of pathogenic fungi and rising ambient temperatures have been discussed by some mycologists (Garcia-Solache and Casadevall, 2010; Casadevall et al., 2020), and positive correlations have been observed between the presence of fungal pathogens in river systems and water pollution levels (De Almeida, 2005; Brandão et al., 2010; Medeiros et al., 2012; Stone et al., 2012; Postma, 2016). Thus, a holistic approach is

needed to investigate the combinatorial effects of both water pollution and global warming on the emergence of fungal pathogens.

2.4.1. Global warming and emerging mycoses

Fungi are well known infectious agents of various organisms, including plants and mammals as well as amphibians (Garber, 2001). *Batrachochytrium dendrobatidis* is a good example of a notorious causative agent of chytridiomycosis among many frog species. Since the emergence of this fungus, researchers have contended that the decline in frog species due to this pathogen is linked to the global rise in temperature (Longcore et al., 1999; Wake and Vredenburg, 2008; Fisher et al., 2009). Although the correlation analysis linking global warming to the decline of amphibian species has been queried by some (Rohr et al., 2008), many other researchers have emphasised the role of rising temperatures in species extinction (Pounds et al., 2006; Alford et al., 2007; D'Amen and Bombi, 2009). These studies employed statistical models to determine correlations between rising temperatures and declining species numbers (Pounds et al., 2006; Alford et al., 2007; Rohr et al., 2008; D'Amen and Bombi, 2009), but failed to consider other biological factors. Recently, however, researchers have begun to study the specific relationship between host and pathogen during climate changes (Cohen et al., 2017; Neely et al., 2020). The thermal mismatch theory was formulated, which states that as environmental conditions deviate from the optimal conditions required for host survival, susceptibility towards infectious agents will increase (Chen et al., 2011; Cohen et al., 2017). Although this theory is limited to cold-adapted hosts, it has provided opportunities to discover important traits of the associated pathogens. For example, it was found that microbial symbionts, specifically pathogens, have a broader thermal breadth than their hosts (Neely et al., 2020), due to the higher mass-specific metabolic rates of smaller organisms, thus allowing for a more rapid adaptation to environmental change (Cohen et al., 2017). Understanding these and similar microbial characteristics provides opportunities to determine how environmental fungi may adapt to climate change and evolve to survive the conditions of a mammalian host. It must be noted, however, that little research has been conducted on intraspecies differences in thermal breadth, between pathogenic fungi recovered from natural environments and that of laboratory or clinical strains. Such studies may provide better insight into how environmental stressors like elevated temperatures could facilitate adaptation.

Garcia-Solache and Casadevall (2010) presented an important hypothesis averring that mammalian fungal infections will increase as a result of global warming. This hypothesis requires an understanding of how pathogens adapt to specifically overcome the restrictions associated with the mammalian body. In humans, fungal infections have mainly occurred in individuals with immune deficiencies, while relatively few cases were recorded among immunocompetent persons (Badiee and Zare, 2017). This is largely owing to the complex and effective immune systems unique to jawed vertebrates, which provide extensive protection against fungal pathogens (Shoham and Levitz, 2005). In addition, an equally effective preventative mechanism is the thermal restriction zone. Endothermic animals can regulate body temperature in response to infection during which basal temperatures increase in a process known as fever. Microorganisms such as unicellular- and filamentous fungi have a relatively low tolerance to high temperatures and therefore deteriorate during fever conditions (Robert and

Casadevall, 2009). The high thermal susceptibility, in addition to the intolerance of the already high body temperature, effectively prevents fungal pathogens from frequently causing invasive infections. Thus, with the increase in ambient temperatures, and the potential ability of fungi to adapt to increased environmental temperatures, global warming threatens to expedite the emergence of mycoses by introducing more thermally tolerant fungi.

Two major restrictions prevent an exponential increase of mycoses cases in humans: a complex, highly adaptable immune system (Blanco and Garcia, 2008) and elevated body temperatures. The law of parsimony (Sober, 1981; McMeekin et al., 2008) would dictate that investigating the microorganism's ability to adapt to the thermal restrictions in mammals could provide a better indication of potential virulence than investigating its ability to evade host immunity. Therefore, this review will continue by discussing available fundamental knowledge on fungal thermotolerance.

2.4.2. Thermally tolerant fungi

For microorganisms to be considered thermotolerant, they should be able to withstand temperatures that exceed the population's optimal growth temperature (Robert et al., 2015). The upper thermal limit can range from 35°C to 62°C depending on the type of microorganism (Tansey and Brock, 1972; Maheshwari et al., 2000). Many bacterial species have been identified as thermally tolerant and some groups have also been recognised as thermophilic. Most fungi, however, have failed to attain this characteristic and remain susceptible to higher temperatures (Robert and Casadevall, 2009). Exceptions to this include some industrially important yeasts that tolerate high temperatures, which is essential for the success of some processes. Understanding the mechanisms of thermotolerance in these yeasts is crucial for industries to optimise bread and wine fermentation as well as biofuel production (Parapouli et al., 2020). These mechanisms have thus been scrupulously investigated due to their economic value. Conversely, studies investigating thermotolerant fungi associated with infection and disease are lacking.

Robert et al. (2015) investigated thermotolerance among a wide range of yeast species (CBS Culture Collection, Netherlands) and highlighted that many were able to grow at temperatures above 35°C. Although these thermotolerant species represented a polyphyletic group of fungi, it was noted that thermotolerance was significantly more common among the ascomycetous yeasts than among the basidiomycetes. However, the maximum temperature of growth (T_{max}) for basidiomycetous yeast species has increased over the past decades. This suggests that basidiomycetes could be increasingly implicated in future novel fungal infections. Regardless, increasing T_{max} trends were observed for all yeasts investigated over the past few decades, potentially due to adaptation to the global change in temperature (Robert et al., 2015). Environmental stressors, such as temperature, are common agents in the evolution of microorganisms (Baquero, 2009) and it was contended that acquired thermotolerance among fungi could increase the incidence of infections (Araújo et al., 2017). In knowing the original environmental habitats of potentially pathogenic fungi and gaining a fundamental comprehension of their thermotolerant nature, researchers could in future determine the risk of these microorganisms becoming emerging pathogens (Araújo et al., 2017; Jackson et al., 2019). Well-known examples of

such thermotolerant fungi that are contended to have emerged from the environment as pathogens are *Candida auris*, as well as the pathogenic cryptococci and aspergilli.

2.4.2.1. Candida auris

Candida auris is a multidrug-resistant pathogen, which is also known for its nosocomial transmission (Rhodes and Fisher, 2019). The simultaneous rise of infections caused by four genetically discrete clades of *C. auris,* in distinct geographical regions, has raised questions regarding the cause of the emergence of this pathogen (Kean et al., 2020). It was proposed that *C. auris* could be the model organism explaining emergence of novel mycoses as a result of global warming (Casadevall et al., 2020). *Candida auris* sp. nov. was first isolated from a hospitalised patient's ear (Satoh et al., 2009), an organ that represents cooler environments compared to the rest of the human body. It was therefore contended that the yeast could have gradually gained temperature tolerance while inhabiting the ear, possibly enabling invasive infection and subsequently causing many outbreaks of *C. auris*-related candidiasis (Casadevall et al., 2020; Jackson et al., 2019; Kean et al., 2020). Alarmingly, the frequency of these outbreaks and severity of *C. auris* infections has highlighted this yeast's epidemic potential (Meis and Chowdhary, 2019). Recently, cases of *C. auris* infections have increasingly been reported among patients suffering from Covid-19 (Rodriguez, et al., 2020; Almeida et al., 2021; Villanueva-Lozano et al., 2021).

With little knowledge available on the natural origin of *C. auris*, determining the cause of this yeast's emergence remains cumbersome. Studies have suggested that *C. auris* is of environmental origin and emerged as a pathogen only after being introduced to healthcare systems by patients carrying the yeast (Casadevall et al., 2020; Kean et al., 2020). Some hypothesise that, as observed for certain pathogenic cryptococcal species (Moschetti et al., 2017), *C. auris* may have initially inhabited birds (body temperature of 42°C) where it consequently attained its thermotolerance. The migration of these animals to areas where they are in close contact with humans could also explain the emergence of *C. auris* at distinct geographical regions (Moschetti et al., 2017; Kean et al., 2020). Another proposed habitat for this yeast is wetland systems (Casadevall et al., 2019) that are known to have anaerobic zones, in which *C. auris* would be able to exist. This species also tolerates elevated salt concentrations, which may explain why *C. auris* was recently isolated from a salt marsh (Arora et al., 2021). Except for one isolate, most of the environmental isolates were found to be antifungal-resistant and grew well at 37°C and 42°C. Subsequent phylogenetic analyses revealed the existence of single nucleotide polymorphism differences between the environmental isolates and clinical isolates belonging to the same clade. The authors suggested that, once introduced into anthropogenic habitats, *C. auris* is capable of rapid adaptation to obtain both antifungal resistance and thermotolerance. They concurred, however, that more research is required to obtain a better understanding of the genetic diversity of this species in natural habitats. To date this species has not been detected in any other aquatic environment; however, frequent isolations of *Candida* spp. from aquatic environments (Table 1) as well as the recent findings of Arora et al. (2021) indicate that *C. auris* will potentially be found in more of these environments.

2.4.2.2. Cryptococcus *spp.*

Of the wide diversity of *Cryptococcus* spp. identified so far, infection is mainly caused by representatives of the *Cryptococcus gattii / Cryptococcus neoformans* species complex (Hagen et al., 2015; May et al., 2016). Their ability to withstand the mammalian core body temperature and to evade the immune response are key characteristics that facilitate systemic infections in humans and other mammals (Perfect, 2006) and it is known that cryptococcosis is one of the leading causes of mortality among immunocompromised individuals (UNAIDS, 2021). This infection is acquired after exposure to pathogenic cryptococci present in natural environments (May et al., 2016). Common natural habitats for these microorganisms are decaying plant material, such as trees, as well as the bodies and excreta of avian species (Ellis and Pfeiffer, 1990; Ellis and Pfeiffer, 1992; Lazera et al., 1996; Nielsen et al., 2007). The latter suggests that birds can serve as vectors for these cryptococci and subsequently distribute the pathogen to urban areas where interaction with humans might occur. These yeasts are introduced to the mammalian body through inhalation of aerosolised propagules (Perfect, 2006).
Recently, transcriptional regulation and signalling pathways were uncovered in pathogenic cryptococci that increase the virulence of these yeasts; many of these pathways are also up regulated at higher temperatures (Juvvadi et al., 2014; Chatterjee and Tatu, 2017; Oliveira et al., 2020; Toplis et al., 2020; Bosch et al., 2021). Typical virulence factors observed in these yeasts include capsule enlargement and melanin production, which enable successful evasion of host immunity. Some of these virulence factors were also observed in non-pathogenic species (Petter et al., 2001; Watkins et al., 2017). For example, the basidiomycetous yeast, *Saitozyma podzolica* (syn. *Cryptococcus podzolicus)*, produces virulence factors but is unable to cause disease in mammals due to its sensitivity towards higher temperatures (Petter et al., 2001). With the increased T_{max} observed among basidiomycetes (Robert et al., 2015), it is plausible that more non-pathogenic species of the basidiomycetous yeasts will cause fungal diseases in future in the event of acquired thermotolerance. Similar phenomena may also be observed for other fungal species; however, without establishing the level of thermotolerance of these potential pathogens, one cannot determine the risk of such a fungus becoming an emergent pathogen.

2.4.2.3. Aspergillus *spp.*

For most fungi, the thermal limit for survival is low and rarely breaches the basal temperatures of mammalian species (Robert and Casadevall, 2009). An exception, however, is the upper limit of some species of *Aspergillus*, a genus of filamentous fungi of which some representatives cause respiratory diseases among both immunocompromised and immunocompetent individuals (Kousha et al., 2011; Badiee and Zare, 2017). Members of this genus, such as *A. fumigatus*, can survive up to 70°C and actively grow at 37°C (Albrecht et al., 2010). The ability to grow at such high temperatures would suggest that this organism might be considered thermophilic; however, *A. fumigatus* and other species within this genus are capable of rapidly growing at mesophilic temperatures, allowing them to be predominant in natural environments (Paulussen et al., 2017). This characteristic distinguishes *Aspergillus* from thermophilic groups and delineates aspergilli as thermotolerant, with a broad thermal range. The ability to grow at mammalian body temperature enables the above-mentioned aspergilli, such as *A. fumigatus*, to infect humans even during fever conditions (Bhabhra and Askew, 2005). Aspergillosis is known for manifesting in the respiratory system, similar to what is seen for

cryptococcosis. The spores of *Aspergillus* spp. are ubiquitous in nature, found in soil, air (both in natural environments and in buildings) as well as in natural water sources (O'Gorman and Fuller, 2008; Paulussen et al., 2017). Human contact with these fungi is therefore inevitable and poses a great risk to individuals predisposed to fungal infection because of defective immune systems. Additionally, antifungal resistance among *Aspergillus* spp. continues to complicate the treatment of aspergillosis. This resistance has also been observed in environmental isolates that have not yet been implicated in clinical situations (Hoda et al., 2019). Such intrinsic resistance towards antifungals adds further pathogenic potential to these aspergilli and poses a great threat to immunocompromised individuals who encounter these thermotolerant fungi in the environment.

2.4.3. Molecular aspects of thermotolerance

Temperature-related stress largely affects the physiology of an organism (Buckley and Huey, 2016). Alterations in a cell responding to elevated temperatures can be studied by investigating proteomic and genomic functionality under stressful conditions. In doing so, researchers identified stress response pathways that overlap among distinct fungal groups (Tereshina, 2005; Fuchs and Mylonakis, 2009; Leach et al., 2012; Juvvadi et al., 2014). The most common response pathway that is associated with thermotolerance is the heat shock response (HSR; Tereshina, 2005), which responds to sudden high increases in external temperature. It is a complex adaptation mechanism that focuses on downregulating housekeeping genes and upregulating cytoprotective genes in order to prevent or restore any damage due to the imposing heat shock (Verghese et al., 2012). The products and cofactors of these regulatory processes are proteins or chaperones known as heat shock proteins (HSPs). Their functions, in addition to gene regulation, include the denaturing, folding, refolding and transport of cytosolic proteins affected by the external stress (Tereshina, 2005). Additionally, HSPs assist in the transcriptional regulation of the cell wall integrity (CWI) and environmental stress response (ESR) pathways (Fuchs and Mylonakis, 2009; Verghese et al., 2012), both of which play a role in thermotolerance. The ESR involves many other signalling transductions responding to external changes in the microorganism's environment such as oxidative-, osmotic-, and pH stress (Verghese et al., 2012). The HSR, for instance, represents a subset within the ESR, since all of the genes involved in this pathway are included in the ESR regulon. The CWI pathway, on the other hand, harbours many other functions separate to environmental stress, despite having overlapping pathways with both the ESR and HSR (Dhar et al., 2013). Nevertheless, multiple regulatory proteins and genes in the CWI pathway have been identified and linked to thermotolerance. Within all these pathways and stress responses, a plethora of proteins and transcriptional factors all cooperate and cross-communicate to provide the microorganism with the proper machinery to adapt to thermal stress (Fuchs and Mylonakis, 2009; Verghese et al., 2013; Dhar et al., 2013).

2.4.3.1. Long term thermotolerance and microevolution

Yeasts and other fungi adapt to environmental stress using several mechanisms which depend on the degree of stimulation as well as the duration thereof (Causton et al., 2001; Berry and Gasch, 2008; Dhar et al., 2013; Pereira et al., 2018). As for cells undergoing heat shock, the harsh stimulus is perceived, and various signals are sent to the rest of the cell to initiate repair as well as prevent further

anticipated damage caused by the heat shock (Tereshina, 2005). During this process, housekeeping genes are paused while the cell rapidly responds to the external threat via HSR upregulation. However, during prolonged exposure to stressful conditions other signalling transductions occur simultaneously which aid in long-term adaptation, including regulatory factors of the CWI pathway and ESR (Causton et al., 2000; Berry and Gasch, 2008; Fuchs et al., 2009; Chen et al., 2012; Dunayevich et al., 2018; Sanz et al., 2018). Although most studies focused on industrially important yeasts, some researchers have investigated the molecular signalling constituents of thermotolerance in clinically relevant fungi (Argüelles, 1997; Juvvadi et al., 2003; Chang et al., 2004; Nichols et al., 2007; Chen et al., 2012; Chen et al., 2013; Chow et al., 2017; Yang et al., 2017; Brandão et al., 2018; Jung et al., 2018; So et al., 2018; Bloom et al., 2019). While the regulatory mechanisms of thermotolerance may vary among fungi, the underlying response pathways are similar (Alonso-Monge et al., 2009; Brown et al., 2020).

Generally, signalling pathways are induced by stimuli received through proteins located on the surface of a cell (Levin, 2005). These proteins or sensors can differ among species and rarely have singular roles. In fungi, precursors that form part of the CWI pathway mostly initiate signalling linked to thermotolerance (Verna et al., 1997; Zu et al., 2001; Verghese et al., 2012; Huang et al., 2018). The messages that these precursors receive are sent to other proteins that are part of various pathways, including the mitogen-activated protein kinase (MAPK)-, RAS-cAMP- and calcineurin signalling pathways (Fuchs et al., 2009; Parts et al., 2011; Verghese et al., 2012; Juvvadi et al., 2014). These pathways are also involved in many other cellular processes and are therefore not exclusively responsible for thermal stress adaptation (Causton et al., 2001; Berry and Gasch, 2008; Fuchs et al., 2009; Chen et al., 2012; Dunayevich et al., 2018; Sanz et al., 2018). Particular genes and proteins that form part of these pathways were linked to thermotolerance. However, the genome and transcriptome of yeasts were investigated only at severe heat shock conditions which might not provide an adequate representation of adaptation to gradual temperature increase (Fuchs et al., 2009; Parts et al., 2011; Verghese et al., 2012; Juvvadi et al., 2014). To gain a better perspective of fungi adapting to prolonged temperature increases, the genetic changes that occur over generations should be investigated.

Huang et al. (2018) carried out experimental evolution on a laboratory strain of *S. cerevisiae* by initiating adaptation through a stepwise increase in temperature, after which the genome was sequenced to investigate acquired thermotolerance. Considering that global temperatures increase gradually (IPCC, 2018), the approach of Huang and co-workers might provide an adequate representation of microevolution in fungi occurring in natural environments with temperature increases. The goal of their study was to experimentally evolve a yeast to obtain a high temperature growth phenotype (Htg^+), as well as determining major contributing factors by investigating the mutations that occurred during the experimental evolution (Huang et al., 2018). The authors found mutations such as single nucleotide variants (SNVs), insertions and deletions (INDELs) and segmental duplications / deletions that could be associated with the Htg^+ phenotype. Many of these mutations were nonsynonymous which altered the amino acid sequence of some proteins. Moreover, the mutations were multiple and sometimes parallel, increasing the significance of the mutation and thus the probability of association with the Htg^+ phenotype. As a result of their study, genetic mutations were identified that can contribute to long-term fungal thermotolerance. In addition, a combination of non-essential gene mutations was examined and

it was determined that a genetically modified thermotolerant strain can be attained (Huang et al., 2018). Although this might seem like a breakthrough for industrial purposes (Amore and Faraco, 2012), it becomes increasingly concerning when considering the implications that global warming might have on fungal microevolution and consequently the emergence of novel fungal pathogens.

2.4.3.2. The dirty river, the fungus and the heat

So far, studies aimed at investigating microevolutionary changes in clinically relevant fungi have mainly focused on virulence and physiological reactions within the host (Magditch et al., 2012; Wartenberg et al., 2014; Ene et al., 2018). However, the combined contribution of pollution and global warming on fungal evolution has not been studied. Apart from the regulatory pathways or physiological reactions to environmental changes, little is known regarding fungal adaptation on a genetic level (Causton et al., 2001; Dhar et al., 2013). Moreover, studies often fail to consider the microevolutionary effects that result from interactions with other biotic and abiotic factors, e.g. other organisms, including plant life, and environmental conditions. The latter, for example, might include anthropogenic pollutants and climate change. Studies investigating pollution-associated microevolution are lacking despite the valuable insight that can be gained from understanding the effects of pollution on fungal growth. This is especially concerning since the pollutants may include antifungals released into the rivers (Chitescu et al., 2015). Thus, during the process of acquiring thermotolerance with the aid of global warming, fungi in polluted aquatic environments could potentially gain resistance towards antifungals, due to the presence of these compounds in surface waters. Future emerging pathogens could therefore be resistant to treatment before even causing infections. Pollution, such as organic pollution, also promotes the growth of fungi by serving as a rich source of nutrients (Wen et al., 2017), which might allow some species to outcompete more fastidious microorganisms and consequently disturb the natural mycobiome (Ortiz-Vera et al., 2018). Certain pollutants may also initiate virulence phenotypes in fungi, e.g. as seen for persistent organic pollutants (POPs) such as pentachlorophenol (Martins et al., 2018). Increasing anthropogenic activities resulting in polluted river water could therefore potentially cause a rise in fungal numbers, particularly pathogenic species. Considering the wide range of factors that may impact on fungi in natural environments, it is prudent to employ not only genomics but also more traditional techniques, such as biochemical, ecological, genetic, morphological, and ontological approaches (Naranjo-Ortiz and Gabaldón, 2019), to delineate the complex synergetic effect of climate change and increased pollution levels on fungal biology.

2.5. CONCLUSIONS

With the ever-increasing urbanisation to accommodate the equally growing population, increased environmental temperatures and pollution of rivers will have detrimental outcomes on human health (Fig. 1). South Africa is a developing country experiencing rapid urbanisation in informal communities with municipalities struggling to keep up and failing to implement proper sanitation infrastructure, causing communities to be dependent on natural water sources for their livelihoods (Colvin et al., 2016). Although little research has been conducted for South Africa, there is growing evidence that a wide diversity of fungi, including opportunistic species that can tolerate the mammalian body temperature, occur in South African rivers. The extent of the risk posed by waterborne fungi to community health

therefore needs to be determined, especially in view of the fact that opportunistic yeasts, such as *Candida* spp., that infect individuals suffering from HIV / AIDS appear to be common in polluted rivers. Furthermore, rising river water temperatures will most probably induce increased thermotolerance amongst waterborne fungi. Worryingly, the fungal metabolism can adapt to these increases and current evidence indicates that more fungi will become thermotolerant as global temperatures rise. This, together with the fact that sewage-polluted rivers contain elevated concentrations of fungi, points to an imminent increase in the incidence of mycoses in the not-so-distant future. Important to note is that non-clinical isolates implicated in mycoses might represent antifungal-resistant fungi due to the various chemicals derived from agricultural-, industrial- and anthropogenic waste that end up in surface waters. However, few studies have been conducted to elucidate the resistance acquired from antifungal exposure in water sources. Nonetheless, the combinatorial effects of pollution (organic or inorganic) and global warming could be drivers in fungal microevolution and potentially lead to a rise in emergent pathogenic fungi, for which there is a limited range of anti-fungal drugs available. Efforts to better record the occurrence and delineate the ecology and antifungal resistance of clinically relevant fungi in South African river systems should therefore be a priority.

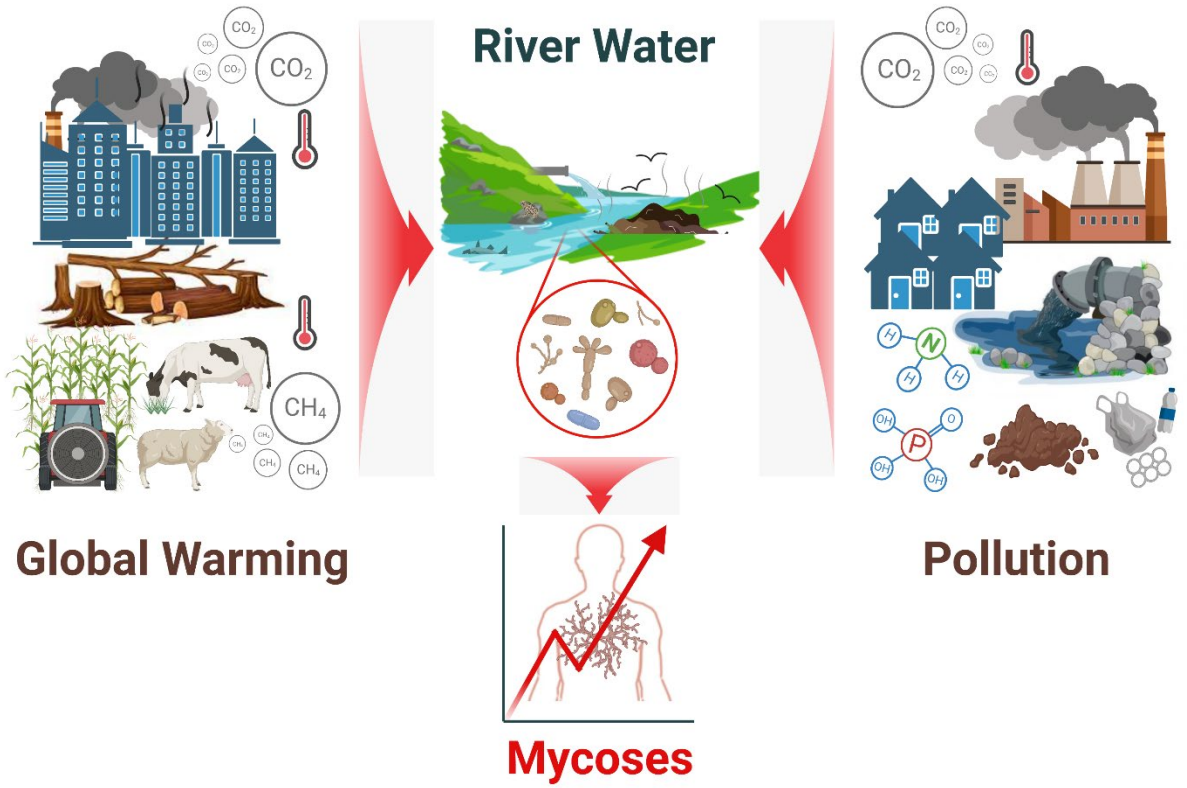

Figure 2.1: The illustration above signifies the contributing factors that may have an impact on humans susceptible to mycoses. Many individuals are dependent on river water due to rapidly growing urban areas with poor sanitation infrastructure. As pollution enhances the proliferation of fungi in the water and induces adaptation to chemicals such as antifungals, global warming simultaneously promotes long-term thermotolerance which aids in fungal pathogenesis. Together the increased temperatures and polluted water may lead to an increase of mycoses cases among immunocompromised people. This figure was created using BioRender.com

2.6. ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from The Water Research Commission, Pretoria, South Africa (WRC Project No. 2019/2020-00137). The National Research Foundation (NRF) is thanked for the personal funding of Heidi Steffen. Further thanks are given to fellow researchers in the Botha Laboratory (Department of Microbiology, Stellenbosch University) for general advice and insights given during the write up of this review article.

2.7. AUTHOR CONTRIBUTIONS

Heidi Steffen contributed to write up and conceptualisation. Alfred Botha, Gideon Wolfaardt and Caylin Bosch contributed to conceptualisation and revision.

CHAPTER 3: POTENTIALLY PATHOGENIC FUNGI IN THE PLANKENBRUG / EERSTERIVIER SYSTEM WITHIN THE STELLENBOSCH REGION

3.1. INTRODUCTION

Nine percent of South Africans are directly reliant on natural water sources for drinking and domestic use (Colvin et al., 2016). Unfortunately, these waters are often polluted with sewage, agricultural, industrial, and other anthropogenic waste which poses a threat to human health (Glińska-Lewczuk et al., 2016; Liyanage & Yamada, 2017; Cullis et al. 2019; Herbig & Meissner, 2019). A major contributor to this threat is the presence of infectious microbial species in the water (Bezuidenhout, 2013; Tubatsi et al., 2015). While the bacteria, viruses and protists that are associated with water pollution have been extensively studied, only a modicum of information is available on the occurrence of opportunistic and pathogenic fungi in surface waters (Monapathi et al., 2020a; Steffen et al., 2022).

