Assessing the abundance of *Cryptosporidium* and *Giardia* spp. in borehole water close to eMbalenhle wastewater treatment plant and associated human health impacts.

by

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Submitted in accordance with the requirements

For the degree of

MASTER OF SCIENCE

DEPARTMENT OF ENVIRONMENTAL SCIENCE

At the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: Dr. T.N Mhlongo

(MARCH 2023)

DECLARATION

I Busisiwe Fezile Makhombothi declare that the dissertation: Assessing the abundance of *Cryptosporidium* and *Giardia* spp. in borehole water close to eMbalenhle wastewater treatment plant and associated impacts, which I am submitting for the degree of Masters in Environmental Science at the University of South Africa, is my work and that all the quoted work and sources have been acknowledged using references.

I also declare that I have submitted the dissertation to originality checking software and that it is within the accepted requirements for originality.

I further declare that this work has not been previously submitted elsewhere, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethical approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

23 March 2023

Signature **Date**

(BF Makhombothi)

ACKNOWLEDGMENTS

I am grateful to God for making it possible for me to complete my research.

My sincere gratitude goes to my supervisor Dr. T.N Mhlongo for the support, guidance, and encouragement in this journey. I mostly appreciate the faith you had in my abilities even when I was in doubt, there are few supervisors like you out there.

To UNISA (University of South Africa), thank you for granting me bursary assistance for the past two years.

Many thanks to Macsteel for assisting with a grant for laboratory analysis.

To Aquadoc Laboratory, thank you for your assistance with the laboratory analysis.

Govan Mbeki Municipality, Water and Sanitation department, thank you for allowing me to conduct sampling at eMbalenhle Wastewater Treatment Works.

Final thanks go to my family, my fiancé, and my daughter for their love, understanding, and belief in me.

DEDICATION

This dissertation is dedicated to my daughter Buliswa, my mother Zandile Makhombothi, and my fiancé Sibusiso Rasmeni.

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LIST OF ABBREVIATIONS

DWA	Department of Water Affairs
DWS	Department of Water and Sanitation
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
EPA	Environmental Protection Agency
GMM	Govan Mbeki Municipality
ISO 17025:2017	General requirements for the competence of testing and calibration laboratories
MP COGTA	Mpumalanga Cooperative Governance and Traditional Affairs
SABS	South African Bureau of Standards
SANS	South African National Standards
TSS	Total Suspended Solids
TDS	Total Dissolved Solids
US EPA	United States Environmental Protection Agency

WHO	World Health Organization
WWTW	Wastewater Treatment Works
WWTP	Wastewater Treatment Plants

RESEARCH MANUSCRIPT TO BE PUBLISHED IN 2023

The MSc dissertation will yield 2 publications of which the first one is in the process of finalization and submission to a journal. The title and authors are added below.

1. Makhombothi, B., Mhlongo T.N. and Zikalala, S. (proposed publication year is 2023). Prevalence of *Cryptosporidium* and *Giardia* spp. in groundwater at eMbalenhle wastewater treatment plant, Mpumalanga Province, South Africa.

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ABSTRACT

Groundwater quality is often an overlooked aspect and due to this oversight, groundwater remains vulnerable to contamination by such sources as wastewater treatment plants (WWTPs). Even though most WWTPs in South Africa are monitoring the contamination of groundwater for physicochemical and some microbiological parameters, contamination by *Cryptosporidium* and *Giardia* spp. are often disregarded. Using groundwater contaminated with enteric pathogens like *Cryptosporidium* and *Giardia* oo(cyst) for consumption, irrigation, and recreational activities lead to gastrointestinal illness in humans and animals. This research aimed to assess the abundance of *Cryptosporidium* and *Giardia* spp. in borehole water close to the eMbalenhle wastewater treatment plant and the associated impacts. Groundwater samples were collected from three boreholes located inside the eMbalenhle wastewater treatment plant in the period autumn to the summer season. The samples were collected and analyzed for the physicochemical and microbiological indicator parameters including the presence of *Cryptosporidium* and *Giardia* oo(cyst). The selected parameters were evaluated and compared with the South African National Standard for Drinking Water SANS241:2015, South African Water Guidelines (Irrigation, Recreational Activities, and Aquatic Ecosystem), and guidelines for drinking water as stipulated by the World Health Organization (WHO). The correlation between the physicochemical and microbiological parameters with the abundance of the oo(cyst) was determined using Microsoft Excel correlation analysis.

Some physicochemical parameters of boreholes exceeded the recommended limits except the range of such parameters as total suspended solids (TSS) (21-157 mg/L), nitrates (17-20 mg/L), phosphate (0.2 mg/L), and ammonia (0.1- 0.2 mg/L), which were below the SANS241:2015 specification of 1.5 mg/L. The results for (pH, conductivity, and chloride) showed a degree of conformance with all the standards. The *E. coli* and coliforms were present in all the boreholes throughout the sampling seasons, with *E. coli* in the range 7-900 cfu/100 mL and fecal coliform in the range 9 – 1100 cfu/100 mL. The highest counts were recorded in autumn. *Cryptosporidium* and *Giardia* (oo)cysts were detected by immunofluorescence and immunomagnetic separation. An analysis of groundwater samples from all the boreholes for *Cryptosporidium* detected oo(cysts) in autumn in one borehole. An

analysis for *Giardia* on all samples from the two seasons (autumn and summer) showed the presence of a *Giardia* cyst. In autumn, all boreholes tested positive for *Giardia* cysts and the highest count for the cysts were recorded in the same period. In summer cysts were detected in only one borehole. The findings of the study can be used by the Govan Mbeki Municipality to improve its existing groundwater quality and monitoring strategy. The microbial parameters exceeded acceptable limits and proved the borehole water to be unsuitable for human consumption, irrigation, recreational activities, and aquatic ecosystem.

Keywords: *Cryptosporidium* and *Giardia* oo(cyst), *Escherichia coli*, fecal coliform, enteric pathogens, groundwater, wastewater treatment.

CHAPTER 1.

INTRODUCTION

1.1. Background

Freshwater resources are increasingly becoming limited as water has become a scarce commodity (Gleick & Cooley, 2021; Dungeni & Momba, 2010). Groundwater is an alternative to potable water to balance the demand for available freshwater (Boretti & Rosa, 2019). Knowing the water quality of any source of water to be used for human consumption is key. Poor water quality has resulted in a 6.3% percentage of waterborne illnesses globally (Manetu & Karanja, 2021; Galal-Gorchev, 1993). Groundwater contamination occurs through the soil surface and seepages from waste disposal facilities, industries, wastewater treatment works (WWTWs), and cemeteries disseminating microbial contamination into groundwater (Li et al., 2021; Tredoux et al., 2004). Water leaching into the soil becomes a suitable transport medium for a host of micro-organisms including *Cryptosporidium* and *Giardia* spp. Leading to these organisms ending up in groundwater aquifers (Daniels et al., 2018a).

Determining groundwater quality is crucial-especially if the water is utilized for human consumption or irrigation of agricultural fields. Outbreaks of foodborne illness linked to the consumption of fruits and vegetables have often implicated viruses, parasites, but most importantly micro-organisms such as *Escherichia coli, Salmonella, Campylobacter jejuni, and Vibrio cholera* (Bintsis, 2017 ; Davis & Kendall, 2012). The origin of these organisms and viruses has been shown to be irrigation water. Due to the ability of vegetable roots and leaves to absorb and adsorb microorganisms from contaminated water used for irrigation, pathogenic organisms may accumulate on the plants leading to these organisms infecting humans when these vegetables are consumed without thorough cooking (Alegbeleye & Sant'Ana, 2022; Davis & Kendall, 2012; Pachepsky et al., 2011). *Cryptosporidium* and *Giardia* spp. have also shown the capability of penetrating the soil through irrigation by contaminated water leading to these organisms ending up in fruits and vegetables that are consumed raw (Javanmard et al., 2020; Pachepsky et al., 2011).