The lack of information on clinically relevant fungi in aquatic environments can be attributed to the limited number of fungal taxa identified as pathogenic species (Robert & Casadevall, 2009). Most fungi are incapable of infection due to the human body's advanced immune system and thermal restriction zone. The latter is of concern as thermotolerance among fungal species is a major virulence trait, and it was contended that rising environmental temperatures could gradually drive acquired thermotolerance among naturally occurring fungi (Garcia-Solache & Casadevall, 2010; Casadevall et al., 2020; Steffen et al., 2022), which would eventually render these fungi clinically relevant. Apart from selecting for thermotolerance no standard procedure has been established to isolate clinically relevant, opportunistic or pathogenic fungal species from the environment.

Understanding the ecology of clinically relevant fungi in the natural environment is essential for the well-being of the immunocompromised population suffering from HIV / AIDS, who are most susceptible to fungal infections (Ellis et al., 2000). Although this target group could globally be considered minor, the proportion of the immunocompromised population is likely underestimated due to the exclusion of individuals with immunosuppressive conditions such as diabetes, cancer, obstructive pulmonary disease, and hospitalization (Low & Rotstein, 2011; Fernández-Ruiz et al., 2017; Schwartz & Denning, 2019). Nevertheless, the proportion of individuals in Southern Africa living with HIV / AIDS is dauntingly large compared to other countries (UNAIDS, 2021). In addition, the availability of antifungal drugs to treat mycoses among this population is mostly restricted to Amphotericin B and Fluconazole (Kneale et al., 2016). Another worrying phenomenon is the increasing reports of antifungal resistance, against these drugs, among clinically relevant fungi (Pfaller et al., 2010; Pfaller et al., 2012; Silva et al., 2012). It is clear these microorganisms occurring in the environment pose a threat to public health; however, very little information exists on the risk of infection when being exposed to harmful fungi.

In developing countries, including South Africa, immunosuppressed individuals may reside in rural settlements that lack proper water infrastructure, thus increasing dependency on nearby surface waters such as rivers (Colvin et al., 2016). The presence of pathogenic fungi within rivers could therefore place these communities at high risk of contracting infections, which are becoming increasingly difficult to treat as a result of antifungal resistance. The frequent detection of antifungal resistant strains from South African rivers raises further concern because of the high risk of severe infections and fatal outcomes caused by these microorganisms (Monapathi et al., 2017; Monapathi et al., 2018; Monapathi et al., 2021). The aim of this study was therefore to evaluate the risk of potentially pathogenic fungi present in polluted water from a South African river system in close proximity to human activity. The objectives of the study were as follows: i) to investigate the fungal diversity of the river water subjected to different levels of pollution and to isolate, enumerate and identify potentially pathogenic yeasts using thermally selective isolation; ii) investigate the antifungal resistance profiles of the isolated yeasts; and iii) conduct a provisional quantitative risk assessment on yeast infections following river water ingestion. Overall, we provide insight into fungal diversity and the prevalence of certain yeast pathogens in polluted river water, and demonstrate the potential risks associated with river water use in developing countries.

3.2. MATERIALS AND METHODS

3.2.1. Study area

River water sampling was conducted in and around Stellenbosch, Western Cape, South Africa. Ten sampling sites were selected forming part of the Eersterivier River Catchment (ERC). Tributaries such as Plankenbrug river, Krom river and the Veldwagters river were included in this study (Figure 3.1), representing waters frequently met with human influence due to their proximity to urban and industrial areas.

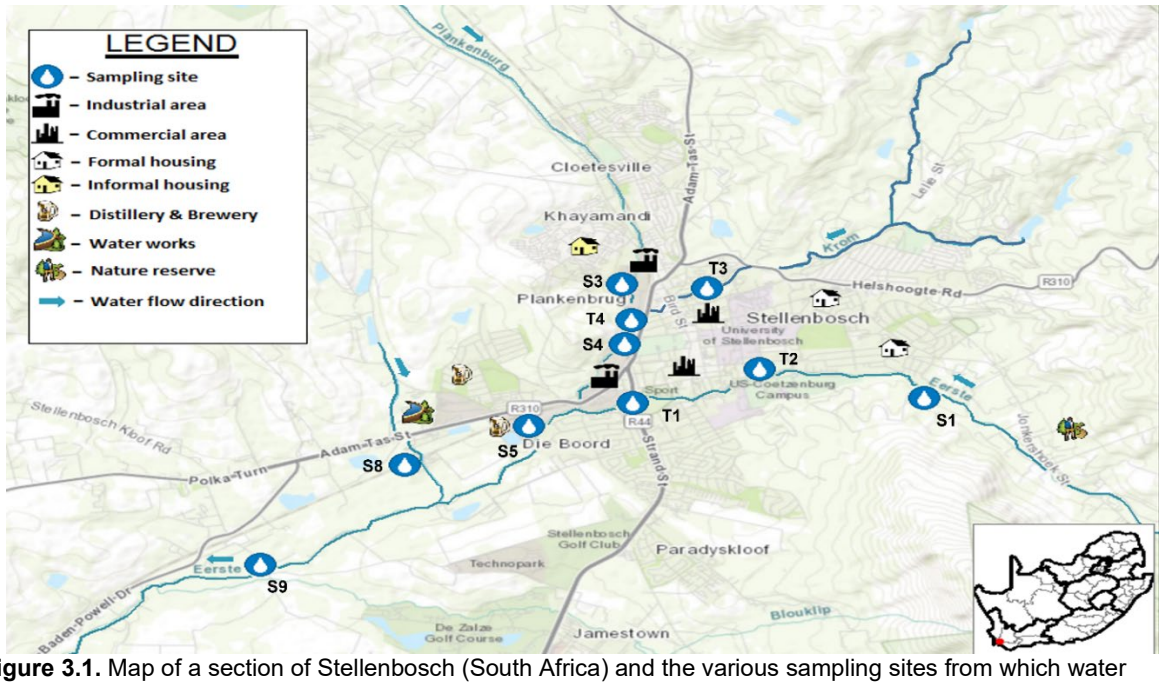

Figure 3.1. Map of a section of Stellenbosch (South Africa) and the various sampling sites from which water samples were collected for analysis. This map was constructed using South Africa's ArcGIS online software (https://www.esri.com/en-us/arcgis/products/arcgis-online/capabilities/make-maps).

3.2.2. Sampling and water quality analysis

Samples were collected every second month (when feasible) for a period of one year to encompass all seasons (Table 3.1). Thirty 200 mL water samples (three samples per site) were collected at approximately 30 cm depth (when feasible) and poured into sterilized 200 mL Schott bottles. Additional water samples were collected in August 2021 as required for metagenomic analyses. Measurement of pH, conductivity and temperature was carried out on site using a multi-parameter instrument (PCTestr35™; Eutech, Paisley, UK) and dissolved oxygen (DO) was measured using a DO meter (ProODO; YSI, Yellow Springs, USA). Chemical oxygen demand (COD) and ammonia (NH_3) concentration were determined in the laboratory using a kit (Supeclo, Bellefonte, USA) and the phenol-hypochlorite assay (Weatherburn, 1967), respectively. COD and NH_3 measurements were carried out within 6-10 hours of sampling. Faecal contamination was evaluated by determining the numbers of *Escherichia coli* and coliforms using dilution plates prepared with membrane lactose glucuronide agar (MLGA; Sigma-Aldrich, St. Louis, USA) and an incubation period of 18 h at 37°C.

Table 3.2. Sampling dates and details.

Sampling Date	Season	Notes
7 September 2020	Spring	No data obtained for *E. coli* and coliform counts
18 February 2021	Summer	No COD data obtained: values estimated using site average
15 April 2021	Autumn	N.A.
19 June 2021	Winter (heavy rainfall*)	Access to site S9 was restricted, no data obtained for this site
14 August 2021	Winter (heavy rainfall*)	Additional water samples used for metagenomic analyses
27 October 2021	Spring	Heavy rainfall* occurrence in the seven days prior sampling
15 January 2022	Summer	N.A.

* Based on data obtained from weather forecasting websites (http://www.weather.lcao.co.za/; https://www.worldweatheronline.com/).

3.2.3. Metagenomics: sample preparation and analyses

River water samples collected in one sampling event (Table 3.1) were aseptically filtered through membrane filters (0.45µm pore size; GN-6 Metricel® filters, Pall Corporation, New York, US). Preparation for DNA extraction followed a method described by Waso and colleagues (2016) during which the biological matter on the membrane was dislodged using citrate buffer and vortexing. Cells were harvested by centrifugation and the resulting cell pellets were suspended in 200 µL phosphate-buffered saline (PBS). The samples were subsequently treated with ethidium monoazide (EMA) as described previously (Reyneke et al., 2017) to exclude the detection of DNA from non-viable cells. Total DNA was extracted using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corporation, Irvine, USA), and sent for PacBio metagenomic sequencing at Inqaba Biotec (Pretoria, South Africa). Analysis of ITS1F gene amplicons was performed on the Sequel system by PacBio (www.pacb.com). Raw subreads were processed through the SMRTlink (v8.0.0.80529) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). To enable further insights into the fungal communities across the river sites, the data was analysed both individually and in two groups, one considered highly polluted (HP) and the other less polluted (LP). The grouping was based on the river water's overall average water quality parameters DO, COD, NH_3, and conductivity as described above (**Table S1 & Figure S1**).

The raw sequence data were analysed using Mothur (v.1.44.1), following a tutorial (available at http://www.mothur.org/wiki/), with some modifications for the fungal ITS region (Schloss, 2009). In short,

sequences that were between 100-1000 bps in length, with an average quality score of 25 and higher, no ambiguous bases, and containing homopolymer regions shorter than eight base pairs, were selected for further analysis. Chimeric sequences and sequences with errors were removed to ensure that only high-quality sequences were classified using the latest available UNITE reference database (Abarenkov et al., 2021). Sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity and classified using a cut-off value of 80%. All samples were normalized to contain the same number of sequences.

3.2.4. Isolation and enumeration of yeasts

Up to 100 mL water from each sample was filtered (polycarbonate filter-holder; Sartorius Stedim Biotech, Göttingen, Germany) through a nitrocellulose membrane (0.45 µm; Sartorius Stedim Biotech) that was subsequently placed onto Sabouraud dextrose agar (SDA; Atlas, 2010) supplemented with 500 mg chloramphenicol (Sigma-Aldrich). Agar plates were incubated at 37°C for 20-24 hours after which yeast colonies were counted and recorded.

3.2.5. Identification of yeasts

Up to six yeast isolates per sampling site were randomly selected from observable yeast colonies on isolation plates using the Harrison's Disc method (Harrigan & McCance, 1976). Following pure culture preparation, yeast isolates were streaked onto yeast malt extract agar (YMA; Atlas, 2010) to be identified using sequence analysis of taxonomic informative gene sequences that were amplified using the polymerase chain reaction (PCR). For this purpose, a single yeast colony of each isolate was suspended in 50 µL sterile milliQ, after which the cells were disrupted by incubating at 99°C for 5 minutes to obtain DNA. The D1-D2 domain of the 28S rDNA from each isolate was subsequently amplified with the forward primer F63 5'-CATATACAATAAGCGGAGGAAAAG-3' and the reverse primer LR3 5'-GTCCGTGTTTCAAGACGG-3' using an Applied Biosystems 2720 thermal cycler. Reaction tubes contained the following components: 12.5 µL Taq Ready Master Mix (New England Biolabs, Ipswich, USA), 1.25 µL of each of the primers, 8 µL milliQ water and 2 µL DNA. PCR conditions were as follows: initial denaturation step (94°C for 5 min), 25 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s) and extension (72°C for 30 s) and lastly a single final extension (72°C for 7 min). PCR products were evaluated using gel electrophoresis prior to being submitted for Sanger Sequencing at the Central Analytical Facilities (Stellenbosch University, South Africa). Sequences were analysed using FinchTV (Finch TV 1.4.0, Geospiza, Inc.) prior to BLAST identification on the NCBI database (http://www.ncbi.nlm.nih.gov/). Briefly, the 28S ribosomal RNA sequences (LSU) from the fungi type and reference material database on NCBI were compared to the amplified sequences of the isolates, and hits with the highest maximum score and an E value of zero were recognised as positive identifications. The identities of the isolates were subsequently confirmed by drafting phylogenetic trees using MEGA11 (version 11; Tamura et al., 2021; **Figures S2-6**).

3.2.6. Sequence accession numbers

The D1/D2 rDNA gene sequences of the yeast isolates identified during this study were deposited in the Genbank database under the following accession numbers: OK618557-OK618604, OK618606-

OK618664, OL721678-OL721730, OL721839-OL721863, OL774708-OL774765 and OM883947-OM883970.

3.2.7. Storage and quality control of yeast isolates

Stock cultures of the identified yeast isolates were stored at −80°C in 25% (v/v) glycerol and deposited in the yeast culture collection of the Department of Microbiology, Stellenbosch University (Stellenbosch, South Africa). Quality control of the yeast isolate collection was conducted by recording colony morphology on a chromogenic medium, i.e. molybdate agar (**Table S2**; MBA; MacLaren, 1960). It must be noted that the chromogenic agent solution used in this medium was 6.75% (w/v) phosphomolybdic acid (PMA; $H_3PMo_{12}O_{40;}$ analytical reagent, Sigma-Aldrich), which resulted in the same concentration of molybdenum in the medium as was obtained by others using the original chromogenic agent ($20MoO_3 \bullet 2H_3PO$; MacLaren, 1960). Changes in the yeast cultures were detected by selecting cultures to prepare yeast cell suspensions in physiological saline solution (single colony suspensions), which was then spotted onto the chromogenic medium and incubated at 37°C for 5 days. The colony pigment and morphology of both the top and underside of the agar plates where subsequently recorded to evaluate any change that may have occurred in the working as well as stock cultures.

3.2.8. Antifungal susceptibility of yeasts

Fluconazole (FLU; Sigma-Aldrich) and amphotericin B (AmB; EMD Millipore Corp., Burlington, USA) were tested against the clinically relevant yeasts identified during the study. The minimum inhibitory concentration (MIC) of yeast strains was determined using the broth microdilution method as stipulated by the Clinical and Laboratory Standards Institute (M27-A2; NCCLS, 2002). Antifungal concentrations ranged from 0.063-64 mg/L and 0.031-16 mg/L for FLU and AmB, respectively. All strains were subjected to antifungal susceptibility testing in triplicate and incubated for 48 hours at 35°C. The FLU MIC was determined as the lowest concentration at which ≥50% of growth was inhibited, whereas the AmB MIC was determined as the lowest concentration which inhibited ≥99% of growth.

3.2.9. Statistical analysis

3.2.9.1. *Water quality and potentially pathogenic yeasts*

All data collected from sampling events were statistically analysed using XLSTAT (version 2021.4, Addinsoft Inc., New York, USA) to determine means, standard errors, and correlations between parameters. Due to the non-normality of the data (as determined by a Shapiro-Wilk test) the Kruskal-Wallis and multiple pairwise comparisons (Steel-Dwass-Critchlaw-Flinger method) tests were used to evaluate significant differences between sampling sites. Significance was determined at a confidence level of 95% ($p<0.05$). Furthermore, multivariate correlations were investigated between microbiological and physicochemical data through redundancy analysis (RDA). A permutation test was included to confirm whether response variables and explanatory variables were linearly related.

3.2.9.2. *Metagenomics*

All statistical analyses were performed in Mothur (v.1.44.1) and R (v.4.1.0, R Core Team 2021) using the microeco-package (Liu et al., 2021). Non-parametric Kruskal-Wallis H-tests were calculated for all

alpha diversity metrics. Multidimensional scaling plots (Principal Coordinate Analysis; PCoA) were drawn in R using the Bray-Curtis dissimilarity matrix. Further statistical evaluations of the PCoA plots were conducted using Permutational Multivariate Analysis of Variance (PERMANOVA). Spearman correlation tests were performed between environmental measures and PCoA ordinations based on the Bray-Curtis dissimilarity matrix. Differences in pathogenic abundance between pollution groups was assessed using the Mann-Whitney test. For all statistical evaluations, a p-value of 0.05 was considered significant.

3.2.10. Development of yeast quantitative microbial risk assessment (QMRA)

Various parameters are used when conducting QMRA, namely contaminant concentrations, exposure routes, microorganism survival probability and contaminant concentration at which 50% of a population are killed (http://qmrawiki.canr.msu.edu/). The latter two parameters are determined using clinical data obtained from species-specific pathogenicity studies in animal hosts. Studies focusing on these characteristics of pathogenic fungi including yeasts are, however, either lacking or insubstantial. Nevertheless, the pathogenicity of *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Clavispora lusitaniae* (syn. *Candida lusitaniae*), *Pichia kudriavzevii* (syn. *Candida krusei*) and *Meyerozyma guilliermondii* (syn. *Candida guilliermondii*) has been investigated (Hasenclever et al., 1959; Wingard et al., 1982; Fromtling et al., 1987; Arendrup et al., 2002). Thus, we used data from these studies to calculate the pathogenic potential (PP) of the yeast species as described by Casadevall (2017) to be used as a proxy for the dose response model parameters. We then employed the @RISK software by Palisade (version 8; New York, USA), and the PP of the yeasts (as absolute values), to determine the risk of yeast infection after exposure to the river water.

3.2.10.1. *Exposure assessment*

Ingestion was identified as the direct exposure route for infection of pathogenic yeasts occurring in river water. Species specific exposure doses (1) were calculated, assuming an average ingestion rate (Kahn & Stralka, 2009), using the following equation:

$$d_{exposure} = v_{ingestion} \times C_{contaminant} \quad (1)$$

The total daily ingestion of the water source is represented by the volume of ingestion ($v_{ingestion}$), while the species-specific yeast concentration is represented by the contaminant concentration ($C_{contaminant}$). The latter was calculated by first determining total thermotolerant yeast counts on the filter disks that were incubated on the SDA plates, then by identification of random isolates from the disks (**Tables S3-9**). Yeast cell concentrations were subsequently calculated (with the assumption that one yeast colony would represent 1×10^6 cells; Joseph & Hall, 2004) and log-transformed to normalize the data (McDonald, 2014; **Table S10**). Furthermore, yeast concentration results obtained from HP and LP sites were pooled, respectively.

3.2.10.2. *Dose response parameters*

Since no dose response model, as well as the parameters to be used in the model, exist for pathogenic yeasts, the above-mentioned PP was used as a proxy to determine the probability of infection. The PP was calculated according to the method of Casadevall (2017) using the following equation:

$$PP = \frac{F_s}{I} \times 10^M \quad (2)$$

The term (F_s) refers to the fraction of affected individuals that show symptoms of infection after being exposed to a representative of a specific pathogen. Therefore, data obtained by others on the fungal burden, i.e. the fraction of infected kidneys in murine models after being intravenously exposed to representatives of the relevant yeast species, was used for F_s. (Arendrup et al., 2002). The equation $F_s = 1$ was used for a yeast species where previous studies reported the lethal dose that killed 50% of the population (LD_{50}) after being exposed to the particular yeast species (Hasenclever et al., 1959; Wingard et al., 1982; Fromtling et al., 1987). The term (I) represents the infecting inoculum, i.e. the cell concentration that was intravenously injected into the animal model to induce infection. The fraction of mortality (M) represents the death outcomes among infected individuals. Using the abovementioned parameters, and data available from literature (Hasenclever et al., 1959; Wingard et al., 1982; Fromtling et al., 1987; Arendrup et al., 2002), we determined the PP of seven clinically relevant yeasts (Table 1).

Table 3.3: Dose response parameters of clinically relevant yeasts.

Species	PP‡	Study type(s)	References
Candida albicans	2.44×10^{-4}	LD_{50} and Fungal burden	Hasenclever et al. (1959); Wingard et al. (1982); Arendrup et al. (2002)
Candida glabrata	3.80×10^{-6}	Fungal burden	Arendrup et al. (2002)
Candida parapsilosis	2.18×10^{-6}	Fungal burden	Arendrup et al. (2002)
Candida tropicalis	1.93×10^{-4}	LD_{50} and Fungal burden	Hasenclever et al. (1959); Wingard et al. (1982); Fromtling et al. (1987); Arendrup et al. (2002)
Clavispora lusitaniae	2.14×10^{-6}	Fungal burden	Arendrup et al. (2002)
Meyerozyma guilliermondii	1.76×10^{-8}	Fungal burden	Arendrup et al. (2002)
Pichia kudriavzevii	3.70×10^{-8}	Fungal burden	Arendrup et al. (2002)

‡ Pathogenic Potential – represents a mean and was calculated as described by Casadevall (2017)
LD_{50} – Lethal Dose at which 50% of the population died

To determine the daily risk of fungal infection ($P_{infection,d}$; Weiskerger & Brandão, 2020) a modification of the exponential dose response model (3) was used in this study, whereby k was replaced by PP (4):

$$P_{infection} = 1 - e^{-k(d_{exposure})} \quad (3)$$

$$P_{infection,d} = 1 - e^{-PP(d_{exposure})} \quad (4)$$

The annual risk of infection ($P_{infection,a}$) was subsequently determined to evaluate the health risks faced by individuals who are dependent on a natural water source for drinking. The following equation was used:

$$P_{infection,a} = 1 - [1 - P_{infection,d}]^{365} \quad (5)$$

The following assumptions were made while conducting the QMRA during this study: (a) individuals drinking natural surface water do so on a daily basis, (b) wild type and clinical yeast strains would react

similarly upon exposure to host, (c) isolation at 37°C mimics the immediate stress a yeast is exposed to inside the host, (d) translocation of the yeast from the intestines into the bloodstream occurs post-ingestion in order to cause infection (Hirayama et al., 2020; Zhai et al., 2020), and (e) the murine model adequately mimics human host conditions. For the purpose of this study, risk of infection was determined for river water of varying quality and could not be compared to previous risk estimations, since no such estimations existed at the time of this study.

3.3. RESULTS

3.3.1. Water quality analysis

Several biological and physicochemical parameters of the river water, some of which are known pollution indicators, were found to differ between the sampling sites (Figures 3.2 and 3.3) The mean values obtained for the physicochemical parameters that were measured over the experimental period are presented in Figure 3.2. Water temperature ranged from 12.4-26.4°C and site S8 showed significantly higher temperatures than site S1 ($p<0.05$). The average pH of the river water was found to be 7.94 and no statistical differences were observed between any of the sampling sites. Dissolved oxygen was lowest at sites S3 (1.26-5.47 mg/L) and S4 (0,79-8,48 mg/L). All sampling sites (except S4) showed significantly higher ($p<0.05$) DO values than site S3. A DO range of 3.16-8.46 mg/L was observed for site S8 which had significantly higher O_2 concentrations than sites S1, T1, T2, T3, S3 ($p<0.001$), S5 ($p<0.01$) and S9 ($p<0.05$). Three sites (S1, T1, T2) showed mean DO values >10 mg/L ranging from 10.28-10.37 mg/L.

The conductivity measurements at all ten sites ranged from 81 to 944 µS/m, with the highest mean observed at site S3 (787 µS/m) and the lowest at site S1 (92 µS/m). The largest variation was observed at site S4 with a mean conductivity ranging from 155 to 856 µS/m. Mean conductivity was <110 µS/m at sites S1, T1 and T2; >200 µS/m at sites S9, S5, T3 and T4; and >600 µS/m at sites S3, S4 and S8. Significant differences were observed between most sites (Figure 3.2C; $p<0.05$). Chemical oxygen demand at all sites ranged from 1-117 mg/L and site S3 showed the highest mean of 79.17 mg/L, whereas S1 had the lowest mean of 27.08 mg/L. Site S3 showed significantly higher COD values than all sites ($p<0.001$) except site S4. The latter site also presented significantly higher COD values than sites S1, T1 and T3. Furthermore, site S1 also showed significantly lower COD values than sites S8 and S9 ($p<0.05$). Ammonia concentrations ranged from 0.001 to 1.050 mg/L, with the highest means observed for sites S3 (0.365 mg/L) and S4 (0.143 mg/L). Concentrations <0.05 mg/L were observed for sites S1, T1, T2, T3, T4 and S9, whereas concentrations >0.05 mg/L were observed for sites S4, S3, S5 and S8. The mean NH_3 concentration of site S3 was significantly higher than that of all the other

sites ($p<0.01$) except for site S4. Furthermore, site S4 had significantly higher NH_3 concentrations than what was observed at sites S1, S9, T1, T2, T3 and T4 ($p<0.05$). The microbiological load and hence the level of faecal pollution was determined by quantifying the numbers of *Escherichia coli* and total coliforms in the river water (Figure 3.3). *Escherichia coli* was detected at all sampling sites and ranged from 1.70×10 to 2.74×10^6 CFU/100mL. The highest mean *E. coli* concentrations were observed at site S3 (4.64×10^5 CFU/100mL) and the lowest mean was observed at site S1 (1.88×10^2 CFU/100mL).

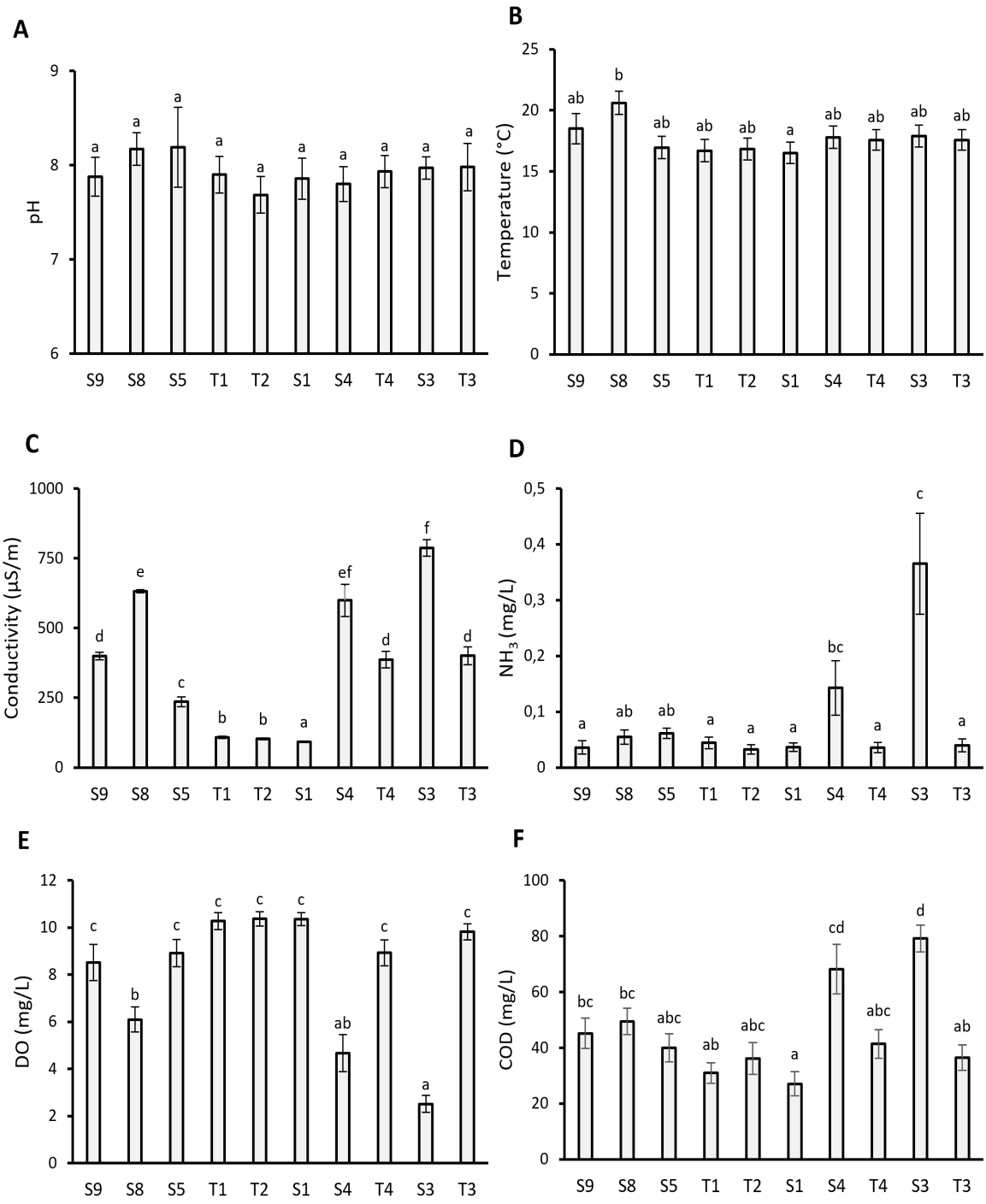

Figure 3.2. Physicochemical parameters of river water measured at the different sampling sites, Stellenbosch, South Africa: **A**) pH, (**B**) temperature, (**C**) conductivity, (**D**) NH_3 concentration, (**E**) DO and (**F**) COD. The bars represent the site averages of seven sampling events during which both dry and rainy seasons are included. Error bars represent standard error of means (SEM) and statistically significant differences ($p<0.05$) between sampling sites are denoted with different lettering above bars (bars that share letters are not significantly different from each other).

Similarly, the coliform counts ranged from 1.02×10^2 to 5.87×10^6 CFU/100mL and the highest mean of 1.13×10^6 CFU/100mL was observed at site S3, whereas site S1 showed the lowest mean (4.80×10^2 CFU/100mL). Both *E. coli* and coliform counts obtained at site S3 was significantly higher ($p<0.05$) than that of the other sites. Site S1 had significantly lower *E. coli* concentrations than all sites ($p<0.05$)

excluding sites S9, T1 and T2. Similar results were observed for coliform counts, with the exception that site S1 also showed significantly lower values than site S9.

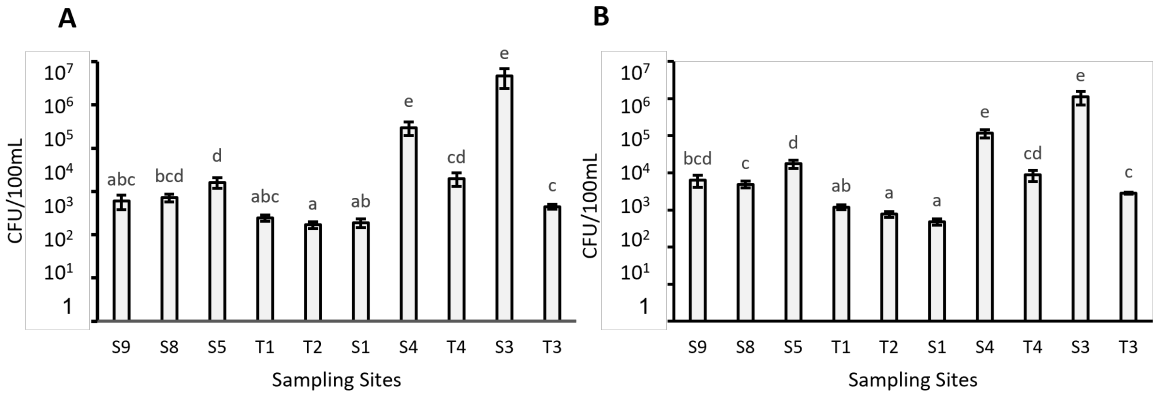

Figure 3.3. Microbiological load (colony forming units (CFU)/100 mL) of the river water at the different sampling sites as determined by enumeration of (**A**) *E. coli* and (**B**) coliforms. The bars represent the means of six sampling events during which both dry and rainy seasons were included. Error bars represent SEM and significant differences ($p<0.05$) between sampling sites are denoted with different lettering above bars (bars that share letters are not significantly different from each other).

3.3.2. Fungal diversity

The relationships of fungal community composition (represented by relative abundance as determined from metagenomic data) among different sites is presented in Figure 3.4.

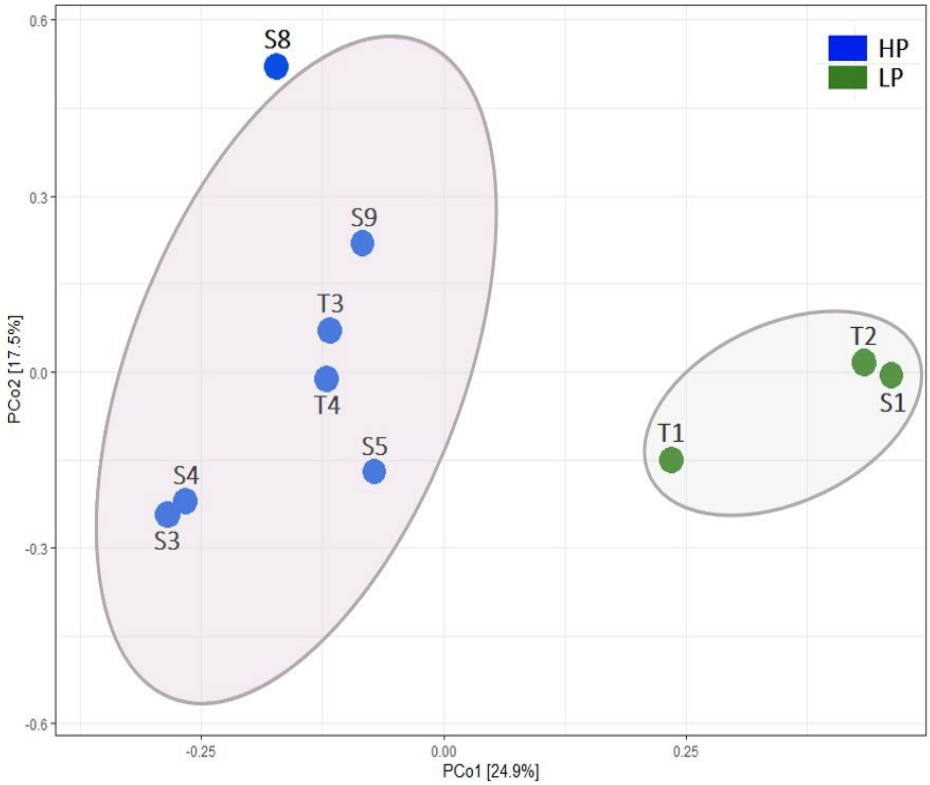

Figure 3.4. Principal coordinate analysis plot (PCoA) of the fungal communities in water samples from river sites of varying levels of pollution: less polluted (LP); highly polluted (HP).

The fungal communities of less polluted (LP) and highly polluted (HP) sites differed significantly (p=0.007).

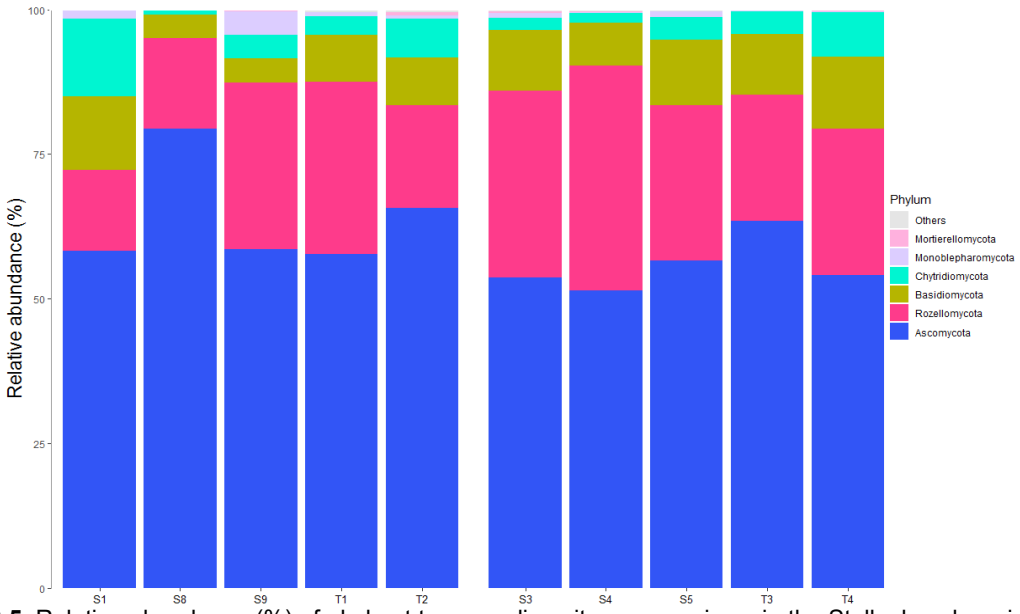

Figure 3.5. Relative abundance (%) of phyla at ten sampling sites across rivers in the Stellenbosch region (Cape Town, South Africa).

Metagenomic analyses indicated that the most predominant phylum detected at all sampling sites was Ascomycota (Figure 3.5). Three other major phyla were also detected, namely Rozellomycota (previously Cryptomycota), Basidiomycota, and Chytridiomycota (Figure 3.5). Twenty genera among these phyla, that may harbour opportunistic and pathogenic fungal strains, were detected at an overall relative abundance of 0.91% (Figure 3.6). A significantly higher (p=0.038) relative abundance of pathogen representing genera was observed at HP sites compared to LP sites (Figure 3.6).

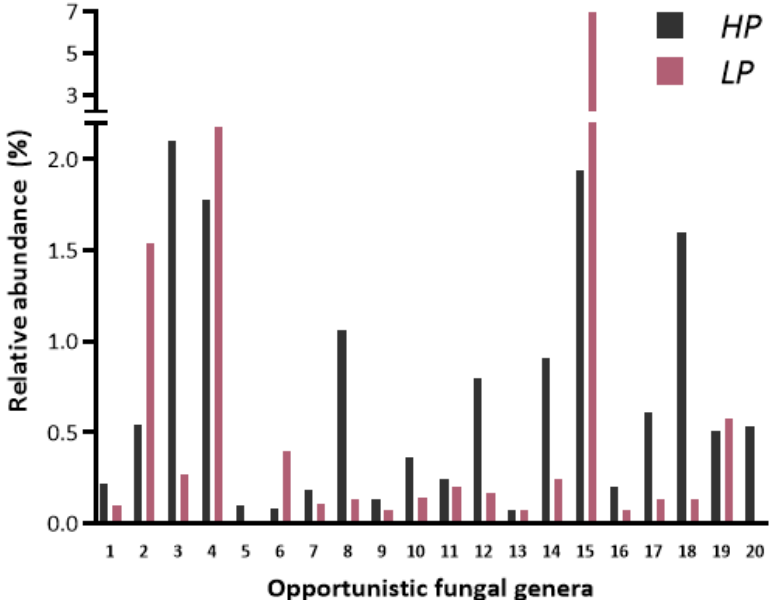

Figure 3.6. Results obtained using metagenomic analysis: Average relative abundance (%) of genera which harbour opportunistic and pathogenic species, detected in river water of varying quality: HP, highly polluted; LP, less polluted. Genera: (1) *Acremonium,* (2) *Aspergillus,* (3) *Candida,* (4) *Cladosporium,* (5) *Clavispora,* (6) *Coniochaeta,* (7) *Cryptococcus,* (8) *Cutaneotrichosporon,* (9) *Cyberlindnera,* (10) *Debaryomyces,* (11) *Exophiala,* (12) *Fusarium,* (13) *Meyerozyma,* (14) *Microsphaeropsis,* (15) *Penicillium,* (16) *Pichia,* (17) *Rhodotorula,* (18) *Saccharomyces,* (19) *Talaromyces* and (20) *Trichosporon*.

3.3.3. Occurrence of thermotolerant yeasts in river water

Thermotolerant yeast concentrations in the river water were determined by enumerating yeast colonies on filter disks that were incubated at 37°C on chloramphenicol-supplemented SDA. The mean yeast concentration (CFU/100mL) for each site is presented in Figure 3.7. The experimental period encompassed all seasons (Table 3.1). Mean yeast concentrations did not differ significantly between any of the sampling sites (Figure 3.7). However, significantly lower yeast concentrations were observed during September 2020, February 2021, and April 2021 compared to the observed yeast concentrations of the remaining sampling months (Table 3.1). Aside from a slightly negative correlation to COD ($r=-0.103$), overall thermotolerant yeast concentrations did not correlate with any of the other measured physicochemical parameters in this study.

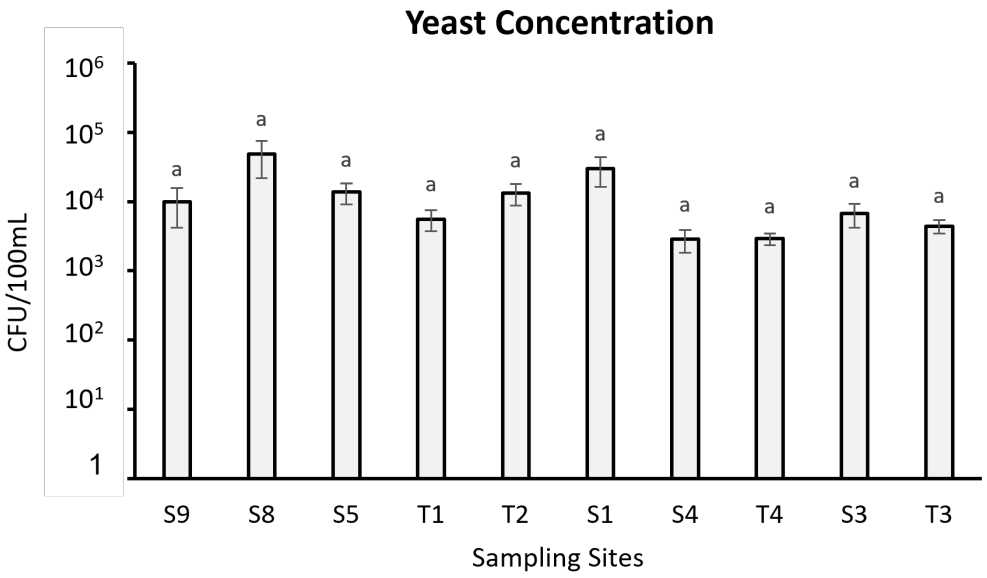

Figure 3.7. Yeast numbers (CFU/100mL) in river water as determined after enumeration at 37°C. The bars represent the means of seven sampling events including months with high and low rainfall. Error bars represent SEM and significant differences ($p<0.05$) between sampling sites are denoted with different lettering above bars.

3.3.4. Yeast identification

Thermotolerant representatives of 42 different species were isolated from the river water during the experimental period; 41 of these species were ascomycetous fungi, while *Cutaneotrichosporon mucoides* (syn. *Trichosporon mucoides*) is known to be a basidiomycetous yeast (Table 3.2). Species that were frequently isolated (>10 isolates) were *M. guilliermondii* (syn. *C. guilliermondii*), *P. kudriavzevii* (syn. *C. krusei*), *C. glabrata*, *Clavispora lusitaniae* (syn. *Candida lusitaniae*) and *Saccharomyces cerevisiae*. Sites T1, T2 and S1 show the highest species richness (*S*=12), whereas the lowest was observed at site S3 (*S*=5). Members of *M. guilliermondii* were frequently isolated from all sites.

Several clinically relevant yeasts were isolated representing well-known pathogens (Table 3.3): *Candida albicans*, *C. glabrata*, *Candida tropicalis*, *Lodderomyces elongisporus* (syn. *Candida parapsilosis*), and *P. kudriavzevii* (Cooper, 2013). Opportunistic pathogenic species isolated from the

Table 3.3. Yeast species identified in river water of Stellenbosch, South Africa

Species	Pathogenicity [a]	Sampling site	Number of isolates [b]
Candida albicans	P	S9, S8	4
Candida blankii	OP	T2	1
Candida ethanolica	NP	S9, S5	3
Candida glabrata	P	S9, S8, S5, T1, S1, S4, T4, S3, T3	20
Candida melibiosica	OP	S1	1
Candida pseudoaaseri	OP	S4	1
Candida pseudoglaebosa	NP	S4	1
Candida pseudolambica	NP	T4	1
Candida silvanorum	NP	T1, S1	2
Candida tropicalis	P	S5, S4, T3	6
Clavispora lusitaniae (syn. *Candida lusitaniae*)	OP	S9, S5, T1, T4, S3, T3	11
Coniochaeta hoffmannii	NP (P, canine)	T1	1
Cutaneotrichosporon mucoides (syn. *Trichosporon mucoides*)	OP	T2, S1	2
Cyberlindnera jadinii (syn. *Candida utilis*)	OP	S9, S8, S5	7
Cyberlindnera rhodanensis	NP (P, bovine)	S4	1
Debaryomyces subglobosus	NP	S1	1
Hanseniaspora guilliermondii	NP	T1	1
Hanseniaspora pseudoguilliermondii	NP	T1, T4	4
Hanseniaspora thailandica	NP	T2	1
Kazachstania bovina (syn. *Candida bovina*)	NP (P, bovine)	S1	2
Kazachstania servazzii (syn. *Saccharomyces servazzii*)	NP	T1	1
Kluyveromyces marxianus (syn. *Candida kefyr*)	OP	S8, S3	2
Kodamaea ohmeri (syn. *Yamadazyma ohmeri*)	OP	T1, T4	2
Kuraishia molischiana (syn. *Candida molischiana*)	NP	T2	1
Lodderomyces elongisporus (syn. *Candida parapsilosis*)	P	T2	1
Meyerozyma carpophila (syn. *Candida carpophila*)	NP	T2	1
Meyerozyma caribbica (syn. *Candida fermentati*)	OP	T4	1
Meyerozyma guilliermondii (syn. *Candida guilliermondii*)	OP	S9, S8, S5, T1, T2, S1, S4, T4, S3, T3	221[c]
Ogataea henricii	NP	T2	1
Pichia bruneiensis	NP	T2	1
Pichia kluyveri	OP	S9, S5, S1	4
Pichia kudriavzevii (syn. *Candida krusei*)	P	S9, S5, T1, S1, S4, T4, S3, T3	64
Pichia manshurica	NP	S9, S8, T3	4
Pichia occidentalis	NP	S9	2
Saccharomyces cerevisiae	OP	S9, S5, T1, T2, T3	12
Saccharomycopsis fibuligera	NP	T2	2
Saturnispora silvae (syn. *Candida silvae*)	NP	T2	2
Schwanniomyces polymorphus	NP	S1	3
Suhomyces ambrosiae (syn. *Candida ambrosiae*)	NP	S1	1
Trichosporiella flavificans (syn. *Candida flavificans*)	NP	T1	1
Wickerhamomyces onychis	OP	S8	1
Yamadazyma mexicana	NP	S1, T4	3

P – Pathogen; OP – Opportunistic pathogen; NP – Non-pathogen.

[a] Based on clinical cases reported in literature of the species (Al-Sweih et al., 2011; Kurtzman, 2011; Pfüller et al., 2011; Cooper, 2013; Rizzitelli et al., 2016; Aslani et al., 2018; Nobrega de Almeida et al., 2018; Hirayama et al., 2018; Treguier et al., 2018; Lim et al., 2020; Seth-Smith et al., 2020).

[b] Randomly selected from the chloramphenicol-supplemented SDA isolation medium (Harrigan & McCance, 1976).

[c] Represent isolates that were identified as *M. guilliermondii* based on MBA morphology as observed in **Table S2.**

river water were members of *Candida blankii*, *Candida melibiosica*, *Candida pseudoaaseri*, *C. lusitaniae*, *Cutaneotrichosporon mucoides* (syn. *Trichosporon mucoides*), *Cyberlindnera jadinii* (syn. *Candida utilis*), *Kluyveromyces marxianus* (syn. *Candida kefyr*), *Kodamaea ohmeri* (syn. *Yamadazyma ohmeri*), *Meyerozyma caribbica*, *M. guilliermondii* (syn. *Candida guilliermondii*), *Pichia kluyveri*, *S. cerevisiae*, and *Wickerhamomyces onychis* (Al-Sweih et al., 2011; Kurtzman, 2011; Pfüller et al., 2011; Cooper, 2013; Rizzitelli et al., 2016; Aslani et al., 2018; Nobrega de Almeida et al., 2018; Hirayama et al., 2018; Treguier et al., 2018; Lim et al., 2020; Seth-Smith et al., 2020).

Colony morphology on MBA, used for quality control of the yeast isolate collection, is presented in **Table S2.** Representatives of some species were unable to grow on MBA and these species were therefore omitted from the results presented in **Table S2.** These species are *C. lusitaniae*, *K. marxianus*, *Kazachstania servazzii*, *Pichia bruneiensis*, *Candida pseuodolambica*, *Yamadazyma mexicana*, *Pichia occidentalis*, *Candida ethanolica*, *C. pseudoaaseri*, and *Saturnispora silvae*.

3.3.5. Multivariate correlation analysis

Redundancy analysis was carried out on microbiological and physicochemical data collected during this study. The microbiological data included *E. coli* and total coliform counts, total thermotolerant yeast concentrations, as well as the concentrations of the most frequently (>10) isolated yeast species. The analysis involved multiple linear regression and principal component analysis to construct an ordination biplot indicating the associations between the different parameters (Figure 3.8). The biplot collectively represents 87.14% of the variation in the data. Variation was highest for microbiological data.

Conductivity and COD values were weakly associated with *P. kudriavzevii* whereas *S. cerevisiae* concentrations appear to have a strong positive association with pH (Figure 3.8). However, Pearson correlation analysis revealed that the latter association is weak. The pollution indicators NH_3, COD, and conductivity clustered with *E. coli*, coliform, *C. lusitaniae*, and *C. glabrata* concentrations (Figure 3.8). These microbial parameters, including *P. kudriavzevii*, further had a moderate to strong negative association with DO which was confirmed with correlation analyses. Conversely, total thermotolerant yeasts numbers and *M. guilliermondii* concentration were found to be negatively associated with NH_3, COD, and conductivity whereas it had a weak positive association with DO. Similar results were observed following Pearson correlation analysis. Furthermore, a possible trend was observed between thermotolerant yeast concentration and DO values for some sampling sites (**Figures S7-9**).

3.3.6. Antifungal susceptibility

Yeast isolates recovered from the different sampling sites and identified as representatives of clinically relevant species were subjected to antifungal susceptibility testing. Consequently, the FLU (Table 3.4) and AmB (Table 3.5) MICs were determined for these yeasts. Two yeast isolates representing *Candida blankii* and *Pichia kluyveri* had MICs of 16mg/L and 32mg/L, respectively (dose-dependent/intermediate category; Pfaller et al., 2010). Furthermore, most isolates identified as *P. kudriavzevii* had FLU MICs of 16 mg/L and 32 mg/L (dose-dependent category; Pfaller et al., 2010).

Potential FLU resistance was observed for one *C. albicans* isolate (S8), one *Cutaneotrichosporon mucoides* isolate (T2), one *M. guilliermondii* isolate (T3), four *P. kluyveri* isolates (S9, S8, S5, and S1), and four *P. kudriavzevii* isolates (S4, T4, and S3) (**Tables S11-12**). All isolates tested during our study were susceptible to AmB. The highest AmB MIC (4mg/L) was observed for a *C. glabrata* isolate (site S8), two isolates of *P. kudriavzevii* (S5 and S4), and one *C. tropicalis* isolate (T4; Table 3.5)

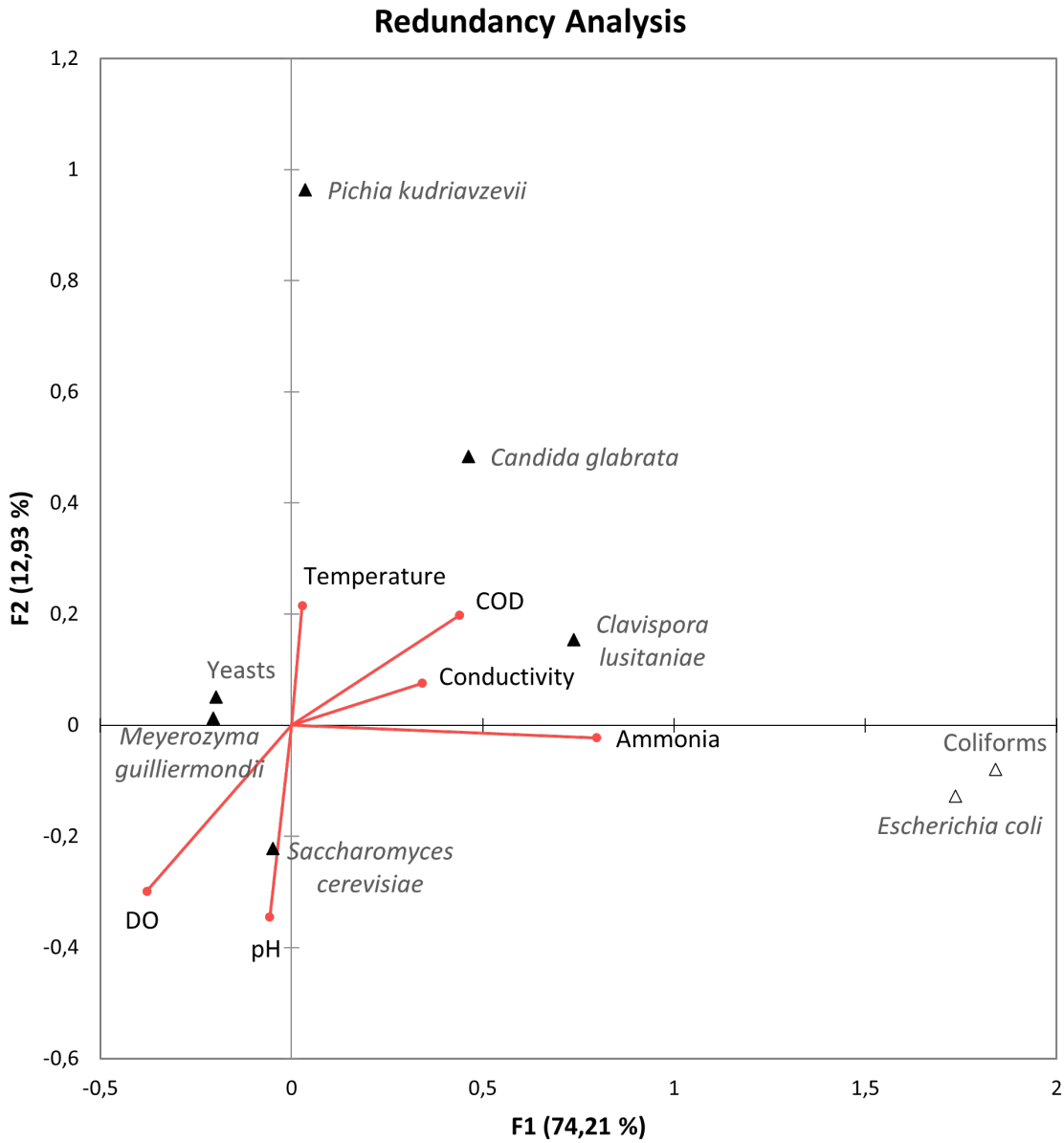

Figure 3.8. Redundancy analysis of (▲) total thermotolerant yeast concentrations, *Candida glabrata, Clavispora lusitaniae, Meyerozyma guilliermondii*, *Pichia kudriavzevii* and *Saccharomyces cerevisiae* counts, (∆) *E. coli* and coliform concentrations, as well as the (●) pH, temperature, DO, conductivity, COD and NH_3 levels in the water. Both axes collectively represent 87.13% of the variability in the data. A permutation test confirmed a linear relationship between response and explanatory variables.