Cryptosporidium transmission route is, directly and indirectly, manifested in humans and animals. The direct route is the fecal-oral route and the indirect route is through contact with *Cryptosporidium* oocysts in the environments and contaminated hosts such as water and food (Robertson et al., 2020; Thomson et al., 2017; McLauchlin et al., 2000; Toze, 1997). *Giardia* is primarily transmitted through the fecal-oral route as a 10-12 µm long *Giardia* cyst due to poor hygiene infrastructure and occasionally through oral-anal contact during a sexual encounter (Daniels et al., 2018; Lyimo et al., 2014). Literature has shown that waterborne infectious diseases such as hemolytic uremic syndrome, cryptosporidiosis, giardiasis, hepatitis A, salmonella, and cholera are affecting communities, especially rural communities without water and sanitation services (Magana-Arachchi & Wanigatunge, 2020; Delahoy et al., 2018; Ezeh et al., 2014; Lyimo et al., 2014; Andersson & Bohan, 2001). Groundwater has, for a long time, been considered safe. However, with time it has become evident that it is as much susceptible to contamination as surface water (Al-Hashimi, 2021; Bartram & Pedley, 1996).

The World Health Organization (WHO) has issued guidelines and standards for groundwater quality intended for human consumption, these include the limits for the concentrations of *Cryptosporidium and Giardia* spp. for risk assessment and water-borne outbreak investigations (WHO, 2017; WHO, 2007; Galal-Gorchev, 1993; Sayato, 1989). In South Africa, the National Norms and Standards provide details on the frequency of monitoring groundwater, especially where intended for human consumption (Department of Water and Sanitation, 2017). However, for *Cryptosporidium and Giardia* spp. the monitoring standards are only for treated water from the water supply systems South African National Standard (SANS) 241:2015, and South African guideline for domestic, nothing for groundwater abstractions for human consumption and irrigation (Sigudu et al., 2014; DWAF, 1996c). This has led to gaps in groundwater monitoring of *Cryptosporidium and Giardia* spp. the health risk assessment, and the impacts associated with these protozoans.

Though the amount of groundwater contributing to freshwater reserves may be 0.61% compared to 80% from wastewater return flows and the present 2.5% surface water globally, there is a need to understand the presence of *Cryptosporidium* and *Giardia* spp. in groundwater as a contaminant (UNEP et al., 2008). With the water shortages that are being

experienced in areas like Cape Town and Port Elizabeth, to mention a few, groundwater is now considered the future source of fresh water in South Africa. This study will, therefore, determine the presence of *Cryptosporidium* and *Giardia* spp. contamination at eMbalenhle WWTW boreholes, and its significant health impacts.

1.2. Problem statement

Cryptosporidium and *Giardia* spp. have mainly been associated with waterborne outbreaks. It is estimated that *Cryptosporidium* is second only to rotavirus in the cause of moderate-to-severe gastrointestinal diseases during the early years (0-24 months) of human life (Dhal et al., 2022 ; Putignani & Menichella, 2010). The *Cryptosporidium* and *Giardia* spp*.* parasites cause cryptosporidiosis and giardiasis infections resulting in gastrointestinal illness which can be life-threatening without medical treatment (Wang et al., 2023; Squire & Ryan, 2017).

WHO indicated that more than 58 million children are affected by diarrhea globally (Fradette et al., 2022; Boschi-Pinto et al., 2008). According to zoonotic studies of *Cryptosporidium*, malnutrition and the prevalence of the Human Immuno-Deficiency Virus (HIV) exacerbate cryptosporidiosis and giardiasis infections in African countries (Robertson et al., 2020).In South Africa 18. 7% of the population is HIV positive, therefore, creating a suitable environment for the spread and prevalence of opportunistic pathogens such as *Cryptosporidium* and *Giardia* spp. (Stats SA, 2020). The other population group at risk of these pathogens are individuals with compromised immunity, transplant recipients, and chemotherapy patients (Deksne, 2022; Duhain, 2012; Grundlingh & De Wet, 2004).

In India, 50% of rural households are dependent on untreated groundwater as their primary source of drinking and cooking, *Cryptosporidium* and *Giardia* spp. has been identified as a major course of child diarrhea morbidity in the area, even at low concentrations (Daniels et al., 2018). In London, North Thames, cryptosporidiosis epidemiology has transpired even though the quality of groundwater complied with the water treatment specifications, the outbreak was due to drinking water from the borehole (Willocks et al., 1998). Even though *Cryptosporidium* and *Giardia* spp*.* have been identified in groundwater studies, little attention has been paid to their monitoring (Fradette et al., 2022). Consequently, no standardized or

regulated method for monitoring *Cryptosporidium* and *Giardia* spp. in groundwater as well as the health hazards associated with these protozoans.