Table 3.4. Fluconazole minimum inhibitory concentration (MIC) modes and ranges of clinically relevant yeast species isolated from river water sites.

	Fluconazole MIC Mode *(Range)* (mg/L)									
	Sampling Sites									
Yeasts	**S9**	**S8**	**S5**	**T1**	**T2**	**S1**	**S4**	**T4**	**S3**	**T3**
Candida albicans	**0.25**[a]	*(0.25-64)*[c]	-	-	-	-	-	-	-	-
Candida blankii	-	-	-	-	**16**[a]	-	-	-	-	-
Candida glabrata	**4** *(2-4)*	**2** *(2-8)*	*(1-4)*[b]	**2**[a]	-	**2**[a]	**4** *(2-4)*	**1**[a]	**4** *(2-4)*	**4**[a]
Candida melibiosica	-	-	-	-	-	**0.25**[a]	-	-	-	-
Candida pseudoaaseri	-	-	-	-	-	-	**0.5**[a]	-	-	-
Candida tropicalis	-	-	*(0.25-0.5)*[b]	-	-	-	**0.25**[a]	-	-	*(0.5-1)*[b]
Clavispora lusitaniae	**0.25**	-	**0.5**[a]	*(0.5-1)*	-	-	-	*(0.13-0.25)*[b]	**0.25**	**0.25**[a]
Cutaneotrichosporon mucoides	-	-	-	-	**>64**[ac]	**4**[a]	-	-	-	-
Cyberlindnera jadinii	-	**1** *(1-4)*	**2** *(1-2)*	-	-	-	-	-	-	-
Kluyveromyces marxianus	-	**1**[a]	-	-	-	-	-	-	**0.25**[a]	-
Kodamaea ohmeri	-	-	-	**4**[a]	-	-	-	**2**[a]	-	-
Lodderomyces elongisporus	-	-	-	-	**0.13**[a]	-	-	-	-	-
Meyerozyma caribbica	-	-	-	-	-	-	-	**1**[a]	-	-
Meyerozyma carpophila	-	-	-	-	**4**[a]	-	-	-	-	-
Meyerozyma guilliermondii	**1**[a]	**4** *(2-4)*	**4**	**4** *(2-4)*	**4** *(2-8)*	**4**	**4**	**4** *(2-4)*	**4**	**4** *(4-64)*[c]
Pichia kluyveri	**>64**[c]	-	**32**[a]	-	-	**>64**[ac]	-	-	-	-
Pichia kudriavzevii	-	-	**32**	**32**	-	**32**	**32** *(32-64)*[c]	**32** *(32-64)*[c]	**32** *(8-64)*[c]	**32** *(16-32)*
Wickerhamomyces onychis	-	**2**[a]	-	-	-	-	-	-	-	-

[a] - Represents only one isolate; [b] - No mode; [c] - Include potentially resistant isolates

Table 3.5. Amphotericin B minimum inhibitory concentration (MIC) modes and ranges of clinically relevant yeast species isolated from river water sites.

	Amphotericin B MIC Mode *(Range)* (mg/L)									
	Sampling Sites									
Yeasts	**S9**	**S8**	**S5**	**T1**	**T2**	**S1**	**S4**	**T4**	**S3**	**T3**
Candida albicans	**1**[a]	**1**	-	-	-	-	-	-	-	-
Candida blankii	-	-	-	-	**1**[a]	-	-	-	-	-
Candida glabrata	**1** *(1-2)*	**1** *(0.5-4)*	**2**	**2**[a]	-	**1**[a]	**2**	**1**[a]	**1** *(1-2)*	**2**[a]
Candida melibiosica	-	-	-	-	-	[a]	-	-	-	-
Candida pseudoaaseri	-	-	-	-	-	-	**0.5**[a]	-	-	-
Candida tropicalis	-	-	*(0.5-2)*[b]	-	-	-	**2**[a]	-	-	*(0.5-4)*[b]
Clavispora lusitaniae	**1** *(0.5-1)*	-	**1**[a]	*(0.5-1)*[b]	-	-	-	*(0.5-1)*[b]	**0.5**[a]	**0.5**[a]
Cutaneotrichosporon mucoides	-	-	-	-	**1**[a]	**0.5**[a]	-	-	-	-
Cyberlindnera jadinii	-	**0.5**	**1** *(1-2)*	-	-	-	-	-	-	-
Kluyveromyces marxianus	-	**1**[a]	-	-	-	-	-	-	**1**[a]	-
Kodamaea ohmeri	-	-	-	**0.5**[a]	-	-	-	**0.5**[a]	-	-
Lodderomyces elongisporus	-	-	-	-	**0.13**[a]	-	-	-	-	-
Meyerozyma caribbica	-	-	-	-	-	-	-	**1**[a]	-	-
Meyerozyma carpophila	-	-	-	-	**1**[a]	-	-	-	-	-
Meyerozyma guilliermondii	**1**[a]	**0.5** *(0.5-1)*	**0.5**	**1** *(0.5-2)*	**1**	**0.5**	**0.5**	**0.5** *(0.5-1)*	*(0.5-1)*[b]	**0.5** *(0.5-2)*[b]
Pichia kluyveri	**0.5**	-	**0.25**[a]	-	-	**0.5**[a]	-	-	-	-
Pichia kudriavzevii	-	-	**2** *(2-4)*	**1**	-	**1**	**0.5** *(0.5-4)*[b]	**1** *(0.5-1)*	**2** *(0.5-2)*	**1** *(0.5-1)*
Wickerhamomyces onychis	-	**1**[a]	-	-	-	-	-	-	-	-

[a] - Represents only one isolate; [b] - No mode

3.3.7. QMRA

The risk of obtaining a yeast infection via river water ingestion was estimated for six clinically relevant species: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. lusitaniae*, *M. guilliermondii*, and *P. kudriavzevii*. The annual probabilities of infection via ingestion for four of the six yeast species are presented in Figure 3.9; *C. albicans* and *C. tropicalis* were detected only in HP sites and were therefore excluded from this figure.

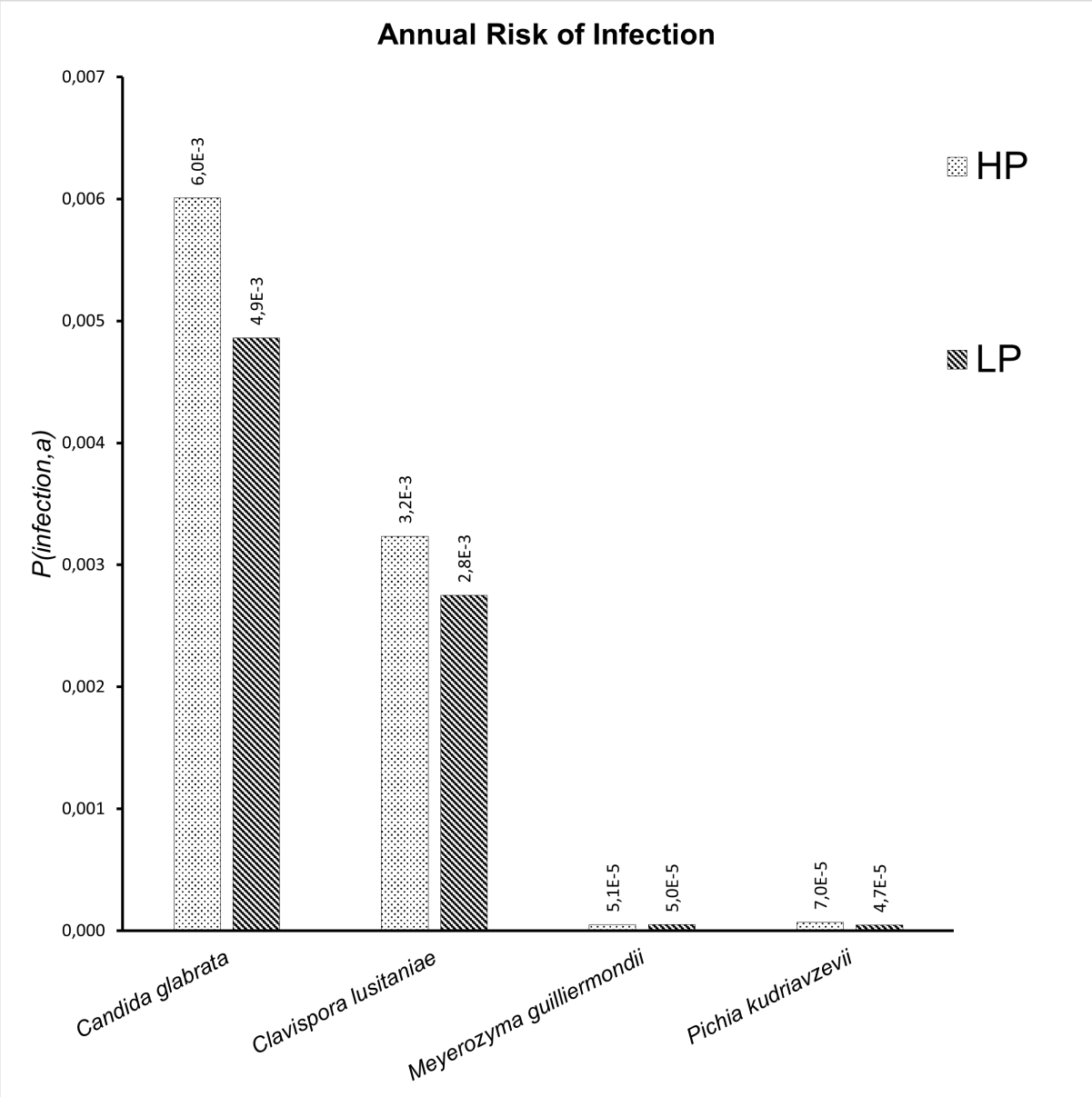

Figure 3.9. Estimated annual risk of yeast infection from drinking water of less polluted (LP) and highly polluted (HP) river water, South Africa.

The annual risk of obtaining *C. albicans* and *C. tropicalis* infections through drinking river water (HP sites) was 0.26 (25.98%) and 0.23 (23.29%) respectively. These values were considerably higher than those of *C. glabrata, C. lusitaniae*, *M. guilliermondii* and *P. kudriavzevii* (Figure 3.9). Among the four species presented in Figure 3.9, *C. glabrata* posed the greatest threat. On the other hand, the lowest

risk of infection was observed for *M. guilliermondii* followed by *P. kudriavzevii*. Furthermore, risk of infection from ingesting river water from LP sites was lower than that of ingesting water from HP sites.

3.4. DISCUSSION

The pollution levels of the Eerste and Plankenbrug rivers in the Western Cape Province, South Africa, have been under investigation for the past three decades during which several research groups assessed the water quality (Barnes, 2004; Ngwenya, 2006; Ackermann, 2010; Britz et al., 2013; Postma, 2016). The results of these studies indicated that the upstream sections of the Eerste river were unpolluted, while the downstream sections were contaminated due to confluence with the notoriously polluted Plankenbrug river (Figure 3.1). While some of these observations were similar to our study's findings, it is important to note that our results indicated that the waters upstream of the Eerste river were polluted, albeit moderately. The *E. coli* and coliform levels in these waters exceeded the limits for drinking water set by the South African National Standard (SANS) 241 drinking water guideline, as well as the limits set by guidelines of the Department of Water Affairs and Forestry (DWAF) for domestic water use (DWAF, 1996; SANS, 2015). Both sets of guidelines are frequently referred to when investigating South African polluted waters. In addition to microbial load, these guidelines list certain pollution indicator parameters such as NH_3 concentration, conductivity, and COD, all of which allowed us to discriminate between the less polluted upstream section of the Eerste river (LP sites), and the highly polluted waters of Plankenbrug, Krom, and downstream Eerste river sections (HP sites; **Table S1 & Figure S1**).

Exposure to polluted waters has negative effects on human health, especially due to harmful microorganisms proliferating in the nutrient rich conditions (Schwarzenbach et al., 2010). Various disease-causing bacteria were previously identified in Plankenbrug and surrounding rivers, illuminating the health risks associated with these waters (Barnes, 2004; Ackerman, 2010). The presence of pathogenic fungi in the rivers, however, was only considered by a few (Stone et al., 2013; Postma, 2016). Using metagenomics and culture-based methods our study revealed the presence of various pathogenic fungal species (Table 3.3; **Table S13**), in addition to many strains that could only be identified up to genus-level (Figure 3.6). Relatively few of the pathogenic species identified using the metagenomic analysis represented filamentous fungi while most represented ascomycetous yeasts; however, these observations relied on the highest quality of DNA for species-specific identification, and therefore excluded any DNA possibly damaged or lost during extraction protocols. Therefore, it is possible that more representatives of opportunistic fungal species could have been present in the rivers, but the resolution of the metagenomic analysis was not high enough to identify these fungi in the water. This contention is supported by the fact that numerous fungal genera, known to harbour pathogenic species, were detected with the metagenomic analysis (Figure 3.6), and fewer yeast species were detected using this method than with the selective isolation procedure for culturable thermotolerant yeasts (Table 3.3; **Table S13**).

Interestingly, some of the fungal genera that were also detected through culture-based methods, showed higher relative abundance at HP sites (Figure 3.6) compared to LP sites: i.e. *Candida*, *Clavispora*, *Cutaneotrichosporon*, *Cyberlindnera*, *Debaryomyces*, *Pichia*, and *Saccharomyces*.

Moreover, fungal communities from HP sites differed significantly from that of LP sites (Figure 3.4), supporting claims that these environments affect fungal diversity (Ortiz-Vera et al., 2018). The association between fungal presence and water pollution has been an ongoing investigation without drawing definite conclusions. Some researchers found yeast numbers to correlate with pollution indicators (Medeiros et al., 2012; Brandão et al., 2010; Pietryczuk et al., 2018; Monapathi et al., 2020b), while others observed no such correlations (Samah et al., 2014; Monapathi et al., 2017). The results obtained during our study indicated that total thermotolerant yeast concentrations also did not correlate with any indicator parameters. In fact, either negative or independent associations were observed between thermotolerant yeast concentrations and pollution indicators (Figure 3.8). The most probable reason for this is that high concentrations of yeasts were observed at all sites irrespective of pollution level (**Figure S10**).

The increased yeast concentrations correlated strongly with the presence of *M. guilliermondii*, an ascomycetous opportunistic yeast that dominated the thermotolerant culturable yeast populations in the water and was sometimes almost exclusively isolated from the isolation medium (Savini et al., 2011; **Figures S11-13**). Apart from the possibility that increased dissolved oxygen and rainfall occurrences promote growth of this species (**Figure S7-9**), very little is understood of this phenomenon. Additional experimentation did however indicate that the growth rate of *M. guilliermondii* is considerably higher when the cultures thereof is being agitated on a rotary shaker, as opposed to conditions when the growth medium is kept static (**Figure S14**). The conditions on a rotary shaker could mimic turbulent and oxygen rich waters which are most frequently observed during rainy seasons, providing a possible explanation for the increased numbers of *M. guilliermondii*. Nevertheless, many other environmental factors may play a role in the bloom of *M. guilliermondii* during the rainy season, such as the versatile metabolism of this yeast which could provide competitive advantages under these conditions (Mo et al., 2021). Studying the physiology and niche specific interactions of fungal species in such environments could be enlightening.

Important to note, however, is that the presence of pathogenic yeast species such as *C. glabrata* and *Clavispora lusitaniae* (syn. *Candida lusitaniae*) did associate with polluted river water; positive correlations were revealed between the concentrations of these species and conductivity, COD, and NH_3 levels whereas negative correlations were observed toward DO (Figure 3.8). Additionally, the genus *Clavispora* was not detected in LP sites using metagenomic analysis, further motivating the indicator potential of *C. lusitaniae* (Figure 3.6). Previous studies observed similar results for pathogenic fungi associated with polluted ground water and rivers (Samah et al., 2014; Pietryczuk et al., 2018; Monapathi et al., 2020b). We therefore contend that while total thermotolerant yeast counts cannot be considered as pollution indicators, particular yeast species which inevitably associate with pollution factors should be considered, similar to the bacterial indicator *Escherichia coli*, and its established association with faecal matter (Saxena et al., 2015).

The presence of renown pathogenic yeast species such as *C. albicans*, *C. tropicalis*, *C. glabrata*, *P. kudriavzevii*, and *L. elongisporus* (syn. *Candida parapsilosis*) in the rivers of Stellenbosch is cause for concern. Additionally, reports of antifungal resistant strains of *C. glabrata*, *C. tropicalis*,

C. parapsilosis, and *P. kudriavzevii* (syn. *C. krusei*) in clinical samples recovered from Stellenbosch residents (2018-2019) were alarming (Louw et al., unpublished). Antifungal resistance is frequently involved in detrimental mycoses outcomes and have had devastating effects on livelihoods (Perlin et al., 2017). We found resistance towards one of the most affordable antifungals (FLU; Kneale et al., 2016) among isolates representing *C. albicans*, *C. mucoides*, *M. guilliermondii*, *P. kluyveri* and *P. kudriavzevii* (Table 3.4). These isolates, excluding representatives of *C. mucoides*, were recovered from HP sites (Table 3.4), which suggested that the isolates either acquired antifungal resistance due to the presence of pharmaceuticals in these polluted waters, or via sewage pollution originating from infected individuals. It remains unclear which of these explanations holds true and further investigation is therefore required.

Our study further illuminated the threats regarding the presence of potentially pathogenic yeasts in river water, by determining the annual risk of infection via ingestion (posed by six clinically relevant yeasts) through a provisional QMRA (Figure 3.9). It was observed that species with higher pathogenic potential presented considerably higher risk of infection despite low concentrations. It also became evident during this study that increased risk of infection associates with contaminated river water (Figure 3.9). While these estimations remain rudimentary due to a lack of fungal QMRA studies and epidemiological data (Weiskerger & Brandão, 2017), they highlight the health threats faced by immunocompromised individuals when being exposed to polluted waters.

3.5. CONCLUSION

South Africa has a disproportionally large population of immunocompromised people, primarily due to the number of individuals living with HIV / AIDS (UNAIDS, 2021). In addition, about 32% of South Africa's population live in rural / informal areas (https://www.macrotrends.net) and are frequently dependent on natural surface water for domestic purposes, including drinking (Colvin et al., 2016; Edokpayi et al., 2018). Understanding the risks these individuals face daily is of dire importance, our study highlights these issues through identifying the pathogenic fungi present in the river water. Moreover, our study revealed that antifungal resistant strains of some yeast species occur in these waters and provide confirmation that polluted waters pose a greater mycoses-related health threat than clean waters do, thereby highlighting the crucial need for environmental remediation. There is still much left to investigate regarding environmental fungi: the ecology and interactions of pathogenic fungi in polluted surface waters, the origins of antifungal resistance as well as the epidemiology and pathogenesis of opportunistic fungi. Exploring these research fields would aid in future risk assessments, knowledge dissemination, and risk management—all of which are crucial to ensure the safety of susceptible individuals.

CHAPTER 4: GENERAL CONCLUSIONS, RECOMMENDATIONS AND FUTURE RESEARCH

4.1. GENERAL CONCLUSIONS

This study underscored the latent threat to public health posed by opportunistic, clinically relevant fungi occurring in South African river systems. The overarching aim of the study was to expand on the knowledge of opportunistic and pathogenic fungi present in rivers and to highlight the associated health risks (Chapter 1). The first objective was to review existing literature on the presence of pathogenic fungi in rivers (Chapter 2). Subsequently, the presence of clinically relevant fungi in a South African urban river system, as well as the risks posed by these fungi, were reported on in Chapter 3.

The literature review in Chapter 2 revealed that opportunistic and pathogenic fungi can be found in all environments, however, their presence in aquatic ecosystems remain relatively unexplored. Literature also showed that although pathogenic fungi were frequently detected in polluted water sources, the direct relationship between these fungi and pollutants has not been elucidated. The literature also highted the lack of surveys on the presence of pathogenic filamentous fungi in river systems. Fungal identification in these cases were often limited to genus-level, restricting the recognition of pathogenic filamentous fungi present in these environments. Conversely, various unicellular fungi were detected in surface waters, and notorious pathogenic yeast species were recurrently observed in rivers of South Africa and other countries across the globe. The dominant opportunistic and pathogenic yeast species found during these studies were *Candida albicans*, *Candida glabrata*, *Candida. parapsilosis*, *Candida tropicalis*, *Candida lusitaniae*, *Meyerozyma guilliermondii* and *Pichia kudriavzevii*, all of which are ascomycetes. Basidiomycetous yeasts were seldomly detected in rivers, and when they were found, they were identified as either *Cryptococcus albidus* (syn. *Naganishia albida*), *Cryptococcus laurentii* (syn. *Papiliotrema laurentii*) or *Rhodotorula mucilaginosa*.

Furthermore, Chapter 2 explored the roles of thermotolerance in pathogenesis and how fungi might attain this trait in response to rising environmental temperatures because of climate change. It was highlighted that the inability of many fungal species to survive elevated temperatures would be the primary obstacle, excluding the immune system, preventing them to infect and cause harm to the human body. It was also argued that fungal species harbouring virulence traits which cannot survive at mammalian body temperatures, might gain advantage through acquired thermotolerance. The increasing environmental temperatures could therefore drive microevolution among fungal species, resulting in the emergence of novel pathogens. It was stated that existing pathogenic as well as newly thermotolerant opportunistic species might proliferate in a nutrient-rich environment such as polluted river water.

Rivers are used as primary water sources for many individuals in informal settlements, especially in developing countries such as South Africa. The water may be used for drinking, cooking, ablutions, and other domestic uses. Exposure to polluted river water through these activities could therefore pose

health risks. Experimental evidence for these risks was presented in Chapter 3, which focused on the presence of opportunistic and pathogenic fungal species in highly polluted (HP) and less polluted (LP) river water. Both high-throughput sequencing and culture-based methods were used to detect pathogenic fungal species. The latter method involved thermally selective isolation of yeast species on agar medium followed by random selection. The metagenomic analysis reported on in this chapter revealed that fungal communities differed significantly between HP and LP environments. This finding, together with the correlations observed between the pathogenic yeast species (*C. glabrata* and *C. lusitaniae*) and pollution indicators, indicated that some fungi might be associated with polluted water. However, the total number of thermotolerant yeasts did not correlate with any of the pollution indicator parameters used in this study. The predominance of *M. guilliermondii*, which correlated with high yeast concentrations, further raised questions regarding the impact of non-anthropogenic (natural) factors on the growth of fungi in river ecosystems.

While it was important to determine the fungal diversity of the polluted river water, further investigation into the health risks these fungi pose was necessary. Thus, Chapter 3 also reports on the antifungal susceptibility profiles of the culturable yeasts recovered from the river water. While the majority of the yeast isolates were found to be susceptible to the two most readily available antifungals (FLU and AmB), some isolates did present resistance towards FLU. These isolates were representatives of *C. albicans*, *C. mucoides*, *M. guilliermondii*, *P. kluyveri*, and *P. kudriavzevii*. Exposure to such antifungal resistant yeasts could be detrimental to immunocompromised individuals, especially considering the inaccessibility of alternative antifungals in low-income countries. Using the results obtained during this study, as well as pre-clinical data obtained from literature, a provisional QMRA was subsequently performed to determine the probability of yeast infection via river water ingestion. The assessment ultimately revealed that the probability of becoming infected is higher when ingesting more polluted water and that certain yeast species pose a greater threat due to their higher pathogenic potentials.

4.2. RECOMMENDATIONS AND FUTURE RESEARCH

An increase in mycosis cases is anticipated, due to a rapid growing population, increasing pollution, and increased environmental temperatures as a result of climate change. Future studies are therefore required to obtain more comprehensive data on the fungal taxa in polluted rivers, as well as the ecophysiology of the clinically relevant fungi in these waters.

4.2.1. Detection of pathogenic fungi

Detection and enumeration of microorganisms in natural environments are essential to investigate the microbial diversity and observe any causes for imbalances within the microbial communities of these environments. Metagenomic analysis of the mycobiome (Chapter 3) revealed that pollution influences fungal communities in the water. These methods also allowed us to detect pathogenic species. The resolution of the metagenomic analysis, however, was mostly limited to genus-level, while the culture-based techniques employed during this study could confidently identify up to species-level. DNA recovery and sequencing techniques with better resolution should be attempted in future (Lindahl et al., 2013). Moreover, our metagenomic study evaluated the fungal communities of only one sampling

event's data. In future, samples from different seasons should be included and separately analysed, during which seasonal occurrences might be observed.

Thermally selective isolation of yeasts provided higher resolution with regard to the detection of pathogenic species than did the high throughput sequencing conducted during our study. This was owing to the fact that thermotolerance, used as selective measure during the isolation procedure, is one of the main pathogenic traits among fungi (Robert & Casadevall, 2009). However, many other non-pathogenic thermotolerant species of yeasts and filamentous fungi may also be present in these environments. These less pathogenic / non-pathogenic species might have been able to outcompete the species of interest during isolation. This could mean that slower growing pathogenic species were not recorded. Nevertheless, the isolation of these non-pathogenic thermally tolerant fungi from the polluted river water should be viewed as an indication of their potential virulence in future clinical cases.

The thermal selective procedure used during this study selected for both pathogenic and non-pathogenic fungi. Thus, it is clear that more selective procedures should be developed to improve the selective enumeration and identification of pathogenic species in this habitat. To develop a method for selective isolation of yeast species for instance, comprehensive information regarding the physiology of these microbes is needed. An example of such an approach is the selective medium that was developed to identify *C. auris* strains (Das et al., 2021). To achieve their goal, the authors tested various environmental stressors against *C. auris* strains to determine the conditions and medium composition that would promote growth of only those strains that represent *C. auris*. Another approach would be to detect species specific molecular markers in the metagenome extracted from the water samples (Tu et al., 2014). The process would be similar to the methods employed for metagenomic analyses in Chapter 3, except it would involve higher specificity during sequencing. A disadvantage of using such molecular techniques is, however, that physiological studies of the detected fungal species cannot be conducted: The microbe's virulence factors, antifungal susceptibility profile, and growth characteristics would therefore remain unexplored.

4.2.2. Quantification of pathogenic fungi

Since no selective pressure was exerted on the viable mycobiome when high-throughput sequencing was used to analyse the metagenome (Chapter 3) the method did provide a fairly unbiased view of the relative abundance of the different fungal taxa (mesophilic and thermotolerant) in the rivers. It was anticipated that the mesophilic fungi would predominate, considering the average temperature of the river water. Unsurprisingly, the relative abundances of pathogenic fungi were therefore much lower and did not necessarily coincide with the frequency at which pathogenic yeasts were detected using the thermally selective isolation procedure. To the best of our knowledge, no universal genetic marker has been reported for thermotolerance among fungal species. The quantification of thermotolerant (presumably opportunistic and pathogenic) fungi using molecular techniques therefore requires extensive evaluation before it is deemed feasible. Currently a combination of molecular and culture-based techniques serves as the best option; however, as observed in our study and that of others' (Langarica-Fuentes et al., 2014), differences between the results of the two techniques are inevitable.

In our study we determined the concentrations of pathogenic yeasts using the calculated frequency at which species were randomly isolated. While these results enabled us to investigate the respective associations between different species and the environmental parameters, the calculations included estimations that were most likely skewed by the bloom of *M. guilliermondii* witnessed during four of the sampling months. Future studies should therefore only focus on the quantification of clinically relevant species of interest such as those with higher pathogenic potential: *C. albicans*, *C. tropicalis*, *C. neoformans*, and *A. fumigatus* (Casadevall, 2017). However, very few selective media are available for the selective enumeration of these species. As an alternative, a chromogenic medium can be used to preliminarily identify yeast species following enumeration, isolation, and culturing on a relatively non-selective medium, as was done in this study albeit with limited success (Chapter 3). This method, however, is time-consuming and still requires routine analyses of taxonomic informative gene sequences for quality control.

Considering that target-gene sequencing and real-time quantification options are available, quantification of known pathogenic species is currently more feasible using molecular techniques than the above-mentioned culture-based techniques, (Ogata et al., 2015; Martínez-Murcia et al., 2018; Shirvani et al., 2020; Wang et al., 2020). These molecular techniques are closed format technologies due to the pre-existing knowledge of the microbial diversity of the environment (Zhou et al., 2015). Without prior knowledge of the ecology of pathogenic species, gathered either through culture-based or metagenomic methods, molecular quantification can be cumbersome. Our study therefore serves as foundation for future investigations into the quantification of pathogenic species in river water.

4.2.3. Pollution and pathogenic fungi

The results from our metagenomic analysis indicated that fungal communities differ significantly between less polluted and highly polluted river water; and a possible correlation exists between physicochemical pollution indicators and the pathogenic yeast species, *C. glabrata* and *C. lusitaniae*. These observations, together with the results of others (Samah et al., 2014; Pietryczuk et al., 2018; Monapathi et al., 2021), suggest that either these fungi are present within the water due to anthropogenic pollution or that the pollutants within the water offer a means for proliferation of these taxa. Nevertheless, we did detect pathogenic yeast species and moderately high bacterial loads at sampling sites that were assumed to be clean. One such sampling site (S1), representing the best quality water compared to other sites, was situated near formal housing and nature trails: a possible source of anthropogenic pollution. In future, sampling sites free of anthropogenic influences should be included in studies, to serve as pollution-free control groups. However, it should be noted that these control sites would likely represent upstream mountain rivers that could potentially have vastly different natural microbial compositions due to climatic and physicochemical differences (Zeglin, 2015).

The predominance of *M. guilliermondii* in the polluted river water during and following the rainy season was peculiar and warrants further investigation. Due to the lack of a pollution-free control site as well as limited tests for pollution indicators, our study cannot confirm that *M. guilliermondii* (an opportunistic pathogen) is not associated with polluted environments. However, it remains unclear how this yeast was capable of growing to such high concentrations other than its high growth rate that seemed to

associate with agitation. Such an occurrence is important to understand; should other species with higher pathogenic potential have similar growth characteristics. It is therefore crucial to study the niche-specific physiology and interactions of predominant yeasts present in aquatic environments.

In addition to studying the interactions between fungal species, inter-kingdom interactions between bacterial and fungal communities are of interest. The increased nutrient levels in polluted water also promote the growth of bacterial species which possibly interact with pathogenic yeast species. Some of these interactions can be cause for concern, such as quorum sensing between *Pseudomonas aeruginosa* and *C. albicans* (Bandara et al., 2020), especially since it was reported that *P. aeruginosa* induces FLU resistance in *C. albicans*. Moreover, studies reporting on biofilm associated yeasts in river systems, where inter-kingdom interactions are most likely to occur, are lacking.

4.2.4. Emerging pollutants

Antifungals are among the many emerging environmental pollutants currently gaining attention (Richardson & Kimura, 2017). Our study investigated the antifungal susceptibility profiles of isolated yeast species but did not quantify the levels of antifungals present in the river water. Doing so could allow researchers to determine whether the presence of an antifungal might promote resistant phenotypes among species. Risk assessments, however, have previously been conducted to determine the effects of antifungals in surface waters on both human health and antifungal resistance (Assress et al., 2020). Similar studies could be beneficial and enlightening when conducted in tandem with isolation and antifungal susceptibility testing of fungal species.

4.2.5. Quantitative microbial risk assessment

We reported on a provisional QMRA for yeast infection via river water ingestion. Various assumptions were made in order to perform this assessment, and therefore many limitations exist. The results from this assessment suggested that probability of infection relies more upon the pathogenic potential than the concentration of yeasts in the exposure dose. While it is logical that lower concentrations of a more virulent species are required to elicit infection, these estimations might not be entirely reliable for inter-species comparisons of infection risks. Moreover, the clinical data used to determine the PP of *C. glabrata*, *C. parapsilosis*, *C. lusitaniae*, *M. guilliermondii* and *P. kudriavzevii* originated from one study only.

The lack of clinical studies that provide insight into both the survival and pathogenesis of yeast species became obvious as our study progressed. Moreover, existing studies focussing on *C. albicans* and *C. tropicalis* date back to the 1950's. As exemplified by Weiskerger and Brandão (2020) extensive research is required in the clinical fields of yeast research to continue QMRA of yeast infections. Future research into these fields is crucial to standardise QMRA, which in turn is an essential component in raising awareness for mycoses. Currently, estimated fungal disease burdens provide the only encouragement to investigate mycoses. However, these estimations do not represent the actual disease burden since most cases are unreported due to many individuals lacking the proper funds for treatment or accessibility to such health facilities (Ibe, 2021). Quantifiable risks of fungal infection would provide validated and reputable motivation to prioritise fungi in epidemiology as well as develop

improved disease diagnostics and proper treatment solutions (Weiskerger & Brandão, 2020). Risk assessments also allow for relevant risk management and communication to the public (Brown & McClure, 2006).

4.2.6. The way forward

In South Africa, one of the major contributing factors to river water pollution is inadequate sewage treatment operations. More than half of the wastewater treatment operations of South Africa release water of unacceptable standard, which end up in water bodies such as rivers (Herbig, 2019). Moreover, nearly 9% of South Africans directly rely on water from streams, rivers, and wells (Colvin et al., 2016). It is, therefore, clear that polluted river water poses health risks to humans. However, our understanding of the quantifiable risk that fungal pathogens in these waters pose is, however, limited. Nevertheless, our study illuminated the risks, but more importantly the knowledge gaps that indisputably require attention.

The suggested steps needed to be taken to raise awareness, through QMRA, of clinically relevant fungi in polluted source water are:

1) standardise the quantification methods for established pathogenic species such as *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. lusitaniae*, *P. kudriavzevii*, and any others frequently recovered from the environment;

2) investigate alternative exposure routes that might introduce these fungi to its host, such as dermal contact leading to oral exposure (mouth to hand movements) in addition to performing community surveys detailing the usage of nearby water sources in rural settlements;

3) validate the pathogenic potentials of these and other fungi of interest by conducting pre-clinical trials, to establish accurate and reliable dose response model parameters.

It is important to note that “back-to-basics” principles are followed when executing these steps above. Modern day technologies have come very far and have allowed researchers to unravel the smallest of details in science; however, knowledge might get lost in translation when skipping crucial basic investigations, such as classic virulence assays which were used to determine dose-response model parameters of most pathogens known to us today—excluding fungi (Peleg, 2020). It is foreseen that a well-established knowledge foundation, laid using reputable data, would reduce the assumptions made in calculations and increase the validity of future QMRAs. This will enable researchers to investigate fungal pathogenic potential more elaborately using transcriptomic analyses and surrogate biomarkers during environmental and clinical studies (Brul et al., 2012; Kämmer et al., 2020; Thompson et al., 2022). Considering the current global deterioration of the environment because of anthropogenic activities, in-depth studies into the occurrence and pathogenicity of fungi in polluted river systems are expedient.

REFERENCES

ABARENKOV K, ZIRK A, PIIRMANN T, PÖHÖNEN R, IVANOV F, NILSSON RH and KÕLJALG U (2021) UNITE mothur release for eukaryotes 2. Version 10.05.2021. UNITE Community. https://doi.org/10.15156/BIO/1265957

ACKERMANN A (2010) Assessment of microbial loads of the Plankenburg and Berg rivers and the survival of *Escherichia coli* on raw vegetables under laboratory conditions. Thesis (MSc Food Sc) University of Stellenbosch, 2010. http://scholar.sun.ac.za/handle/10019.1/5320?show=full.

ALBRECHT D, GUTHKE R, BRAKHAGE AA and KNIEMEYER O (2010) Integrative analysis of the heat shock response in *Aspergillus fumigatus*. *BMC Genomics* **11** (1). https://doi.org/10.1186/1471-2164-11-32

ALFORD RA, BRADFIELD KS and RICHARDS SJ (2007) Ecology: Global warming and amphibian losses. *Nature* **447** (7144) 3-6. https://doi.org/10.1038/nature05940

ALMEIDA F, RODRIGUES ML and COELHO C (2019) The still underestimated problem of fungal diseases worldwide. *Front. Microbiol.* **10** (FEB) 1-5. https://doi.org/10.3389/fmicb.2019.00214

ALMEIDA JND, FRANCISCO EC, HAGEN F, BRANDÃO IB, PEREIRA FM, PRESTA DIAS PH, DE MIRANDA COSTA MM, DE SOUZA JORDÃO RT, DE GROOT T and COLOMBO AL (2021) Emergence of *Candida auris* in Brazil in a COVID-19 intensive care unit. *J. Fungi* **7** (3) 220. https://doi.org/10.3390/jof7030220

ALONSO-MONGE R, ROMÁN E, ARANA DM, PLA J and NOMBELA C (2009) Fungi sensing environmental stress. *Clin. Microbiol. Infect.* **15** (SUPPL. 1) 17-19. https://doi.org/10.1111/j.1469-0691.2008.02690.x

AL-SWEIH N, KHAN ZU, AHMAD S, DEVARAJAN L, KHAN S, JOSEPH L and CHANDY R (2011) *Kodamaea ohmeri* as an emerging pathogen: A case report and review of the literature. *Med. Mycol. J.* **49** (7) 766-770. https://doi.org/10.3109/13693786.2011.572300.

AMORE A and FARACO V (2012) Potential of fungi as category I Consolidated BioProcessing organisms for cellulosic ethanol production. *Renew. Sustain. Energ. Rev.* **16** (5) 3286-3301. https://doi.org/10.1016/j.rser.2012.02.050

ARASTEHFAR A, CARVALHO A, VAN DE VEERDONK FL, JENKS JD, KOEHLER P, KRAUSE R, CORNELY OA, PERLIN D, LASS-FLÖRL C and HOENIGL M (2020) COVID-19 associated pulmonary aspergillosis (CAPA)—from immunology to treatment. *J. Fungi* **6** (2) 91. https://doi.org/10.3390/jof6020091

ARAÚJO GRDS, DE SOUZA W and FRASES S (2017) The hidden pathogenic potential of environmental fungi. *Future Microbiol.* **12** (16) 1533-1540. https://doi.org/10.2217/fmb-2017-0124

ARENDRUP M, HORN T and FRIMODT-MØLLER N (2002) In vivo pathogenicity of eight medically relevant *Candida* species in an animal model. *Infection* **30** (5) 286-291. https://doi.org/10.1007/s15010-002-2131-0.

ARGÜELLES JC (1997) Thermotolerance and trehalose accumulation induced by heat shock in yeast cells of *Candida albicans*. *FEMS Microbiol. Lett.* **146** (1) 65-71. https://doi.org/10.1016/S0378-1097(96)00455-7

ARORA P, SINGH P, WANG Y, YADAV A, PAWAR K, SINGH A, PADMAVATI G, XU J, and CHOWDHARY A. (2021) Environmental isolation of *Candida auris* from the coastal wetlands of Andaman Islands, India. *Mbio* 12e03181-20. https://doi.org/10.1128/mBio.03181-20

ARROYO MG, FERREIRA AM, FROTA OP, BRIZZOTTI-MAZUCHI NS, PERESI JTM, RIGOTTI MA, MACEDO CE, SOUSA AFLD, ANDRADE DD and ALMEIDA MTGD (2020) Broad diversity of fungi in hospital water. *Sci. World J.* **2020**. https://doi.org/10.1155/2020/9358542

ARVANITIDOU M, KANELLOU K and VAGIONA DG (2005) Diversity of Salmonella spp. and fungi in northern Greek rivers and their correlation to fecal pollution indicators. *Environ. Res.* **99** (2) 278-284. https://doi.org/10.1016/j.envres.2005.01.002

ARVANITIDOU M, KANELLOU K, KATSOUYANNOPOULOS V and TSAKRIS A (2002) Occurrence and densities of fungi from northern Greek coastal bathing waters and their relation with faecal pollution indicators. *Water Res.* **36** (20) 5127-5131. https://doi.org/10.1016/S0043-1354(02)00235-X

ASLANI N, JANBABAEI G, ABASTABAR M, MEIS JF, BABAEIAN M, KHODAVAISY S, BOEKHOUT T and BADALI H (2018) Identification of uncommon oral yeasts from cancer patients by MALDI-TOF mass spectrometry. *BMC Infect. Dis.* **18** (1) 1-11. https://doi.org/10.1186/s12879-017-2916-5.

ASSRESS HA, NYONI H, MAMBA BB and MSAGATI TAM (2020) Occurrence and risk assessment of azole antifungal drugs in water and wastewater. *Ecotoxicol. Environ. Saf.* Elsevier Inc., **187** (October 2019) 109868. https://doi.org/10.1016/j.ecoenv.2019.109868.

ASSRESS HA, SELVARAJAN R, NYONI H, NTUSHELO K, MAMBA BB and MSAGATI TAM (2019) Diversity, co-occurrence and implications of fungal communities in wastewater treatment plants. *Sci. Rep.* **9** (1) 1-15. https://doi.org/10.1038/s41598-019-50624-z

ATLAS RM (2004) Handbook of microbiological media. *CRC press.* https://doi.org/10.1201/9781420039726.

BABIČ MN, GUNDE-CIMERMAN N, VARGHA M, TISCHNER Z, MAGYAR D, VERÍSSIMO C, SABINO R, VIEGAS C, MEYER W and BRANDÃO J (2017) Fungal contaminants in drinking water regulation? A tale of ecology, exposure, purification and clinical relevance. *Int. J. Environ. Res. Public Health* **14** (6). https://doi.org/10.3390/ijerph14060636

BADIEE P and ZARE M (2017) Consideration of invasive fungal infections in immunocompetent hosts *Archiv. Clin. Infect. Dis.* **12** (4) 66111. https://doi.org/10.5812/archcid.66111.

BANDARA HMHN, WOOD DLA, VANWONTERGHEM I, HUGENHOLTZ P, CHEUNG BPK and SAMARANAYAKE LP (2020) Fluconazole resistance in *Candida albicans* is induced by Pseudomonas aeruginosa quorum sensing. *Scientific Reports* **10** (1) 1-17. https://doi.org/10.1038/s41598-020-64761-3.

BAQUERO F (2009) Environmental stress and evolvability in microbial systems. *Clin. Microbiol. Infect.* **15** (SUPPL. 1) 5-10. https://doi.org/10.1111/j.1469-0691.2008.02677.x

BARNES JM, SLABBERT MM, HUISAMEN A and WASSERMANN E (2004) *Escherichia coli* as an indicator of microbiological water quality in the Plankenbrug River: what other pathogens does it indicate? *Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference* ISBN: 1-920-01728-3

BARON E, ANAISSIE E, DUMPHY F, MCCREDIE K and FAINSTEIN V (1988) *Hansenula anomala* fungemia. *Clin. Infect. Dis.* **10** (6) 1182-1186. https://doi.org/10.1093/clinids/10.6.1182

BELFORD I (2013) Investigating the removal of *Candida* and other potential pathogens from wastewater via an experimental rhizofiltration system. Doctoral dissertation, Stellenbosch University. https://scholar.sun.ac.za/handle/10019.1/79806?show=full

BENEDICT K, RICHARDSON M, VALLABHANENI S, JACKSON BR and CHILLER T (2017) Emerging issues, challenges, and changing epidemiology of fungal disease outbreaks. *Lancet Infect. Dis.* **17** (12) e403-e411. https://doi.org/10.1016/S1473-3099(17)30443-7

BERECZKI L, BARTHA N, KOCSUBÉ S, SÓKI J, LENGYEL G, TÁLOSI G, MÁDER K, DEÁK J and DÓCZI I (2012) Fungaemia caused by *Candida pulcherrima*. *Med. Mycol.* **50** (5) 522-524. https://doi.org/10.3109/13693786.2011.644590

BERGMAN AG and KAUFFMAN CA (1984) Dermatitis due to *Sporobolomyces* infection. *Archiv. Dermatol.* **120** (8) 1059-1060. https://doi.org/10.1001/archderm.1984.01650440089026

BERRY DB and GASCH AP (2008) Stress-activated genomic expression changes serve a preparative role for impending stress in yeast. *Mol. Biol. Cell* **19** (11) 4580-4587. https://doi.org/10.1091/mbc.e07-07-0680

BEYDA ND, CHUANG SH, JAHANGIR ALAM M, SHAH DN, NG TM, MCCASKEY L and GAREY KW (2013) Treatment of *Candida famata* bloodstream infections: Case series and review of the literature. *J. Antimicrob. Chemotherapy* **68** (2) 438-443. https://doi.org/10.1093/jac/dks388

BEZUIDENHOUT C (2013) A Large-Scale Study of Microbial and Physico-Chemical Quality of Selected Groundwaters and Surface Waters in the North-West Province, South Africa. WRC Report No. 1966/1/13. URL: www.wrc.org.za

BHABHRA R and ASKEW DS (2005) Thermotolerance and virulence of *Aspergillus fumigatus*: Role of the fungal nucleolus. *Med. Mycol.* **43** (SUPPL.1) 87-93. https://doi.org/10.1080/13693780400029486

BICANIC TA and HARRISON TS (2014) Systemic fungal infections. *Medicine* **42** (1) 26-30. https://doi.org/10.1016/j.mpmed.2013.10.006

BLANCO JL and GARCIA ME (2008) Immune response to fungal infections. *Vet. Immunol. Immunopathol.* **125** (1-2) 47-70. https://doi.org/10.1016/j.vetimm.2008.04.020

BLOOM ALM, JIN RM, LEIPHEIMER J, BARD JE, YERGEAU D, WOHLFERT EA and PANEPINTO JC (2019) Thermotolerance in the pathogen *Cryptococcus neoformans* is linked to antigen

masking via mRNA decay-dependent reprogramming. *Nat. Commun.* **10** (1) 1-13. https://doi.org/10.1038/s41467-019-12907-x
BOLIGNANO G and CRISEO G (2003) Disseminated nosocomial fungal infection by *Aureobasidium pullulans* var. *melanigenum*: A case report. *J. Clin. Microbiol.* **41** (9) 4483-4485. https://doi.org/10.1128/JCM.41.9.4483-4485.2003
BONGOMIN F, GAGO S, OLADELE R and DENNING D (2017) Global and multi-national prevalence of fungal diseases—estimate precision. *J. Fungi* **3** (4) 57. https://doi.org/10.3390/jof3040057
BOSCH C, BHANA Z, TOPLIS B, VOLSCHENK H and BOTHA A (2021) Transcriptomic response of *Cryptococcus neoformans* to ecologically relevant nitrogen concentrations. *FEMS Yeast Res*. https://doi.org/10.1093/femsyr/foab028
BOSCH C, TOPLIS B, VREULINK JM, VOLSCHENK H and BOTHA A (2020) Nitrogen concentration affects amphotericin B and fluconazole tolerance of pathogenic cryptococci. *FEMS Yeast Res.* **20** (2) p.foaa010. https://doi.org/10.1093/femsyr/foaa010
BOYD AS, WHELESS L, BRADY BG and ELLIS D (2017) Cutaneous *Yarrowia lipolytica* infection in an immunocompetent woman. *JAAD Case Rep.* **3** (3) 219-221. https://doi.org/10.1016/j.jdcr.2017.02.010
BRANDÃO F, ESHER SK, OST KS, PIANALTO K, NICHOLS CB, FERNANDES L, BOCCA AL, POÇAS-FONSECA MJ and ALSPAUGH JA (2018) HDAC genes play distinct and redundant roles in *Cryptococcus neoformans* virulence. *Sci. Rep.* **8** (1) 1-17. https://doi.org/10.1038/s41598-018-21965-y
BRANDÃO LR, MEDEIROS AO, DUARTE MC, BARBOSA AC and ROSA CA (2010) Diversity and antifungal susceptibility of yeasts isolated by multiple-tube fermentation from three freshwater lakes in Brazil. *J. Water Health* **8** (2) 279-289. https://doi.org/10.2166/wh.2009.170
BRITO-SANTOS F, FIGUEIREDO-CARVALHO MHG, COELHO RA, SALES A and ALMEIDA-PAES R (2017) Tinea capitis by *Microsporum audouinii*: case reports and review of published global literature 2000-2016. *Mycopathologia* **182** (11) 1053-1060. https://doi.org/10.1007/s11046-017-0181-1
BRITZ TJ, SIGGE GO, HUISAMEN N, KIKINE T, ACKERMANN A, LÖTTER M, LAMPRECHT C and KIDD M (2013) Fluctuations of indicator and index microbes as indication of pollution over three years in the Plankenbrug and Eerste rivers, Western Cape, South Africa. *Water SA* **39** (4) 457-466. https://doi.org/10.4314/wsa.v39i4.3.
BROSS JE, MANNING P, KACIAN D and TALBOT GH (1986) Pseudomeningitis caused by *Sporobolomyces salmonicolor*. *Am. J. Infect. Control* **14** (5) 220-223. https://doi.org/10.1016/0196-6553(86)90121-5
BROWN AJP, LARCOMBE DE and PRADHAN A (2020) Thoughts on the evolution of Core Environmental Responses in yeasts. *Fungal Biol.* **124** (5) 475-481. https://doi.org/10.1016/j.funbio.2020.01.003
BROWN GD, DENNING DW, GOW NA, LEVITZ SM, NETEA MG and WHITE TC (2012) Hidden killers: human fungal infections. *Sci. Translational Med.* **4** (165) 165-165. https://doi.org/10.1126/scitranslmed.3004404
BROWN M and MCCLURE P (2006) Microbiological risk assessment for emerging pathogens. *Emerging foodborne pathogens*, pp.130-152. ISBN: 9781855739635
BUCK JD and BUBUCIS PM (1978) Membrane filter procedure for enumeration of *Candida albicans* in natural waters. *Appl. Environ. Microbiol.* **35** (2) 237-242. https://doi.org/10.1128/aem.35.2.237-242.1978
BUCKLEY LB and HUEY RB (2016) How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integr. Compar. Biol.* **56** (1) 98-109. https://doi.org/10.1093/icb/icw004
BUZZINI P, LACHANCE MA and YURKOV A (2017) Yeasts in natural ecosystems: Diversity. *Yeasts Nat. Ecosyst. Div.* (March 2018) 1-499. https://doi.org/10.1007/978-3-319-62683-3
CAGGIANO G, DIELLA G, TRIGGIANO F, BARTOLOMEO N, APOLLONIO F, CAMPANALE C, LOPUZZO M and MONTAGNA MT (2020) Occurrence of fungi in the potable water of hospitals: A public health threat. *Pathogens* **9** (10) 783. https://dx.doi.org/10.3390%2Fpathogens9100783
CARREGA G, RICCIO G, SANTORIELLO L, PASQUALINI M and PELLICCI R (1997) *Candida famata* fungemia in a surgical patient successfully treated with fluconazole. *Eur. J. Clin. Microbiol. Infect. Dis.* **16** (9) 698. https://doi.org/10.1007/bf01708564
CASADEVALL A (2017) The Pathogenic Potential of a Microbe. *mSphere* **2** (1). https://doi.org/10.1128/msphere.00015-17.