1.3. Justification

Monitoring groundwater microbial contamination potential by wastewater treatment plants (WWTPs) has been largely overlooked. The pathogens in groundwater pose a serious threat to public health globally (Odiyo et al., 2020; Kumar et al., 2014). Wastewater treatment sludge may contain pathogens such as *Cryptosporidium* and *Giardia* spp*.* due to the nature of wastewater received with human feces which is inadvertently transmitted to groundwater if not adequately treated. The removal of *Cryptosporidium* and *Giardia* (oo)cyst from wastewater sludge requires thermal sludge treatment processes (Ladeia et al., 2022 ; Rimhanen-Finne et al., 2004).

According to Rimhanen-Finne et al., 2004 *Cryptosporidium* and *Giardia* spp*.* are not removed through conventional unheated anaerobic digesters (Al-Gheethi et al., 2018). In a study by Gericke, it was found that the transmission of protozoans normally occurs as a result of a failure in the water treatment process (Tram et al., 2022). The author further highlighted that conventional wastewater treatment methods are unable to decrease the *Giardia* cysts and *Cryptosporidium* oocysts population in the treated effluent. This could be the case for the eMbalenhle wastewater plant as it is not equipped with heated anaerobic digesters, which are an efficient system to treat *Cryptosporidium* and *Giardia* spp.

Gericke et al., (1995) reported that in their study in South Africa, cryptosporidiosis outbreaks have been reported in areas where water has undergone extensive treatment. Their research outcome indicated that poor operational and disinfection programs in treatment plants were inadequate for the removal of the oocysts, which leads to the endemic occurrences *of Cryptosporidium* and *Giardia* spp. In a study conducted by Jarmey-Swan et al., 2001 in KwaZulu Natal on *Cryptosporidium* and *Giardia* spp. endemic occurrences, the protozoans were identified as causing diarrheal illness in 39% of children < 1 year old, and 38.5% of children between 3-4 years (Jarmey-Swan et al., 2001). This research further identified potable water supply, lack of hygiene and sanitation, and lack of education as the main factors driving the transmission of these vectors.

Although groundwater is monitored in WWTPs for *Helminth* and *Total coliforms* as per Chapter 4 of the National water act, 1998 (Act No .36 of 1998) (National Water Act, 1998), *Cryptosporidium* and *Giardia* spp. are not monitored across most groundwater systems in South Africa (Water Research Commission, 2009; Dungeni & Momba, 2010). This study, therefore, looks at the prevalence of these enteric pathogens in boreholes near the eMbalenhle wastewater treatment plant and seeks to assess the abundance of (oo)cysts and advise on future monitoring of groundwater applicable to the plant. The data from the study will also add to water quality information in provincial and national water systems that will be useful for current and future water developments of standard monitoring procedures for *Cryptosporidium* and *Giardia* spp. in South Africa.

1.4. Objectives

1.4.1. Main objective:

The focus of the study was to evaluate the abundance of *Cryptosporidium* and *Giardia* spp*.* in groundwater at eMbalenhle wastewater works in Secunda, Mpumalanga province in South Africa.

1.4.2. Specific objectives of the study included:

- Collect and analyse groundwater samples to determine the presence of *Cryptosporidium, Giardia* spp., *and Escherichia coli.*
- Analyse physicochemical parameters such as pH, electrical conductivity (EC), nitrates as N, Free, and saline ammonia as N, phosphate as P, chloride as Cl, and total suspended Solids (TSS).
- Determine the potential health impacts of *Cryptosporidium* and *Giardia* spp. on humans.
- Statistically determine any correlations between the presence *of Cryptosporidium* and *Giardia spp.* with TSS, EC, nitrates (NO_3-N), ammonia (NH_3-N), phosphate, chloride, and *Escherichia coli*

- Make output-dependent recommendations about monitoring and the potential health impacts of *Cryptosporidium* and *Giardia* spp. in groundwater systems.