CASADEVALL A (2020) Climate change brings the specter of new infectious diseases. *J. Clin. Invest.* **130** (2) 553-555. https://doi.org/10.1172/JCI135003
CASADEVALL A, KONTOYIANNIS DP and ROBERT V (2019) On the emergence of *Candida auris*: climate change, azoles, swamps, and birds. *MBio* **10** (4) e01397-19. https://doi.org/10.1128/mBio.01397-19
CAUSTON HC, REN B, SANG SEOK KOH, HARBISON CT, KANIN E, JENNINGS EG, TONG IHN LEE, TRUE HL, LANDER ES and YOUNG RA (2001) Remodelling of yeast genome expression in response to environmental changes. *Mol. Biol. Cell* **12** (2) 323-337. https://doi.org/10.1091/mbc.12.2.323
CHAKRABARTI A, SINGH K, NARANG A, SINGHI S, BATRA, R., RAO, K.L.N., RAY, P., GOPALAN, S., DAS, S., GUPTA, V. AND GUPTA, A.K., 2001. Outbreak of *Pichia anomala* infection in the pediatric service of a tertiary-care center in Northern India. *J. Clin. Microbiol.* **39** (5) 1702-1706. https://doi.org/10.1128/jcm.39.5.1702-1706.2001
CHANG YC, TSAI HF, KAROS M and KWON-CHUNG KJ (2004) THTA, a thermotolerance gene of *Aspergillus fumigatus*. *Fungal Genet. Biol.* **41** (9) 888-896. https://doi.org/10.1016/j.fgb.2004.06.004
CHATTERJEE S and TATU U (2017) Heat shock protein 90 localizes to the surface and augments virulence factors of *Cryptococcus neoformans*. *PLoS Neglected Trop. Dis.* **11** (8) 1-20. https://doi.org/10.1371/journal.pntd.0005836
CHEN D, HU M, GUO Y and DAHLGREN RA (2016) Changes in river water temperature between 1980 and 2012 in Yongan watershed, eastern China: Magnitude, drivers and models. *J. Hydrol.* **533** 191-199. https://doi.org/10.1016/j.jhydrol.2015.12.005
CHEN IC, HILL JK, OHLEMÜLLER R, ROY DB and THOMAS CD (2011) Rapid range shifts of species associated with high levels of climate warming. *Science* **333** (6045) 1024-1026. https://doi.org/10.1126/science.1206432
CHEN Y, KONIECZKA JH, SPRINGER DJ, BOWEN SE, ZHANG J, SILAO FGS, BUNGAY AAC, BIGOL UG and NICOLAS MG (2012) Convergent evolution of calcineurin pathway roles in thermotolerance and virulence in *Candida glabrata. G3 Genes Genom. Genet.* **2** (June) 675-691. https://doi.org/10.1534/g3.112.002279
CHEN YL, LEHMAN VN, LEWIT Y, AVERETTE AF and HEITMAN J (2013) Calcineurin governs thermotolerance and virulence of *Cryptococcus gattii*. *G3: Genes Genom. Genet.* **3** (3) 527-539. https://doi.org/10.1534/g3.112.004242
CHITESCU CL, KAKLAMANOS G, NICOLAU AI and STOLKER AAML (2015) High sensitive multiresidue analysis of pharmaceuticals and antifungals in surface water using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory. *Sci. Total Environ.* **532** 501-511. https://doi.org/10.1016/j.scitotenv.2015.06.010
CHOW EWL, CLANCEY SA, BILLMYRE RB, AVERETTE AF, GRANEK JA, MIECZKOWSKI P, CARDENAS ME and HEITMAN J (2017) Elucidation of the calcineurin-Crz1 stress response transcriptional network in the human fungal pathogen *Cryptococcus neoformans*. *PLoS Genet.* **13** (4) 1-29. https://doi.org/10.1371/journal.pgen.1006667
CHRISTIAENS G, HAYETTE MP, JACQUEMIN D, MELIN P, MUTSERS J and DE MOL P (2005) An outbreak of *Absidia corymbifera* infection associated with bandage contamination in a burns unit. *J. Hosp. Infect.* **61** (1) 88. https://doi.org/10.1016/j.jhin.2004.12.011
COELHO MA, ALMEIDA JMF, MARTINS IM, DA SILVA AJ and SAMPAIO JP (2010) The dynamics of the yeast community of the Tagus river estuary: testing the hypothesis of the multiple origins of estuarine yeasts. *Anton. Leeuw.* **98** (3) 331-342. https://doi.org/10.1007/s10482-010-9445-1
COHEN JM, CIVITELLO DJ, VENESKY MD, MCMAHON TA and ROHR JR (2017) Thermal mismatches explain how climate change and infectious disease drove widespread amphibian extinctions. *bioRxiv* 164814. https://doi.org/10.1101/164814
COLES M, COX K and CHAO A (2020) *Candida haemulonii*: An emerging opportunistic pathogen in the United States? *IDCases* **21** e00900. https://doi.org/10.1016/j.idcr.2020.e00900
COLOMBO AL, PADOVAN ACB and CHAVES GM (2011) Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin. Microbiol. Rev.* **24** (4) 682-700. https://doi.org/10.1128/CMR.00003-11
COLVIN C, MURUVEN D, LINDLEY D, GORDEN H and SCHACHTSCHENEIDER K (2016) Water: Facts and futures rethinking South Africa's water future. WWF-SA, Cape Town. 1-100. URL: www.wwf.org.za
COOKE WB, PHAFF HJ, MILLER MW, SHIFRINE M and KNAPP EP (1960) Yeasts in polluted water and sewage. *Mycologia* **52** (2) 210-230. https://doi.org/10.1080/00275514.1960.12024896

COOPER CR (2011) Yeasts pathogenic to humans. Elsevier *Volume 1*. B.V. 9-19. https://doi.org/10.1016/B978-0-444-52149-1.00002-1
CORTEZ KJ, ROILIDES E, QUIROZ-TELLES F, MELETIADIS J, ANTACHOPOULOS C, KNUDSEN T, BUCHANAN W, MILANOVICH J, SUTTON DA, FOTHERGILL A, and co-authors (2008) Infections caused by *Scedosporium* spp. *Clin. Microbiol. Rev.* **21** (1) 157-197. https://doi.org/10.1128/CMR.00039-07
CULLIS JDS, HORN A, ROSSOUW N, FISHER-JEFFES L, KUNNEKE MM and HOFFMAN W (2019) Urbanisation, climate change and its impact on water quality and economic risks in a water scarce and rapidly urbanising catchment: Case study of the Berg River Catchment. *H2Open J.* **2** (1) 146-167. https://doi.org/10.2166/H2OJ.2019.027
D'AMEN M and BOMBI P (2009) Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy. *Biol. Conserv.* **142** (12) 3060-3067. https://doi.org/10.1016/j.biocon.2009.08.004
DAS S, SINGH S, TAWDE Y, CHAKRABARTI A, RUDRAMURTHY SM, KAUR H, SHANKARNARAYAN SA and GHOSH A (2021) A selective medium for isolation and detection of *Candida auris*, an emerging pathogen. *Journal of Clinical Microbiology*, **59** (2) e00326-20. https://doi.org/10.1128/JCM.00326-20
DATTA N, ARENDRUP MC and SAUNTE JP (2015) First report of *Candida palmioleophila* endogenous endophthalmitis. *Acta Ophthalmol.* **93** (6) e517-e518. https://doi.org/10.1111/aos.12662
DE ALMEIDA JMGCF (2005) Yeast community survey in the Tagus estuary. *FEMS Microbiol. Ecol.* **53** (2) 295-303. https://doi.org/10.1016/j.femsec.2005.01.006
DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (1996) South African Water Quality Guidelines: Volume 1: Domestic Water Use. *Volume 1* Second Edition. https://www.dws.gov.za/Groundwater/documents/Pol_saWQguideFRESHDomesticusevol1.pdf.
DHAR R, SÄGESSER R, WEIKERT C and WAGNER A (2013) Yeast adapts to a changing stressful environment by evolving cross-protection and anticipatory gene regulation. *Mol. Biol. Evol.* **30** (3) 573-588. https://doi.org/10.1093/molbev/mss253
DOGGETT, MS (2000) Characterization of fungal biofilms within a municipal water distribution system. *Appl. Environ. Microbiol.* **66** (3) 1249-1251. https://doi.org/10.1128%2Faem.66.3.1249-1251.2000
DUNAYEVICH P, BALTANÁS R, CLEMENTE JA, COUTO A, SAPOCHNIK D, VASEN G and COLMAN-LERNER A (2018) Heat-stress triggers MAPK crosstalk to turn on the hyperosmotic response pathway. *Sci. Rep.* **8** (1) 1-15. https://doi.org/10.1038/s41598-018-33203-6
DUPONT B, BROWN HHC, WESTERMANN K, MARTINS MD, REX JH, LORTHOLARY O and KAUFFMANN CA (2000) Mycoses in AIDS. *Med. Mycol.* **38** (i) 259-267. https://doi.org/10.1080/mmy.38.s1.259.267
DYNOWSKA M (1997) Yeast-like fungi possessing bio-indicator properties isolated from the Łyna river. *Acta Mycol.* **32** (2) 279-286. https://doi.org/10.5586/am.1997.024
EDOKPAYI JN, ROGAWSKI ET, KAHLER DM, HILL CL, REYNOLDS C, NYATHI E, SMITH JA, ODIYO JO, SAMIE A, BESSONG P and DILLINGHAM R (2018) Challenges to sustainable safe drinking water: a case study of water quality and use across seasons in rural communities in Limpopo province, South Africa. *Water* **10** (2) 159. https://doi.org/10.3390/w10020159
EISENBERG ES, ALPERT BE, WEISS RA, MITTMAN N and SOEIRO R (1983) *Rhodotorula rubra* peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. *Am. J. Med.* **75** (2) 349-352. https://doi.org/10.1016/0002-9343(83)91216-0
EL HELOU G and PALAVECINO E (2017) *Candida pararugosa*: First Reported Bloodstream Infection in an Adult. *Cureus* **9** (5). https://doi.org/10.7759/cureus.1283
ELANDER RP (2003) Industrial production of β-lactam antibiotics. *Appl. Microbiol. Biotechnol.* **61** (5) 385-392. https://doi.org/10.1007/s00253-003-1274-y
ELLIS D, MARRIOTT D, HAJJEH RA, WARNOCK D, MEYER W and BARTON R (2000) Epidemiology: Surveillance of fungal infections. *Medical Mycology* **38** 173-182. https://doi.org/10.1080/mmy.38.1.173.182.
ELLIS DH and PFEIFFER TJ (1990) Natural habitat of *Cryptococcus neoformans* var. *gattii*. *J. Clin. Microbiol.* **28** (7) 1642-1644. https://doi.org/10.1128/jcm.28.7.1642-1644.1990
ELLIS DH and PFEIFFER TJ (1992) The ecology of *Cryptococcus neoformans*. *Eur. J. Epidemiol.* **8** (3) 321-325. https://doi.org/10.1007/bf00158562

ENE IV, FARRER RA, HIRAKAWA MP, AGWAMBA K, CUOMO CA and BENNETT RJ (2018) Global analysis of mutations driving microevolution of a heterozygous diploid fungal pathogen. *Proc. Natl Acad. Sci.* **115** (37) E8688-E8697. https://doi.org/10.1073/pnas.1806002115

FERNÁNDEZ-RUIZ M, GUINEA J, PUIG-ASENSIO M, ZARAGOZA O, ALMIRANTE B, CUENCA-ESTRELLA M and AGUADO JM (2017) Fungemia due to rare opportunistic yeasts: Data from a population-based surveillance in Spain. *Medical Mycology* **55** (2) 125-136. https://doi.org/10.1093/mmy/myw055.

FISHER MC, GARNER TW and WALKER SF (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annu. Rev. Microbiol.* **63** 291-310. https://doi.org/10.1146/annurev.micro.091208.073435

FROMTLING RA, ABRUZZO GK and GILTINAN DM (1987) Candida tropicalis infection in normal, diabetic and neutropenic mice. *Journal of Clinical Microbiology* **25** (8) 1416-1420. https://doi.org/10.1128/jcm.25.8.1416-1420.1987.

FUCHS BB and MYLONAKIS E (2009) Our paths might cross: The role of the fungal cell wall integrity pathway in stress response and cross talk with other stress response pathways. *Eukaryotic Cell* **8** (11) 1616-1625. https://doi.org/10.1128/EC.00193-09

GADANHO M, LIBKIND D and SAMPAIO JP (2006) Yeast diversity in the extreme acidic environments of the Iberian pyrite belt. *Microb. Ecol.* **52** (3) 552-563. https://doi.org/10.1007/s00248-006-9027-y

GAFFI (2017) Fungal disease frequency. URL: https://www.gaffi.org/why/fungal-disease-frequency/ (Accessed 28 January 2022).

GARBER G (2001) An overview of fungal infections. *Drugs* **61** (SUPPL. 1) 1-12. https://doi.org/10.2165/00003495-200161001-00001

GARCIA-SOLACHE MA and CASADEVALL A (2010) Global warming will bring new fungal diseases for mammals. *mBio* **1** (1) 1-3. https://doi.org/10.1128/mBio.00061-10

GARG D, MUTHU V, SEHGAL IS, RAMACHANDRAN R, KAUR H, BHALLA A, PURI GD, CHAKRABARTI A and AGARWAL R (2021) Coronavirus disease (Covid-19) associated mucormycosis (CAM): case report and systematic review of literature. *Mycopathologia* **186** (2) 289-298. https://doi.org/10.3410/f.739505445.793585416

GHAJARI A, LOTFALI E, NOROUZI M and ARAB-MAZAR Z (2018) First report of Vulvovaginitis due to *Cryptococcus magnus* in Iran. *Curr. Med. Mycol.* **4** (1) 30. https://dx.doi.org/10.18502%2Fcmm.4.1.32

GLIŃSKA-LEWCZUK K, GOŁAŚ I, KOC J, GOTKOWSKA-PŁACHTA A, HARNISZ M and ROCHWERGER A (2016) The impact of urban areas on the water quality gradient along a lowland river. *Environ. Monit. Assess.* **188** (11) 1-15. https://doi.org/10.1007/s10661-016-5638-z

GOLD JW, POSTON W, MERTELSMANN R, LANGE M, KIEHN T, EDWARDS F, BERNARD E, CHRISTIANSEN K and ARMSTRONG D (1981) Systemic infection with *Trichosporon cutaneum* in a patient with acute leukemia: report of a case. *Cancer* **48** (10) 2163-2167. https://doi.org/10.1002/1097-0142(19811115)48:10<2163::AID-CNCR2820481008>3.0.CO;2-T

GOLDANI LZ, CRAVEN DE and SUGAR AM (1995) Central venous catheter infection with *Rhodotorula minuta* in a patient with AIDS taking suppressive doses of fluconazole. *Journal of Medical and Veterinary Mycology* **33** (4) 267-270. https://doi.org/10.1080/02681219580000531

GOSTINČAR C, GRUBE M and GUNDE-CIMERMAN N (2011) Evolution of Fungal Pathogens in Domestic Environments? *Fungal Biol.* **115** (10) 1008-1018. https://doi.org/10.1016/j.funbio.2011.03.004

GŘLER NC, TOSUN I and AYDIN F (2017) The identification of *Meyerozyma guilliermondii* from blood cultures and surveillance samples in a university hospital in Northeast Turkey: A ten-year survey. *J. Mycol. MÚdicale* **27** (4) 506-513. https://doi.org/10.1016/j.mycmed.2017.07.007

GROSSART HP, VAN DEN WYNGAERT S, KAGAMI M, WURZBACHER C, CUNLIFFE M and ROJAS-JIMENEZ K (2019) Fungi in aquatic ecosystems. *Nat. Rev. Microbiol.* **17** (6) 339-354. https://doi.org/10.1038/s41579-019-0175-8

GUARNER J (2017) Human immunodeficiency virus and fungal infections. *Seminars Diagn. Pathol.* **34** (4) 325-331. https://doi.org/10.1053/j.semdp.2017.04.007

GUINEA J (2014) Global trends in the distribution of *Candida* species causing candidemia. *Clinical Microbiol. Infection* **8** 5-10. URL: https://doi.org/10.1111/1469-0691.12539

GUITARD J, ANGOULVANT A, LETSCHER-BRU V, L'OLLIVIER C, CORNET M, DALLE F, GRENOUILLET F, LACROIX C, VEKHOFF A, MAURY E, and co-authors (2013) Invasive infections due to *Candida norvegensis* and *Candida inconspicua*: Report of 12 cases and review of the literature. *Medical Mycology* **51** (8) 795-799. https://doi.org/10.3109/13693786.2013.807444

GYAURGIEVA OH, BOGOMOLOVA TS and GORSHKOVA GI (1996) Meningitis caused by *Rhodotorula rubra* in an HIV-infected patient. *J. Med. Vet. Mycol.* **34** (5) 357-359. https://doi.org/10.1080/02681219680000611

HA M v., CHOY MS, MCCOY D, FERNANDEZ N and SUH JS (2018) *Candida catenulata* candidemia and possible endocarditis in a cirrhotic patient successfully de-escalated to oral fluconazole. *J. Clin. Pharm. Therapeutics* **43** (6) 910-913. https://doi.org/10.1111/jcpt.12728

HAACK SK, DURIS JW, FOGARTY LR, KOLPIN DW, FOCAZIO MJ, FURLONG ET and MEYER MT (2009) Comparing wastewater chemicals, indicator bacteria concentrations, and bacterial pathogen genes as faecal pollution indicators. *J. Environ. Qual.* **38** (1) 248-258. https://doi.org/10.2134/jeq2008.0173

HAGEN F, KHAYHAN K, THEELEN B, KOLECKA A, POLACHECK I, SIONOV E, FALK R, PARNMEN S, LUMBSCH HT and BOEKHOUT T (2015) Recognition of seven species in the *Cryptococcus gattii/Cryptococcus neoformans* species complex. *Fungal Genet. Biol.* **78** 16-48. https://doi.org/10.1016/j.fgb.2015.02.009

HAGESKAL G, LIMA N and SKAAR I (2009) The study of fungi in drinking water. *Mycol. Res.* **113** (2) 165-172. https://doi.org/10.1016/j.mycres.2008.10.002

HANSEN J, SATO M, HEARTY P, RUEDY R, KELLEY M, MASSON-DELMOTTE V, RUSSELL G, TSELIOUDIS G, CAO J, RIGNOT E and co-authors (2016) Ice melt, sea level rise and superstorms: evidence from paleoclimate data, climate modelling, and modern observations that 2°C global warming could be dangerous. *Atmos. Chem. Phys* **16** 3761-3812. https://doi.org/10.5194/acp-16-3761-2016, 2016.

HARRIGAN WF and MCCANCE ME (1966) Statistical Methods for the Selection and Examination of Microbial Colonies. *Laboratory Methods in Microbiology* 34-35. https://doi.org/10.1016/b978-1-4832-3205-8.50014-3.

HARRISON TS and BROUWER AE (2009) Systemic fungal infections. *Medicine* **37** (12) 660-664. https://doi.org/10.1016/j.mpmed.2009.09.016

HASENCLEVER HF (1959) Comparative pathogenicity of Candida albicans for mice and rabbits. *Journal of bacteriology* **78** (1) 105-109. https://doi.org/10.1128/jb.78.1.105-109.1959.

HAVELAAR AH, FURUSE K and HOGEBOOM WM (1986). Bacteriophages and indicator bacteria in human and animal faeces. *J. Appl. Bacteriol.* **60** (3) 255-262. https://doi.org/10.1111/j.1365-2672.1986.tb01081.x

HERBIG FJW (2019) Talking dirty – effluent and sewage irreverence in South Africa: A conservation crime perspective. *Cogent Social Sciences*, **5** (1). https://doi.org/10.1080/23311886.2019.1701359.

HERBIG FJW (2019) Talking dirty – effluent and sewage irreverence in South Africa: A conservation crime perspective. *Cogent Soc. Sci.* **5** (1). https://doi.org/10.1080/23311886.2019.1701359

HIRAYAMA T, MIYAZAKI T, ITO Y, WAKAYAMA M, SHIBUYA K, YAMASHITA K, TAKAZONO T, SAIJO T, SHIMAMURA S, YAMAMOTO K, and co-authors (2020) Virulence assessment of six major pathogenic Candida species in the mouse model of invasive candidiasis caused by fungal translocation. *Scientific Reports* **10** (1) 1-10. https://doi.org/10.1038/s41598-020-60792-y.

HIRAYAMA T, MIYAZAKI T, YAMAGISHI Y, MIKAMO H, UEDA T, NAKAJIMA K, TAKESUE Y, HIGASHIY, YAMAMOTO Y, KIMURA M, and co-authors (2018) Clinical and microbiological characteristics of *Candida guilliermondii* and *Candida fermentati*. *Antimicrobial Agents and Chemotherapy* **62** (6). https://doi.org/10.1128/AAC.02528-17.

HOANG JK and BURRUSS J (2007) Localized cutaneous *Cryptococcus albidus* infection in a 14-year-old boy on etanercept therapy. *Pediatric Dermatol.* **24** (3) 285-288. https://doi.org/10.1111/j.1525-1470.2007.00404.x

HODA S, AGARWAL H, AHLUWALIA SK and VIJAYARAGHAVAN MVP (2019) Antifungal Resistance Analysis of Environmental Isolates of *Aspergillus* in North India. *J. Pure Appl. Microbiol.* **13** (1) 385-392. https://doi.org/10.22207/jpam.13.1.42

HSUEH PR, TENG LJ, HO SW and LUH KT (2003) Catheter-related sepsis due to *Rhodotorula glutinis*. *J. Clin. Microbiol.* **41** (2) 857-859. https://doi.org/10.1128/JCM.41.2.857-859.2003

HU Y, ZHANG J, LI X, YANG Y, ZHANG Y, MA J and XI L (2013) *Penicillium marneffei* infection: an emerging disease in mainland China. *Mycopathologia* **175** (1) 57-67. https://doi.org/10.1007/s11046-012-9577-0
HUANG CJ, LU MY, CHANG YW and LI WH (2018) Experimental evolution of yeast for high-temperature tolerance. *Mol. Biol. Evol.* **35** (8) 1823-1839. https://doi.org/10.1093/molbev/msy077
HUANG DB, OSTROSKY-ZEICHNER L, WU JJ, PANG KR and TYRING SK (2004) Therapy of common superficial fungal infections. *Dermatol. Therapy* **17** 517-522. https://doi.org/10.1111/j.1396-0296.2004.04055.x
HUNTER-ELLUL L, SCHEPP ED, LEA A and WILKERSON MG (2014) A rare case of Cryptococcus luteolus-related tenosynovitis. *Infection* **42** (4) 771-774. https://doi.org/10.1007/s15010-014-0593-5
HUSSAIN S, SALAHUDDIN N, AHMAD I, SALAHUDDIN I and JOOMA R (1995) Rhinocerebral invasive mycosis: occurence in immunocompetent individuals. *Eur. J. Radiol.* **20** (2) 151-155. https://doi.org/10.1016/0720-048x(95)00644-6
IBE C (2022) The fight against mycoses in Africa: are we making progress? *Clinical Microbiology and Infection* European Society of Clinical Microbiology and Infectious Diseases, **28** (1) 9-12. https://doi.org/10.1016/j.cmi.2021.09.004.
ILEA RC (2009) Intensive livestock farming: Global trends, increased environmental concerns, and ethical solutions. *J. Agric. Environ. Ethics* **22** (2) 153-167. https://doi.org/10.1007/s10806-008-9136-3
IPCC (2018) Summary for Policymakers. *Global Warming of 1.5°C.* In: Masson-Delmotte V, Zhai P, Pörtner H-O, Roberts D, Skea J, Shukla PR, Pirani A, Moufouma-Okia W, Péan C, Pidcock R, Connors S, Matthews JBR, Chen Y, Zhou X, Gomis MI, Lonnoy E, Maycock T, Tignor M, and Waterfield T (eds.) *Global Warming of 1.5°C. World Meteorological Organization, Geneva. 32 pp. URL:* https://www.ipcc.ch/sr15/
IPCC (2021) Summary for Policymakers. In: Masson-Delmotte V, Zhai P, Pirani A, Connors SL, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis MI, Huang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock TK, Waterfield T, Yelekçi O, Yu R and Zhou B (eds.). *Climate Change 2021: The Physical Science Basis.* Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. URL: https://www.ipcc.ch/report/ar6/wg1/ (Accessed 18 March 2022).
IWEN PC, SCHUTTE SD, FLORESCU DF, NOEL-HURST RK and SIGLER L (2012) Invasive *Scopulariopsis brevicaulis* infection in an immunocompromised patient and review of prior cases caused by *Scopulariopsis* and *Microascus* species. *Med. Mycol.* **50** (6) 561-569. https://doi.org/10.3109/13693786.2012.675629
JACKSON BR, CHOW N, FORSBERG K, LITVINTSEVA AP, LOCKHART SR, WELSH R, VALLABHANENI S and CHILLER T (2019) On the origins of a species: What might explain the rise of *Candida auris*? *J. Fungi* **5** (3). https://doi.org/10.3390/jof5030058
JARVIS WR (1995) Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. *Clin. Infect. Dis.* **20** (6) 1526-1530. https://doi.org/10.1093/clinids/20.6.1526
JOSEPH SB and HALL DW (2004) Spontaneous mutations in diploid *Saccharomyces cerevisiae*: more beneficial than expected. *Genetics*, **168** (4) 1817-1825. https://doi.org/10.1534/genetics.104.033761
JOSHI A, SINGH R, SHAH MS, UMESH S and KHATTRY N (2010) Subcutaneous mycosis and fungemia by *Aureobasidium pullulans*: A rare pathogenic fungus in a post allogeneic BM transplant patient. *Bone Marrow Transplant.* **45** (1) 203-204. https://doi.org/10.1038/bmt.2009.111
JUNEJA D, BORAH AK, NASA P, SINGH O, JAVERI Y and DANG R (2012) *Candida sake* candidaemia in non-neutropenic critically ill patients: A case series. *Critical Care Resusc.* **14** (1) 187-191.
JUNG J, MOON YS, YOO JA, LIM JH, JEONG J and JUN JB (2018a) Investigation of a nosocomial outbreak of fungemia caused by *Candida pelliculosa* (*Pichia anomala*) in a Korean tertiary care center. *J. Microbiol. Immunol. Infect.* **51** (6) 794-801. https://doi.org/10.1016/j.jmii.2017.05.005
JUNG KW, LEE KT, AVERETTE AF, HOY MJ, EVERITT J, HEITMAN J and BAHN YS (2018b) Evolutionarily conserved and divergent roles of Unfolded Protein Response (UPR) in the pathogenic *Cryptococcus* species complex. *Sci. Rep.* **8** (1) 1-15. https://doi.org/10.1038/s41598-018-26405-5

JUVVADI PR, KUROKI Y, ARIOKA M, NAKAJIMA H and KITAMOTO K (2003) Functional analysis of the calcineurin-encoding gene cnaA from *Aspergillus oryzae*: Evidence for its putative role in stress adaptation. *Arch. Microbiol.* **179** (6) 416-422. https://doi.org/10.1007/s00203-003-0546-3

JUVVADI PR, LAMOTH F and STEINBACH WJ (2014) Calcineurin as a multifunctional regulator: Unraveling novel functions in fungal stress responses, hyphal growth, drug resistance, and pathogenesis. *Fungal Biol. Rev.* **28** (2) 56-69. https://doi.org/10.1016/j.fbr.2014.02.004

KACZMARSKI EB, LIU YIN JA, TOOTH JA, LOVE EM and DELAMORE IW (1986) Systemic infection with *Aureobasidium pullulans* in a leukaemic patient. *J. Infect.* **13** (3) 289-291. https://doi.org/10.1016/S0163-4453(86)91388-5

KAHN HD and STRALKA K (2009) Estimated daily average per capita water ingestion by child and adult age categories based on USDA's 1994-1996 and 1998 continuing survey of food intakes by individuals. *J Expo Sci Environ Epidemiol* **19** (4) 396-404. https://doi.org/10.1038/jes.2008.29.

KÄMMER P, MCNAMARA S, WOLF T, CONRAD T, ALLERT S, GERWIEN F, HÜNNIGER K, KURZAI O, GUTHKE R, HUBE B and LINDE J (2020) Survival strategies of pathogenic Candida species in human blood show independent and specific adaptations. *mBio*, **11** (5) pp.e02435-20. https://doi.org/10.1128/mbio.02435-20.

KASSAMALI H, ANAISSIE E, RO J, ROLSTON K, KANTARJIAN H, FAINSTEIN V and BODEY GP (1987) Disseminated *Geotrichum candidum* infection. *J. Clin. Microbiol.* **25** (9) 1782-1783. https://doi.org/10.1128/jcm.25.9.1782-1783.1987

KEAN R, BROWN J, GULMEZ D, WARE A and RAMAGE G (2020) *Candida auris*: A decade of understanding of an enigmatic pathogenic yeast. *J. Fungi* **6** (1) 30. https://doi.org/10.3390/jof6010030

KEDRA M (2020) Regional response to global warming: Water temperature trends in semi-natural mountain river systems. *Water* **12** (1). https://doi.org/10.3390/w12010283

KENYON C, BONORCHIS K, CORCORAN C, MEINTJES G, LOCKETZ M, LEHLOENYA R, VISMER HF, NAICKER P, PROZESKY H, VAN WYK M and co-authors (2013) A dimorphic fungus causing disseminated infection in South Africa. *New England J. Med.* **369** (15) 1416-1424. https://doi.org/10.1056/nejmoa1215460

KNEALE M, BARTHOLOMEW JS, DAVIES E and DENNING DW (2016) Global access to antifungal therapy and its variable cost. *Journal of Antimicrobial Chemotherapy* **71** (12) 3599-3606. https://doi.org/10.1093/jac/dkw325.

KOEHLER P, TACKE D and CORNELY OA (2016) Bone and joint infections by *Mucorales*, *Scedosporium, Fusarium* and even rarer fungi. *Crit. Rev. Microbiol.* **42** (1) 158-171. https://doi.org/10.3109/1040841X.2014.910749

KORDOSSIS T, AVLAMI A, VELEGRAKI A, STEFANOU I, GEORGAKOPOULOS G, PAPALAMBROU C and LEGAKIS NJ (1998) First report of *Cryptococcus laurentii* meningitis and a fatal case of *Cryptococcus albidus* cryptococcaemia in AIDS patients. *Med. Mycol.* **36** (5) 335-339. https://doi.org/10.1080/02681219880000521

KOUSHA M, TADI R and SOUBANI AO (2011) Pulmonary aspergillosis: a clinical review. *Eur. Respir. Rev.* **20** (121) 156-174. https://doi.org/10.1183/09059180.00001011

KUJATH P and KUJATH C (2010) Complicated skin, skin structure and soft tissue infections are we threatened by multi-resistant pathogens? *Eur. J. Med. Res.* **15** (12) 544-553. https://doi.org/10.1186/2047-783x-15-12-544

KURTZMAN CP, FELL JW and BOEKHOUT T eds (2011) *The yeasts: a taxonomic study*. Elsevier. ISBN: 978-0-444-52149-1

KWASNIEWSKA, K (1988) Horizontal distribution and density of yeasts and filamentous fungi in Lake St. Clair water. *J. Great Lakes Res.* **14** (4) 438-443. https://doi.org/10.1016/S0380-1330(88)71577-4

LAMOTH F, LOCKHART SR, BERKOW EL and CALANDRA T (2018) Changes in the epidemiological landscape of invasive candidiasis. *J. Antimicrob. Chemotherapy* **73** i4-i13. https://doi.org/10.1093/jac/dkx444

LANGARICA-FUENTES A, HANDLEY PS, HOULDEN A, FOX G and ROBSON GD (2014) An investigation of the biodiversity of thermophilic and thermotolerant fungal species in composts using culture-based and molecular techniques. *Fungal Ecology* Elsevier Ltd, **11** (0) 132-144. https://doi.org/10.1016/j.funeco.2014.05.007.

LAZERA MS, PIRES FDA, CAMILLO-COURA L, NISHIKAWA MM, BEZERRA CCF, TRILLES L and WANKE B (1996) Natural habitat of *Cryptococcus neoformans* var. *neoformans* in decaying

wood forming hollows in living trees. *J. Med. Vet. Mycol.* **34** (2) 127-131. https://doi.org/10.1080/02681219680000191

LEACH MD, KLIPP E, COWEN LE and BROWN AJP (2012) Fungal Hsp90: A biological transistor that tunes cellular outputs to thermal inputs. *Nat. Rev. Microbiol.* **10** (10) 693-704. https://doi.org/10.1038/nrmicro2875

LEAW SN, CHANG HC, BARTON R, BOUCHARA JP and CHANG TC (2007) Identification of medically important *Candida* and non-*Candida* yeast species by an oligonucleotide array. *J. Clin. Microbiol.* **45** (7) 2220-2229. https://doi.org/10.1128/jcm.00543-07

LEVENSON D, PFALLER MA, SMITH MA, HOLLIS R, GERARDEN T, TUCCI CB and ISENBERG HD (1991) *Candida zeylanoides*: Another opportunistic yeast. *J. Clin. Microbiol.* **29** (8) 1689-1692. https://doi.org/10.1128/jcm.29.8.1689-1692.1991

LEYNGOLD I, OLIVI A, ISHII M, BLITZ A, BURGER P, SUBRAMANIAN PS and GALLIA G (2014) Acute chiasmal abscess resulting from perineural extension of invasive sino-orbital aspergillosis in an immunocompetent patient. *World Neurosurg.* **81** (1) 203-e1. https://doi.org/10.1016/j.wneu.2013.08.018

LIM HJ, LEE OJ, KIM SH, SHIN MG and SHIN JH (2020) First Case of Nosocomial Fungemia Caused by Candida melibiosica. *Annals of Laboratory Medicine* **40** (2) 177-179. https://doi.org/10.3343/alm.2020.40.2.177.

LINDAHL BD, NILSSON RH and TEDERSOO L (2013) Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide - Lindahl - 2013 - New Phytologist - Wiley Online Library. URL: http://onlinelibrary.wiley.com/doi/10.1111/nph.12243/full%5Cnpapers2://publication/uuid/AD82815A-95B5-4D45-AB29-5E9C406E8E1F.

LINEMAN M, DO Y, KIM JY and JOO GJ (2015) Talking about climate change and global warming. *PloS ONE* **10** (9) p.e0138996. https://doi.org/10.1371/journal.pone.0138996

LIPKE PN (2018) What we do not know about fungal cell adhesion molecules. *J. Fungi* **4** (2). https://doi.org/10.3390/jof4020059

LIU C, CUI Y, LI X and YAO M (2021) microeco: an R package for data mining in microbial community ecology. *FEMS Micr. Eco.* **97** (2), p 255. https://doi.org/10.1093/femsec/fiaa255

LIU S, XIE Z, LIU B, WANG Y, GAO J, ZENG Y, XIE J, XIE Z, JIA B, QIN P, and co-authors (2020) Global river water warming due to climate change and anthropogenic heat emission. *Glob. Planetary Change* **193** (July) 103289. https://doi.org/10.1016/j.gloplacha.2020.103289

LIU X, WANG J, LIU T, KONG W, HE X, JIN Y and ZHANG B (2015) Effects of assimilable organic carbon and free chlorine on bacterial growth in drinking water. *PLoS ONE* **10** (6) 1-11. https://doi.org/10.1371/journal.pone.0128825

LIYANAGE CP and YAMADA K (2017) Impact of population growth on the water quality of natural water bodies. *Sustainability* **9** (8). https://doi.org/10.3390/su9081405

LO RE V, FISHMAN NO and NACHAMKIN I (2003) Recurrent catheter-related *Rhodotorula rubra* infection. *Clin. Microbiol. Infect.* **9** (8) 897-900. https://doi.org/10.1046/j.1469-0691.2003.00641.x

LOCKHART SR, MESSER SA, PFALLER MA and DIEKEMA DJ (2009) Identification and susceptibility profile of *Candida fermentati* from a worldwide collection of *Candida guilliermondii* clinical isolates. *J. Clin. Microbiol.* **47** (1) 242-244. https://doi.org/10.1128/JCM.01889-08

LOHOUÉ PETMY J, LANDO AJ, KAPTUE L, TCHINDA V and FOLEFACK M (2004) Superficial mycoses and HIV infection in Yaounde. *J. Eur. Acad. Dermatol. Venereol.* **18** (3) 301-304. https://doi.org/10.1111/j.1468-3083.2004.00924.x

LONGCORE JE, PESSIER AP and NICHOLS DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91** (2) 219-227. https://doi.org/10.1080/00275514.1999.12061011

LOUW C, BESTER E, ARCHER E, KAMPER F, WOLFAARDT GM and LOUW TM. 2022. Antimicrobial resistance and antibiotic use in Stellenbosch from 2018 to 2019. SunScholarData repository (unpublished). DOI: 10.25413/sun.17294246

LOW CY and ROTSTEIN C (2011) Emerging fungal infections in immunocompromised patients. *F1000 Med. Rep.* **3** (1) 1-8. https://doi.org/10.3410/M3-14

LUPLERTLOP N, PUMEESAT P, MUANGKAEW W, WONGSUK T and ALASTRUEY-IZQUIERDO A (2016) Environmental screening for the *Scedosporium apiospermum* species complex in public parks in Bangkok, Thailand. *PLoS ONE* **11** (7) 1-10. https://doi.org/10.1371/journal.pone.0159869

MACHIDO D, YAKUBU S and EZEONUEGBU B (2015) Composition of fungal flora in raw refinery effluent, effluent retention pond and a treated effluent recipient river. *J. Appl. Sci. Environ. Manage.* **18** (4) 592. https://doi.org/10.4314/jasem.v18i4.5

MACLAREN JA (1960) The Cultivation of Pathogenic Fungi on a Molybdenum Medium. *Mycologia* **52** (1) 148. https://doi.org/10.2307/3756257.

MADHAVAN T, EISSES J and QUINN EL (1976) Infections due to *Trichosporon cutaneum*, an uncommon systemic pathogen. *Henry Ford Hospital Med. J.* **24** (1) 27-30. https://scholarlycommons.henryford.com/hfhmedjournal/vol24/iss1/5

MAGDITCH DA, LIU TB, XUE C and IDNURM A (2012) DNA mutations mediate microevolution between host-adapted forms of the pathogenic fungus *Cryptococcus neoformans*. *PLoS Pathogens* **8** (10). https://doi.org/10.1371/journal.ppat.1002936

MAGWAZA NM, NXUMALO EN, MAMBA BB and MSAGATI TAM (2017) The occurrence and diversity of waterborne fungi in African aquatic systems: Their impact on water quality and human health. *Int. J. Environ. Res. Public Health* **14** (5). https://doi.org/10.3390/ijerph14050546

MAHESHWARI R, BHARADWAJ G and BHAT MK (2000) Thermophilic fungi: their physiology and enzymes. *Microbiol. Mol. Biol. Rev.* **64** (3) 461-488. https://doi.org/10.1128/mmbr.64.3.461-488.2000

MALCOLM JR, LIU C, NEILSON RP, HANSEN L and HANNAH LEE (2006) Global warming and extinctions of endemic species from biodiversity hotspots. *Conserv. Biol.* **20** (2) 538-548. https://doi.org/10.1111/j.1523-1739.2006.00364.x

MARIER R, ZAKHIREH B, DOWNS J, WYNNE B, HAMMOND GL and ANDRIOLE VT (1978) *Trichosporon cutaneum* endocarditis. *Scan. J. Infect. Dis.* **10** (3) 255-256. https://doi.org/10.3109/inf.1978.10.issue-3.19

MARTÍNEZ-MURCIA A, NAVARRO A, BRU G, CHOWDHARY A, HAGEN F and MEIS JF (2018) Internal validation of GPS™ MONODOSE CanAur dtec-qPCR kit following the UNE/EN ISO/IEC 17025:2005 for detection of the emerging yeast *Candida auris*. *Mycoses* **61** (11) 877-884. https://doi.org/10.1111/myc.12834.

MARTINS C, VARELA A, LECLERCQ CC, NÚÑEZ O, VĚTROVSKÝ T, RENAUT J, BALDRIAN P and SILVA PEREIRA C (2018) Specialisation events of fungal metacommunities exposed to a persistent organic pollutant are suggestive of augmented pathogenic potential. *Microbiome* **6** (1) 208. https://doi.org/10.1186/s40168-018-0589-y

MAY RC, STONE NRH, WIESNER DL, BICANIC T and NIELSEN K (2016) *Cryptococcus*: From environmental saprophyte to global pathogen. *Nat. Rev. Microbiol.* **14** (2) 106-117. https://doi.org/10.1038/nrmicro.2015.6

MCDONALD JH (2014) Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland. https://www.biostathandbook.com/

MCMEEKIN T, BOWMAN J, MCQUESTIN O, MELLEFONT L, ROSS T and TAMPLIN M (2008) The future of predictive microbiology: Strategic research, innovative applications and great expectations. *Int. J. Food Microbiol.* **128** (1) 2-9. https://doi.org/10.1016/j.ijfoodmicro.2008.06.026

MEDEIROS AO, MISSAGIA BS, BRANDÃO LR, CALLISTO M, BARBOSA FAR and ROSA CA (2012) Water quality and diversity of yeasts from tropical lakes and rivers from the Rio Doce basin in Southeastern Brazil. *Braz. J. Microbiol.* **43** (4) 1582-1594. https://doi.org/10.1590/S1517-83822012000400043

MEIS JF and CHOWDHARY A (2020) *Candida auris*—"ten years after." *J. Fungi* **6** (1) 10-11. https://doi.org/10.3390/jof6010002

MERKUR AB and HODGE WG (2002) *Rhodotorula rubra* endophthalmitis in an HIV positive patient. *Brit. J. Ophthalmol.* **86** (12) 1444-1445. http://dx.doi.org/10.1136/bjo.86.12.1444

MICELI MH, DÍAZ JA and LEE SA (2011) Emerging opportunistic yeast infections. *Lancet Infect. Dis.* **11** (2) 142-151. https://doi.org/10.1016/S1473-3099(10)70218-8

MIJITI J, PAN B, DE HOOG S, HORIE Y, MATSUZAWA T, YILIFAN Y, LIU Y, ABLIZ P, PAN W, DENG D, and co-authors (2017) Severe chromoblastomycosis-like cutaneous infection caused by *Chrysosporium keratinophilum*. *Front. Microbiol.* **8** (JAN) 2012-2017. https://doi.org/10.3389/fmicb.2017.00083

MO X, CAI X, HUI Q, SUN H, YU R, BU R, YAN B, OU Q, LI Q, HE S, and co-authors (2021) Whole genome sequencing and metabolomics analyses reveal the biosynthesis of nerol in a multi-stress-tolerant Meyerozyma guilliermondii GXDK6. *Microbial Cell Factories* **20** (1) 1-11. https://doi.org/10.1186/s12934-020-01490-2.

MOAZENI M, ASGARI S and NABILI M (2018) Nosocomial fungal infections: Epidemiology, diagnosis, treatment and prevention. *J. Mazandaran Univ. Med. Sci.* **28** (160) 182-212. http://jmums.mazums.ac.ir/article-1-11237-en.html

MOLINA-LEYVA A, RUIZ-CARRASCOSA JC, LEYVA-GARCIA A and HUSEIN-ELAHMED H (2013) Cutaneous *Cryptococcus laurentii* infection in an immunocompetent child. *Int. J. Infect. Dis.* **17** (12) e1232-e1233. https://doi.org/10.1016/j.ijid.2013.04.017

MONAPATHI M, BEZUIDENHOUT C and RHODE O (2020a) Aquatic yeasts: Diversity, characteristics and potential health implications. *Journal of Water and Health* **18** (2) 91-105. https://doi.org/10.2166/wh.2020.270.

MONAPATHI M, BEZUIDENHOUT C and RHODE O (2020b) Physicochemical parameters and culturable yeast diversity in surface water: A consequence of pollution. *Water SA* **46** (4) 593-601. https://doi.org/10.17159/wsa/2020.v46.i4.9072

MONAPATHI M. E., BEZUIDENHOUT CC and RHODE OHJ (2018) Efflux pumps genes of clinical origin are related to those from fluconazole-resistant Candida albicans isolates from environmental water. *Water Science and Technology* **77** (4) 899-908. https://doi.org/10.2166/wst.2017.607.

MONAPATHI ME, BEZUIDENHOUT CC and RHODE OHJ (2017) Water quality and antifungal susceptibility of opportunistic yeast pathogens from rivers. *Water Sci. Technol.* **75** (6) 1319-1331. https://doi.org/10.2166/wst.2016.580

MONAPATHI ME, OGUEGBULU JC, ADOGO L, KLINK M, OKOLI B, MTUNZI F and MODISE JS (2021) Pharmaceutical Pollution: Azole Antifungal Drugs and Resistance of Opportunistic Pathogenic Yeasts in Wastewater and Environmental Water. *Applied and Environmental Soil Science* **2021** (Figure 1) 1-11. https://doi.org/10.1155/2021/9985398.

MORALES EG, IORIZZO M, LUCCHINI GM and MAINETTI C (2019) *Trichophyton violaceum*: an emerging pathogen in Southern Switzerland. *Dermatology* **235** (5) 434-439. https://doi.org/10.1159/000501271

MORAN GP, COLEMAN DC and SULLIVAN DJ (2012) *Candida albicans* versus *Candida dubliniensis*: why is *C. albicans* more pathogenic? *Int. J. Microbiol.* **2012**. https://doi.org/10.1155/2012/205921

MORRISON J, QUICK MC and FOREMAN MGG (2002) Climate change in the Fraser River Watershed: Flow and temperature climate change in the Fraser River watershed: flow and temperature projections **1694** (June 2002) 230-244. https://doi.org/10.1016/S0022-1694(02)00065-3

MOSCHETTI G, ALFONZO A, FRANCESCA N (2017) Yeasts in Birds. In: Buzzini P, Lachance MA and Yurkov A (eds) *Yeasts in Natural Ecosystems: Diversity*. Springer, Cham. https://doi.org/10.1007/978-3-319-62683-3_14

MUÑOZ P, BOUZA E, CUENCA-ESTRELLA M, EIROS JM, PÉREZ MJ, SÁNCHEZ-SOMOLINOS M, RINCÓN C, HORTAL J and PELÁEZ T (2005) *Saccharomyces cerevisiae* fungemia: An emerging infectious disease. *Clin. Infect. Dis.* **40** (11) 1625-1634. https://doi.org/10.1086/429916

NAGARATHNAMMA T, CHUNCHANUR SK, RUDRAMURTHY SM, VINEETHA KR, RAMAMURTHY K, JOSEPH J and AMBICA R (2017) Outbreak of *Pichia kudriavzevii* fungaemia in a neonatal intensive care unit. *J. Med. Microbiol.* **66** (12) 1759-1764. https://doi.org/10.1099/jmm.0.000645

NARANJO-ORTIZ MA and GABALDÓN T. (2019) Fungal evolution: major ecological adaptations and evolutionary transitions. *Biol. Rev.* **94** (4) 1443-1476. https://doi.org/10.1111/brv.12510

NCCLS (2002) Reference Method for Broth Microdilution Antifungal Susceptibility Testing for Yeasts; Approved Standard – Second Edition. NCCLS document M27-A2. ISBN: 1-56238-469-4.

NEELY WJ, GREENSPAN SE, RIBEIRO LP, CARVALHO T, MARTINS RA, RODRIGUEZ D, ROHR JR, HADDAD CFB, TOLEDO LF and BECKER CG (2020) Synergistic effects of warming and disease linked to high mortality in cool-adapted terrestrial frogs. *Biol. Conserv.* **245** (October 2019) 108521. https://doi.org/10.1016/j.biocon.2020.108521

NEWBOUND M, MCCARTHY MA and LEBEL T (2010) Fungi and the urban environment: A review. *Landscape Urban Plann.* **96** (3) 138-145. https://doi.org/10.1016/j.landurbplan.2010.04.005

NGWENYA F (2006) Water Quality Trends in the Eerste River, Western Cape, 1990-2005. A mini thesis submitted in partial fulfilment of the requirements for the degree of Magister Scientiae, Integrated Water Resources Management in the Faculty of Natural Science, University. *University of the Western Cape* (June) 41. URL: http://hdl.handle.net/11394/2008.

NICHOLS CB, PERFECT ZH and ALSPAUGH JA (2007) A Ras1-Cdc24 signal transduction pathway mediates thermotolerance in the fungal pathogen *Cryptococcus neoformans*. *Mol. Microbiol.* **63** (4) 1118-1130. https://doi.org/10.1111/j.1365-2958.2006.05566.x
NIELSEN K, DE OBALDIA AL and HEITMAN J (2007) *Cryptococcus neoformans* mates on pigeon guano: implications for the realized ecological niche and globalization. *Eukaryotic Cell* **6** (6) 949-959. https://doi.org/10.1128/ec.00097-07
NOBREGA DE ALMEIDA J, CAMPOS S, THOMAZ DY, THOMAZ L, DE ALMEIDA RKG, DEL NEGRO GMB, GIMENES VF, GRENFELL RC, MOTTA AL, ROSSI F and co-authors (2018) *Candida blankii*: An emergent opportunistic yeast with reduced susceptibility to antifungals correspondence. *Emerging Microbes and Infections* **7** (1) 1-4. https://doi.org/10.1038/s41426-017-0015-8
NORDELL B (2003) Thermal pollution causes global warming. *Glob. Planet. Change* **38** (3-4) 305-312. https://doi.org/10.1016/S0921-8181(03)00113-9
NOVAK BABIČ M, ZUPANČIČ J, BRANDÃO J and GUNDE-CIMERMAN N (2018) Opportunistic water-borne human pathogenic filamentous fungi unreported from food. *Microorganisms* **6** (3) 79. https://doi.org/10.3390/microorganisms6030079
NUSSLÉ S, MATTHEWS KR and CARLSON SM (2015) Mediating water temperature increases due to livestock and global change in high elevation meadow streams of the golden trout wilderness. *PLoS ONE* **10** (11) 1-22. https://doi.org/10.1371/journal.pone.0142426
O'GORMAN CM and FULLER HT (2008) Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. *Atmos. Environ.* **42** (18) 4355-4368. https://doi.org/10.1016/j.atmosenv.2008.01.009
OCANSEY BK, PESEWU GA, CODJOE FS, OSEI-DJARBENG S, FEGLO PK and DENNING DW (2019) Estimated burden of serious fungal infections in Ghana. *J. Fungi* **5** (2) 38. https://doi.org/10.3390/jof5020038
OGATA K, MATSUDA K, TSUJI H and NOMOTO K (2015) Sensitive and rapid RT-qPCR quantification of pathogenic *Candida* species in human blood. *Journal of Microbiological Methods* Elsevier B.V., **117** 128-135. https://doi.org/10.1016/j.mimet.2015.07.021.
OLIVEIRA FFM, PAES HC, PECONICK LDF, FONSECA FL, MARINA CLF, BOCCA AL, HOMEM-DE-MELLO M, RODRIGUES ML, ALBUQUERQUE P, NICOLA AM, and co-authors (2020) Erg6 affects membrane composition and virulence of the human fungal pathogen *Cryptococcus neoformans*. *Fungal Genet. Biol.* **140** (March) 103368. https://doi.org/10.1016/j.fgb.2020.103368
ORTIZ-VERA MP, OLCHANHESKI LR, DA SILVA EG, DE LIMA FR, MARTINEZ LR del PR, SATO MIZ, JAFFÉ R, ALVES R, ICHIWAKI S, PADILLA G, and co-authors (2018) Influence of water quality on diversity and composition of fungal communities in a tropical river. *Sci. Rep.* **8** (1) 1-9. https://doi.org/10.1038/s41598-018-33162-y
PAN W, LIAO W, HAGEN F, THEELEN B, SHI W, MEIS JF and BOEKHOUT T (2012) Meningitis caused by *Filobasidium uniguttulatum*: Case report and overview of the literature. *Mycoses* **55** (2) 105-109. https://doi.org/10.1111/j.1439-0507.2011.02054.x
PARAPOULI M, VASILEIADIS A, AFENDRA AS and HATZILOUKAS E (2020) *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* **6** (1) 1-31. https://doi.org/10.3934/microbiol.2020001
PARK JH, OH J, SANG H, SHRESTHA B, LEE H, KOO J, CHO S il, CHOI JS, LEE MH, KIM J, and co-authors (2019) Identification and antifungal susceptibility profiles of *Cyberlindnera fabianii* in Korea. *Mycobiology* **47** (4) 449-456. https://doi.org/10.1080/12298093.2019.1651592
PARTS L, CUBILLOS FA, WARRINGER J, JAIN K, SALINAS F, BUMPSTEAD SJ, MOLIN M, ZIA A, SIMPSON JT, QUAIL MA, and co-authors (2011) Revealing the genetic structure of a trait by sequencing a population under selection. *Genome Res.* **21** (7) 1131-1138. https://doi.org/10.1101/gr.116731.110
PASQUETTI M, MIN ARM, SCACCHETTI S, DOGLIERO A and PEANO A (2017) Infection by *Microsporum canis* in paediatric patients: A veterinary perspective. *Vet. Sci.* **4** (3) 2-7. https://doi.org/10.3390/vetsci4030046
PASTOR FJ and GUARRO J (2008) *Alternaria* infections: laboratory diagnosis and relevant clinical features. *Clin. Microbiol. Infect.* **14** (8) 734-746. https://doi.org/10.1111/j.1469-0691.2008.02024.x
PAULUSSEN C, HALLSWORTH JE, ÁLVAREZ-PÉREZ S, NIERMAN WC, HAMILL PG, BLAIN D, REDIERS H and LIEVENS B (2017) Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microb. Biotechnol.* **10** (2) 296-322. https://doi.org/10.1111/1751-7915.12367

PELEG M (2021) Microbial Dose-Response Curves and Disinfection Efficacy Models Revisited. *Food Engineering Reviews* Food Engineering Reviews, **13** (2) 305-321. https://doi.org/10.1007/s12393-020-09249-6.
PEREIRA T, VILAPRINYO E, BELLI G, HERRERO E, SALVADO B, SORRIBAS A, ALTÉS G and ALVES R (2018) Quantitative operating principles of yeast metabolism during adaptation to heat stress. *Cell Reports* **22** (9) 2421-2430. https://doi.org/10.1016/j.celrep.2018.02.020
PEREIRA VJ, BASÍLIO MC, FERNANDES D, DOMINGUES M, PAIVA JM, BENOLIEL MJ, CRESPO MT and SAN ROMÃO M (2009) Occurrence of filamentous fungi and yeasts in three different drinking water sources. *Water Res.* **43** (15) 3813-3819. https://doi.org/10.1016/j.watres.2009.05.024
PERFECT JR (2006) *Cryptococcus neoformans*: The yeast that likes it hot. *FEMS Yeast Res.* **6** (4) 463-468. https://doi.org/10.1111/j.1567-1364.2006.00051.x
PERLIN DS and WIEDERHOLD NP (2017) Culture-independent molecular methods for detection of antifungal resistance mechanisms and fungal identification. *Journal of Infectious Diseases* **216** (Suppl 3) S458-S465. https://doi.org/10.1093/infdis/jix121.
PETTER R, KANG BS, BOEKHOUT T, DAVIS BJ and KWON-CHUNG KJ (2001) A survey of heterobasidiomycetous yeasts for the presence of the genes homologous to virulence factors of *Filobasidiella neoformans*, CNLAC1 and CAP59. *Microbiology* **147** (8) 2029-2036. https://doi.org/10.1099/00221287-147-8-2029
PFALLER MA, ANDES D, DIEKEMA DJ, ESPINEL-INGROFF A and SHEEHAN D (2010) Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and Candida: Time for harmonization of CLSI and EUCAST broth microdilution methods. *Drug Resistance Updates* Elsevier Ltd, **13** (6) 180-195. https://doi.org/10.1016/j.drup.2010.09.002.
PFALLER MA, DIEKEMA DJ, COLOMBO AL, KIBBLER C, NG KP, GIBBS DL, NEWELL VA, FINQUELIEVICH J, TIRABOSCHI N, ELLIS D and co-authors (2006) *Candida rugosa*, an emerging fungal pathogen with resistance to azoles: Geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program. *J. Clin. Microbiol.* **44** (10) 3578-3582. https://doi.org/10.1128/JCM.00863-06
PFALLER MA, ESPINEL-INGROFF A, CANTON E, CASTANHEIRA M, CUENCA-ESTRELLA M, DIEKEMA DJ, FOTHERGILL A, FULLER J, GHANNOUM M, JONES RN, and co-authors (2012) Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and Candida spp. as determined by CLSI broth microdilution. *Journal of Clinical Microbiology* **50** (6) 2040-2046. https://doi.org/10.1128/JCM.00248-12.
PFÜLLER R, GRÄSER Y, ERHARD M and GROENEWALD M (2011) A novel flucytosine-resistant yeast species, Candida pseudoaaseri, causes disease in a cancer patient. *Journal of Clinical Microbiology* **49** (12) 4195-4202. https://doi.org/10.1128/JCM.05090-11.
PIETRYCZUK A, CUDOWSKI A and HAUSCHILD T (2014) Effect of trophic status in lakes on fungal species diversity and abundance. *Ecotoxicology and Environmental Safety* Elsevier, **109** 32-37. https://doi.org/10.1016/j.ecoenv.2014.07.032.
PIETRYCZUK A, CUDOWSKI A, HAUSCHILD T, ŚWISŁOCKA M, WIĘCKO A and KARPOWICZ M (2018) Abundance and Species Diversity of Fungi in Rivers with Various Contaminations. *Curr. Microbiol.* **75** (5) 630-638. https://doi.org/10.1007/s00284-017-1427-3
PINNA A, CARTA F, ZANETTI S, SANNA S and SECHI LA (2001) Endogenous *Rhodotorula minuta* and *Candida albicans* endophthalmitis in an injecting drug user. *Brit. J. Ophthalmol.* **85** (6) 754-754. http://dx.doi.org/10.1136/bjo.85.6.754-f
POHLE I, HELLIWELL R, AUBE C, GIBBS S, SPENCER M and SPEZIA L (2019) Citizen science evidence from the past century shows that Scottish rivers are warming. *Sci. Total Environ.* **659** 53-65. https://doi.org/10.1016/j.scitotenv.2018.12.325
POSTMA F (2016) Rhizofiltration of urban effluent: Microbial ecology and conceptual treatment mechanisms. Doctoral dissertation, Stellenbosch University. http://scholar.sun.ac.za/handle/10019.1/98274
POTH T, SEIBOLD M, WERCKENTHIN C and HERMANNS W (2010) First report of a *Cryptococcus magnus* infection in a cat. *Med. Mycol.* **48** (7) 1000-1004. https://doi.org/10.3109/13693786.2010.489584
POUNDS JA, BUSTAMANTE MR, COLOMA LA, CONSUEGRA JA, FOGDEN MPL, FOSTER PN, LA MARCA E, MASTERS KL, MERINO-VITERI A, PUSCHENDORF R and co-authors (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439** (7073) 161-167. https://doi.org/10.1038/nature04246

PRINSLOO B, WELDHAGEN GF and BLAINE RW (2003) *Candida famata* central nervous system infection. *S. Afr. Med. J.* **93** (8) 601-602. https://doi.org/10.7196/SAMJ.2288
QADRI SH, AL DAYEL F, STRAMPFER MJ and CUNHA BA (1988) Urinary tract infection caused by *Hansenula anomala*. *Mycopathologia* **104** (2) 99-101. https://doi.org/10.1007/BF00436934\
RADOSAVLJEVIC M, KOENIG H, LETSCHER-BRU V, WALLER J, MALOISEL F, LIOURE B and HERBRECHT R (1999) *Candida catenulata* fungemia in a cancer patient. *J. Clin. Microbiol.* **37** (2) 475-477. https://doi.org/10.1128/jcm.37.2.475-477.1999
RESTREPO A, BAUMGARDNER DJ, BAGAGLI E, JR. CRC, MCGINNIS MR, LÁZERA MS, BARBOSA FH, BOSCO SMG, CAMARGO ZPF, COELHO KIR, and co-authors (2000) Clues to the presence of pathogenic fungi in certain environments. *Med. Mycol.* **38** (s1) 67-77. https://doi.org/10.1080/mmy.38.s1.67.77
REYNEKE B, NDLOVU T, KHAN S and KHAN W (2017) Comparison of EMA-, PMA- and DNase qPCR for the determination of microbial cell viability. *Applied Microbiology and Biotechnology*, *101*(19), 7371-7383. https://doi.org/10.1007/s00253-017-8471-6
RHODES J and FISHER MC (2019) Global epidemiology of emerging *Candida auris*. *Curr. Opin. Microbiol.* **52** 84-89. https://doi.org/10.1016/j.mib.2019.05.008
RICHARDSON SD and KIMURA SY (2017) Emerging environmental contaminants: Challenges facing our next generation and potential engineering solutions. *Environmental Technology and Innovation* Elsevier B.V., **8** 40-56. https://doi.org/10.1016/j.eti.2017.04.002.
RIZZITELLI G, GUANZIROLI E, MOSCHIN A, SANGALLI R and VERALDI S (2016) Onychomycosis caused by *Trichosporon mucoides*. *Int. J. Infect. Dis.* **42** 61-63. https://doi.org/10.1016/j.ijid.2015.11.011
ROBERT V, CARDINALI G and CASADEVALL A (2015) Distribution and impact of yeast thermal tolerance permissive for mammalian infection. *BMC Biol.* **13** (1) 1-14. https://doi.org/10.1186/s12915-015-0127-3
ROBERT VA and CASADEVALL A (2009) Vertebrate endothermy restricts most fungi as potential pathogens. *J. Infect. Dis.* **200** (10) 1623-1626. https://doi.org/10.1086/644642
RODRIGUEZ JY, LE PAPE P, LOPEZ O, ESQUEA K, LABIOSA AL and ALVAREZ-MORENO C (2020) *Candida auris*: A latent threat to critically ill patients with coronavirus disease 2019. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciaa1595
ROHR JR, RAFFEL TR, ROMANSIC JM, MCCALLUM H and HUDSON PJ (2008) Evaluating the links between climate, disease spread, and amphibian declines. *Proc. Natl Acad. Sci. USA* **105** (45) 17436-17441. https://doi.org/10.1073/pnas.0806368105
RUAN SY, CHIEN JY, HOU YC and HSUEH PR (2010) Catheter-related fungemia caused by *Candida intermedia*. *Int. J. Infect. Dis.* **14** (2) e147-e149. https://doi.org/10.1016/j.ijid.2009.03.015
SAMAH AM, SHIMAA RH and RAED SAW (2014) Relative diversity of filamentous fungi and yeasts in groundwater and their correlation to fecal pollution indicators and physicochemical parameters. *International Journal of Current Microbiology and Applied Sciences* **3** (11) 905-919
SANDOVAL-DENIS M, SUTTON DA, FOTHERGILL AW, CANO-LIRA J, GENÉ J, DECOCK CA, DE HOOG GS and GUARRO J (2013) *Scopulariopsis*, a poorly known opportunistic fungus: Spectrum of species in clinical samples and in vitro responses to antifungal drugs. *J. Clin. Microbiol.* **51** (12) 3937-3943. https://doi.org/10.1128/JCM.01927-13
SANS (2015) South African National Standard 241-1: drinking water. Part 1: Microbiological, physical, aesthetic and chemical determinants. 241-2:2015 Drinking water, Part 2: Application of SANS 241-1. Pretoria: Standards South Africa.
SANZ AB, GARCÍA R, RODRÍGUEZ-PEÑA JM and ARROYO J (2018) The CWI pathway: Regulation of the transcriptional adaptive response to cell wall stress in yeast. *J. Fungi* **4** (1) 1-12. https://doi.org/10.3390/jof4010001
SATOH K, MAKIMURA K, HASUMI Y, NISHIYAMA Y, UCHIDA K and YAMAGUCHI H (2009) *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol. Immunol.* **53** (1) 41-44. https://doi.org/10.1111/j.1348-0421.2008.00083.x
SAVINI V, CATAVITELLO C, ONOFRILLO D, MASCIARELLI G, ASTOLFI D, BALBINOT A, FEBBO F, D'AMARIO C and D'ANTONIO D (2011) What do we know about Candida guilliermondii? A voyage throughout past and current literature about this emerging yeast. *Mycoses* **54** (5) 434-441. https://doi.org/10.1111/j.1439-0507.2010.01960.x.

SAXENA G, BHARAGAVA RN, KAITHWAS G and RAJ A (2015) Microbial indicators, pathogens and methods for their monitoring in water environment. *Journal of Water and Health* **13** (2) 319-339. https://doi.org/10.2166/wh.2014.275.
SCHIAVANO GF, PARLANI L, SISTI M, SEBASTIANELLI G and BRANDI G (2014) Occurrence of fungi in dialysis water and dialysate from eight haemodialysis units in central Italy. *J. Hosp. Infect.* **86** (3) 194-200. https://doi.org/10.1016/j.jhin.2013.11.010
SCHLOSS PD, WESTCOTT SL, RYABIN T, HALL JR, HARTMANN M, HOLLISTER EB, LESNIEWSKI RA, OAKLEY BB, PARKS DH, ROBINSON CJ and SAHL JW (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and environmental microbiology*, **75** (23) 7537-7541. https://doi.org/10.1128/AEM.01541-09
SCHWARTZ I and DENNING D (2019) The estimated burden of fungal diseases in South Africa. *J. Infect. Public Health* **12** (1) 124. https://doi.org/10.1016/j.jiph.2018.10.064
SCHWARZENBACH RP, EGLI T, HOFSTETTER TB, VON GUNTEN U and WEHRLI B (2010) Global Water Pollution and Human Health. *Annual Review of Environment and Resources* **35** (1) 109-136. https://doi.org/10.1146/annurev-environ-100809-125342.
SCHWINN A, EBERT J and BRÖCKER EB (1995) Frequency of *Trichophyton rubrum* in tinea capitis. *Mycoses* **38** (1-2) 1-7. https://doi.org/10.1111/j.1439-0507.1995.tb00001.x
SCOPPETTUOLO G, DONATO C, DE CAROLIS E, VELLA A, VACCARO L, LA GRECA A and FANTONI M (2014) *Candida utilis* catheter-related bloodstream infection. *Med. Mycol. Case Rep.* **6** 70-72. https://doi.org/10.1016/j.mmcr.2014.10.003
SCORZONI L, DE LUCAS MP, MESA-ARANGO AC, FUSCO-ALMEIDA AM, LOZANO E, CUENCA-ESTRELLA M, MENDES-GIANNINI MJ and ZARAGOZA O (2013) Antifungal efficacy during *Candida krusei* infection in non-conventional models correlates with the yeast in vitro susceptibility profile. *PLoS ONE* **8** (3). https://doi.org/10.1371/journal.pone.0060047
SETH-SMITH HMB, BÜCHLER AC, HINIC V, MEDINGER M, WIDMER AF and EGLI A (2020) Bloodstream infection with *Candida kefyr*/*Kluyveromyces marxianus*: case report and draft genome. *Clin. Microbiol. Infect.* **26** (4) 522-524. https://doi.org/10.1016/j.cmi.2019.11.014
SFAKIANAKIS A, KRASAGAKIS K, STEFANIDOU M, MARAKI S, KOUTSOPOULOS A, KOFTERIDIS D, SAMONIS G and TOSCA A (2007) Invasive cutaneous infection with *Geotrichum candidum* – Sequential treatment with amphotericin B and voriconazole. *Med. Mycol.* **45** (1) 81-84. https://doi.org/10.1080/13693780600939948
SHARMA V, SHANKAR J and KOTAMARTHI V (2006) Endogeneous endophthalmitis caused by *Sporobolomyces salmonicolor*. *Eye* **20** (8) 945-946. https://doi.org/10.1038/sj.eye.6702051
SHINDE R, MANTUR B, PATIL G, PARANDE M and PARANDE A (2008) Meningitis due *to Rhodotorula glutinis* in an HIV infected patient. *Indian J. Med. Microbiol.* **26** (4) 375-377. https://doi.org/10.4103/0255-0857.43579
SHIRVANI F, YASSIN Z, ERAMI M, LOTFALI E, GHASEMI R and FATTAHI A (2020) Comparison of High-resolution Melting Curve Analysis with Specific Target Gene Sequencing to Identify the Most Common Species of *Aspergillus* and *Fusarium*. *Jundishapur Journal of Microbiology (JJM),[online]*, **13** (12) https://dx.doi.org/10.5812/jjm.110205
SHOHAM S and LEVITZ SM (2005) The immune response to fungal infections. *Brit. J. Haematol.* **129** (5) 569-582. https://doi.org/10.1111/j.1365-2141.2005.05397.x
SIGEE D (2005) *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment.* John Wiley & Sons, Hoboken. https://doi.org/10.5860/choice.43-0305
SILVA S, NEGRI M, HENRIQUES M, OLIVEIRA R, WILLIAMS DW and AZEREDO J (2012) *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: Biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol. Rev.* **36** (2) 288-305. https://doi.org/10.1111/j.1574-6976.2011.00278.x
SKIADA A, PAVLEAS I and DROGARI-APIRANTHITOU M (2017) Rare fungal infectious agents: a lurking enemy. *F1000Research* **6** 1917. https://doi.org/10.12688/f1000research.11124.1
SLÁVIKOVÁ E and VADKERTIOVÁ R (1997) Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. *Anton. Leeuw. Int. J. Gen. Mol. Microbiol.* **72** (2) 77-80. https://doi.org/10.1023/A:1000287005253
SO YS, JANG J, PARK G, XU J, OLSZEWSKI MA and BAHN YS (2018) Sho1 and msb2 play complementary but distinct roles in stress responses, sexual differentiation, and pathogenicity of *Cryptococcus neoformans*. *Front. Endocrinol.* **9** (December) 1-16. https://doi.org/10.3389/fmicb.2018.02958

SOBER E (1981) The principle of parsimony. *Brit. J. Philos. Sci.* **32** (2) 145-156. https://doi.org/10.1093/bjps/32.2.145

SOMBATMAITHAI A, PATTANAPRICHAKUL P, TUCHINDA P, SURAWAN T, MUANPRASART C, MATTHAPAN L and BUNYARATAVEJ S (2015) Tinea capitis caused by *Trichophyton tonsurans* presenting as an obscure patchy hair loss due to daily antifungal shampoo use. *Dermatol. Practical Concept.* **5** (2) 133-135. https://doi.org/10.5826/dpc.0502a27

SOUSA MJ, ARDÖ Y and MCSWEENEY PLH (2001) Advances in the study of proteolysis during cheese ripening. *Int. Dairy J.* **11** (4-7) 327-345. https://doi.org/10.1016/S0958-6946(01)00062-0

SPELLBERG B AND MAERTENS J (2019) Mucormycosis. In: *Principles and Practice of Transplant Infectious Diseases.* 577-589. Springer, New York, NY. https://doi.org/10.1007/978-1-4939-9034-4_34

STEENBERGEN JN, SHUMAN HA and CASADEVALL A (2001) *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc. Natl Acad. Sci.* **98** (26) 15245-15250. https://doi.org/10.1073/pnas.261418798

STEFFEN HC, BOSCH C, SMITH K, WOLFAARDT GM and BOTHA A (2022). Rising Environmental Temperatures and Polluted Surface Waters: The Prelude to the Rise of Mycoses in South Africa. *Water SA* **48** (2) 199-216 / Apr 2022 https://doi.org/10.17159/wsa/2022.v48.i2.3918

STONE W, JONES B-L, WILSENACH J and BOTHA A (2012) External Ecological Niche for *Candida albicans* within Reducing, Oxygen-Limited Zones of Wetlands. *Appl. Environ. Microbiol.* **78** (7) 2443-2445. https://doi.org/10.1128/aem.06343-11

SUGITA T, KAGAYA K, TAKASHIMA M, SUZUKI M, FUKAZAWA Y and NAKASE T (1999) A clinical isolate of *Candida palmioleophila* formerly identified as *Torulopsis candida*. *Jap. J. Med. Mycol.* **40** (1) 21-25. https://doi.org/10.3314/jjmm.40.21

SULEYMAN G and ALANGADEN GJ (2016) Nosocomial fungal infections: epidemiology, infection control, and prevention. *Infect. Dis. Clinics N. Am.* **30** (4) 1023-1052. https://doi.org/10.1016/j.idc.2016.07.008

TAJ-ALDEEN SJ, ABDULWAHAB A, KOLECKA A, DESHMUKH A, MEIS JF and BOEKHOUT T (2014) Uncommon opportunistic yeast bloodstream infections from Qatar. *Med. Mycol.* **52** (5) 549-553. https://doi.org/10.1093/mmycol/myu016

TAMURA K, STECHER G and KUMAR S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **38** (7) 3022-3027. https://doi.org/10.1093/molbev/msab120.

TANG HJ, LAI CC and CHAO CM (2015) Central-line-associated bloodstream infection caused by *Sporobolomyces salmonicolor*. *Infect. Control Hospital Epidemiol.* **36** (9) 1111-1112. https://doi.org/10.1017/ice.2015.158

TANSEY MR and BROCK TD (1972) The upper temperature limit for eukaryotic organisms. *Proc. Natl Acad. Sci.* **69** (9) 2426-2428. https://doi.org/10.1073/pnas.69.9.2426

TERESHINA VM (2005) Thermotolerance in fungi: the role of heat shock proteins and trehalose. *Microbiology* **74** (3) 247-257. https://doi.org/10.1007/s11021-005-0059-y

THOMPSON GR, BOULWARE DR, BAHR NC, CLANCY CJ, HARRISON TS, KAUFFMAN CA, LE T, MICELI MH, MYLONAKIS E, NGUYEN MH, and co-authors (2022) Noninvasive Testing and Surrogate Markers in Invasive Fungal Diseases. *Open Forum Infectious Diseases* **9** (6) 1-16. https://doi.org/10.1093/ofid/ofac112.

TOPLIS B, BOSCH C, SCHWARTZ IS, KENYON C, BOEKHOUT T, PERFECT JR and BOTHA A (2020) The virulence factor urease and its unexplored role in the metabolism of *Cryptococcus neoformans*. *FEMS Yeast Res.* **20** (4) p.foaa031. https://doi.org/10.1093/femsyr/foaa031

TREGUIER P, DAVID M, GARGALA G, CAMUS V, STAMATOULLAS A, MENARD A-L, LENAIN P, CONTENTIN N, LEMASLE E, LANIC H, and co-authors (2018) *Cyberlindnera jadinii* (teleomorph *Candida utilis*) candidaemia in a patient with aplastic anaemia: a case report. *JMM Case Reports* **5** (8) 1-3. https://doi.org/10.1099/jmmcr.0.005160

TROWBRIDGE J, LUDMER LM, RIDDLE VD, LEVY CS and BARTH WF (1999) *Candida lambica* polyarthritis in a patient with chronic alcoholism. *J. Rheumatol.* **26** (8) 1846-1848. https://pubmed.ncbi.nlm.nih.gov/10451090/

TU Q, HE Z and ZHOU J (2014) Strain/species identification in metagenomes using genome-specific markers. *Nucleic Acids Research* **42** (8) 1-12. https://doi.org/10.1093/nar/gku138.

TUBATSI G, BONYONGO MC and GONDWE M (2015) Water use practices, water quality, and households' diarrheal encounters in communities along the Boro-Thamalakane-Boteti river system, Northern Botswana. *Journal of Health, Population and Nutrition* Journal of Health, Population and Nutrition, 1-13. https://doi.org/10.1186/s41043-015-0031-z.

UN WATER (2006) UN World Water Development Report. URL: https://www.unwater.org/publications/water-shared-responsibility (Accessed 11 March 2021).
UNAIDS (2021) Global HIV & AIDS statistics – Fact sheet. URL: https://www.unaids.org/en/resources/fact-sheet (Accessed 25 January 2022).
USAID (2015) Climate Change Information Fact Sheet SOUTHERN AFRICA. URL: https://www.usaid.go south-africa/environment (Accessed 18 March 2021).
VAN VLIET MTH, LUDWIG F, ZWOLSMAN JJG, WEEDON GP and KABAT P (2011) Global river temperatures and sensitivity to atmospheric warming and changes in river flow. *Water Resour. Res.* **47** (2). https://doi.org/10.1029/2010WR009198
VAN WYK DAB, BEZUIDENHOUT CC and RHODE OHJ (2012) Diversity and characteristics of yeasts from water sources in the North West Province, South Africa. *Water Sci. Technol. Water Suppl.* **12** (4) 422-430. https://doi.org/10.2166/ws.2012.009
VEAREY J (2011) Challenging urban health: towards an improved local government response to migration, informal settlements, and HIV in Johannesburg, South Africa. *Glob. Health Action* **4**. https://doi.org/10.3402/gha.v4i0.5898
VERGHESE J, ABRAMS J, WANG Y and MORANO KA (2012) Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiol. Mol. Biol. Rev.* **76** (2) 115-158. https://doi.org/10.1128/mmbr.05018-11
VERGIDIS P, CLANCY CJ, SHIELDS RK, PARK SY, WILDFEUER BN, SIMMONS RL and NGUYEN MH (2016) Intra-abdominal candidiasis: The importance of early source control and antifungal treatment. *PLoS ONE* **11** (4) 1-13. https://doi.org/10.1371/journal.pone.0153247
VERNA J, LODDER A, LEE K, VAGTS A and BALLESTER R (1997) A family of genes required for maintenance of cell wall integrity and for the stress response in *Saccharomyces cerevisiae. Proc. Natl Acad. Sci.* **94** (25) 13804-13809. https://doi.org/10.1073/pnas.94.25.13804
VERONES F, HANAFIAH MM, PFISTER S, HUIJBREGTS MAJ, PELLETIER GJ and KOEHLER A (2010) Characterization factors for thermal pollution in freshwater aquatic environments. *Environ. Sci. Technol.* **44** (24) 9364-9369. https://doi.org/10.1021/es102260c
VERVAEKE S, VANDAMME K, BOONE E, DE LAERE E, SWINNE D and SURMONT I (2008) A case of *Candida lambica* fungemia misidentified as Candida krusei in an intravenous drug abuser. *Sabouraudia* **46** (8) 853-856. https://doi.org/10.1080/13693780802342552
VILLANUEVA-LOZANO H, TREVIÑO-RANGEL RDJ, GONZÁLEZ GM, RAMÍREZ-ELIZONDO MT, LARA-MEDRANO R, ALEMAN-BOCANEGRA MC, GUAJARDO-LARA CE, GAONA-CHÁVEZ N, CASTILLEJA-LEAL F, TORRE-AMIONE G and MARTÍNEZ-RESÉNDEZ MF (2021) Outbreak of *Candida auris* infection in a COVID-19 hospital in Mexico. *Clin. Microbiol. Infect.* **27** (5) 813-816. https://doi.org/10.1016/j.cmi.2020.12.030
WAGNER D, SANDER A, BERTZ H, FINKE J and KERN W v. (2005) Breakthrough invasive infection due to *Debaryomyces hansenii* (teleomorph *Candida famata*) and *Scopulariopsis brevicaulis* in a stem cell transplant patient receiving liposomal amphotericin B and caspofungin for suspected aspergillosis. *Infection* **33** (5-6) 397-400. https://doi.org/10.1007/s15010-005-5082-4
WAKE DB and VREDENBURG VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Natl Acad. Sci.* **105** (Supplement 1) 11466-11473. https://doi.org/10.1073/pnas.0801921105
WALSH TJ and DIXON DM (1989) Nosocomial aspergillosis: environmental microbiology, hospital epidemiology, diagnosis and treatment. *Eur. J. Epidemiol.* **5** (2) 131-142. https://doi.org/10.1007/bf00156818
WANG J, LIU XD and LU J (2012) Urban river pollution control and remediation. *Procedia Environ. Sci.* **13** 1856-1862. https://doi.org/10.1016/j.proenv.2012.01.179
WANG X, GLAWE DA. WELLER DM and OKUBARA PA (2020) Real-time PCR assays for the quantification of native yeast DNA in grape berry and fermentation extracts. *Journal of Microbiological Methods*, *168*, p.105794. http://dx.doi.org/10.1016/j.mimet.2019.105794
WARREN TA, MCTAGGART L, RICHARDSON SE and ZHANG SX (2010) *Candida bracarensis* bloodstream infection in an immunocompromised patient. *J. Clin. Microbiol.* **48** (12) 4677-4679. https://doi.org/10.1128/JCM.01447-10
WARTENBERG A, LINDE J, MARTIN R, SCHREINER M, HORN F, JACOBSEN ID, JENULL S, WOLF T, KUCHLER K, GUTHKE R and KURZAI O (2014) Microevolution of *Candida albicans* in macrophages restores filamentation in a nonfilamentous mutant. *PLoS Genet.* **10** (12) 1004824. https://doi.org/10.1371/journal.pgen.1004824

WASO M, NDLOVU T, DOBROWSKY PH, KHAN S and KHAN W (2016) Presence of microbial and chemical source tracking markers in roof-harvested rainwater and catchment systems for the detection of fecal contamination. *Environmental Science and Pollution Research*, **23** (17) 16987-17001. https://doi.org/10.1007/s11356-016-6895-7

WATKINS R, KING J and JOHNSTON S (2017) Nutritional requirements and their importance for virulence of pathogenic *Cryptococcus* species. *Microorganisms* **5** (4) 65. https://doi.org/10.3390/microorganisms5040065

WEATHERBURN MW (1967) Phenol-Hypochlorite Reaction for Determination of Ammonia. *Analytical Chemistry* **39** (8) 971-974. https://doi.org/10.1021/ac60252a045.

WEIMANN A and ONI T (2019) A systematised review of the health impact of urban informal settlements and implications for upgrading interventions in South Africa, a rapidly urbanising middle-income country. *International Journal of Environmental Research and Public Health* **16** (19) 1-17. https://doi.org/10.3390/ijerph16193608

WEISKERGER CJ and BRANDÃO J (2020) Fungal contaminants in water and sand: A new frontier for quantitative microbial risk assessment. *Current Opinion in Environmental Science and Health* Elsevier Ltd, **16** 73-81. https://doi.org/10.1016/j.coesh.2020.03.001.

WELSH O, VERA-CABRERA L and SALINAS-CARMONA MC (2007) Mycetoma. *Clinics in Dermatology* **25** (2) 195-202. https://doi.org/10.1016/j.clindermatol.2006.05.011

WEN Y, SCHOUPS G and GIESEN N van de (2017) Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. *Nature Publishing Group* (February) 1-9. https://doi.org/10.1038/srep43289

WERTHMAN-EHRENREICH A (2021) Mucormycosis with orbital compartment syndrome in a patient with COVID-19. *Am. J. Emerg. Med.* **42** 264-e5. https://doi.org/10.1016/j.ajem.2020.09.032

WHITBY S, MADU EC and BRONZE MS (1996) *Candida zeylanoides* infective endocarditis complicating infection with the human immunodeficiency virus. *Am. J. Med. Sci.* **312** (3) 138-139. https://doi.org/10.1016/S0002-9629(15)41781-1

WHO (2017) Water sanitation and hygiene (WASH). URL: https://www.who.int/health-topics/water-sanitation-and-hygiene-wash (Accessed 21 April 2021).

WINGARD JR, DICK JD, MERZ WG, SANDFORD GR, SARAL R and BURNS WH (1982) Differences in virulence of clinical isolates of Candida tropicalis and Candida albicans in mice. *Infection and Immunity* **37** (2) 833-836. https://doi.org/10.1128/iai.37.2.833-836.1982.

WIRTH F and GOLDANI LZ (2012) Epidemiology of *Rhodotorula*: An emerging pathogen. *Interdisc. Perspect. Infect. Dis.* **2012**. https://doi.org/10.1155/2012/465717

WONG B, KIEHN TE, EDWARDS F, BERNARD EM, MARCOVE RC, HARVEN EDE and ARMSTRONG D (1982) Bone infection caused by *Debaryomyces hansenii* in normal host: a case report. *Notes* **16** (3) 545-548. https://doi.org/10.1128/jcm.16.3.545-548.1982

YAMAGUCHI MU, RAMPAZZO RDCP, YAMADA-OGATTA SF, NAKAMURA CV, UEDA-NAKAMURA T and DIAS FILHO BP (2007) Yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies. *Braz. Arch. Biol. Technol.* **50** (1) 1-9. https://doi.org/10.1590/S1516-89132007000100001

YANG DH, JUNG KW, BANG S, LEE JW, SONG MH, FLOYD-AVERETTE A, FESTA RA, IANIRI G, IDNURM A, THIELE DJ, and co-authors (2017) Rewiring of signaling networks modulating thermotolerance in the human pathogen *Cryptococcus neoformans*. *Genetics* **205** (1) 201-219. https://doi.org/10.1534/genetics.116.190595

YILMAZ-SEMERCI S, DEMIREL G and TAŞTEKIN A (2017) *Wickerhamomyces anomalus* blood stream infection in a term newborn with pneumonia. *Turk. J. Pediatr.* **59** (3) 349-351. https://doi.org/10.24953/turkjped.2017.03.021

ZEGLIN LH (2015) Stream microbial diversity in response to environmental changes: Review and synthesis of existing research. *Frontiers in Microbiology* **6** (MAY) 1-15. https://doi.org/10.3389/fmicb.2015.00454.

ZHAI B, OLA M, ROLLING T, TOSINI NL, JOSHOWITZ S, LITTMANN ER, AMORETTI LA, FONTANA E, WRIGHT RJ, MIRANDA E, and co-authors (2020) High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. *Nature Medicine* Springer US, **26** (January). https://doi.org/10.1038/s41591-019-0709-7.

ZHOU J, CHEN M, CHEN H, PAN W and LIAO W (2014) *Rhodotorula minuta* as onychomycosis agent in a Chinese patient: First report and literature review. *Mycoses* **57** (3) 191-195. https://doi.org/10.1111/myc.12143

ZHOU J, HE Z, YANG Y, DENG Y, TRINGE SG and ALVAREZ-COHEN L (2015) High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. *MBio*, **6** (1) e02288-14. https://doi.org/10.1128/mbio.02288-14

ZU T, VERNA J and BALLESTER R (2001) Mutations in WSC genes for putative stress receptors result in sensitivity to multiple stress conditions and impairment of Rlm1-dependent gene expression in Saccharomyces cerevisiae. *Mol. Genet. Genomics* **266** (1) 142-155. https://doi.org/10.1007/s004380100537

ZUZA-ALVES DL, SILVA-ROCHA WP and CHAVES GM (2017) An update on *Candida tropicalis* based on basic and clinical approaches. *Front. Microbiol.* **8** (OCT) 1-25. https://doi.org/10.3389/fmicb.2017.01927