1.5. Research questions

- What are the levels of *Cryptosporidium, Giardia* spp., and *Escherichia coli* in the groundwater samples?
- What is the concentration of the physicochemical properties in groundwater from the Embalenhle Wastewater Treatment Plant?
- Are both physicochemical properties and microbiological concentration (*Cryptosporidium, Giardia spp*., *Escherichia coli,* and fecal coliforms*)* within permissible limits, using drinking water standards by WHO and South African Water Guidelines?

1.6. Research limitations

Although the research has more positive outcomes, there were a few limitations that were experienced. One of the limitations was the inability to conduct the laboratory analyses at the UNISA laboratories as per the initial plan. This was due to COVID-19 restrictions which led to delays being experienced thereby necessitating the use of Aquadoc Laboratory for analyses, albeit, at a high cost. Another shortcoming experienced was the delays during the sampling period of the eMbalenhle Wastewater Treatment. There were two instances where the planned sampling was rescheduled due to the plant not working because of vandalism. The samples taken and the frequency of the borehole sampling period were limited due to budget constraints; with more samples and more sampling frequency, the results would have greater accuracy. The delays in the funding caused further delays in sampling. Even though an attempt was made to cover all four seasons, summer could only be covered at the beginning when rainfall was not heavy. As such determining the effect of heavy rains and seepages on groundwater contamination could not be done. Also, the autumn sampling was at the end of the season.

The factors that could influence the findings of the study:

- Sampling delays. The delays in sampling reduced the variables that can be studied i.e. the beginning of summer seasons and end can give more data on the presence of microbes due to rain intensity, groundwater levels, and the water infiltration impact.

- Cost of analysis, limited samples collected. The high cost resulted in limited samples being collected, which condensed the statistical range of the results.

1.7. Ethical consideration

As part of the ethical consideration and ensuring that the research adheres to UNISA policy on Ethics. The Govan Mbeki Municipality, Water and Sanitation Department were consulted and briefed about the details of the research, the length of the study, and how the findings of the study would be disseminated for the benefit of the Municipality. The formal letter to request access to research at eMbalenhle WWTW was sent together with the UNISA Ethics policy consent form. The letter was approved by the Govan Mbeki Municipality Head of the Department of Water and Sanitation. A response letter with the terms and conditions was received (Appendix A and B).

CHAPTER 2.

LITERATURE REVIEW

2.1. Introduction

The chapter gives a concise and thorough review of the means by which microbial contaminants are transported into groundwater in a wastewater treatment. This is achieved by examining and evaluating various literature on groundwater contamination, focusing on *Cryptosporidium* and *Giardia* spp*.* Additionally, the review takes into consideration the objectives highlighted in Chapter one, wastewater treatment process and management.

2.2. Microbial Contamination of groundwater

Microbial contamination is defined as unintended pollution by pathogens such as bacteria, fungi, protozoans, and viruses in water (Sharma & Bhattacharya, 2017). Microbial contamination negatively impacts the environment, including water resources, and has detrimental consequences both economically and in human health. Microbiological pollution is widespread domestically and can lead to waterborne disease outbreaks (Landrigan et al., 2020; Genthe & Kfir, 1992). In the United States, it was discovered that a significant amount of waterborne outbreaks stem from contaminated groundwater that is linked to WWTPs (Pandey et al., 2014).

Groundwater is water found in aquifers which are water deposits underneath a water table, submerged in the rocks and sediments of the earth's surface (Vahdat-Aboueshagh et al., 2021; Chapman, 1996). The formation of groundwater contaminants is through precipitation infiltration and surface runoff (Li et al., 2021; Hamilton, 2005). The water table aquifers are closer to the earth's surface; as such issues like droughts and pollution easily affect them. Groundwater moves slowly in comparison to surface water- the slow movement affects the dilution rate of the contaminants which consequently form a plume within the aquifer (Akhtar et al., 2021; Singhal et al., 2010). The speed at which the contamination is released from the contaminant plume is dependent on the contaminant type, the velocity of the groundwater based on topography, and the density of the plume (Akhtar et al., 2021; Singhal

et al., 2010). Various microbial contaminants can be transmitted in the wastewater treatment plant as indicated in Table 1.

Table 1. Pathogens in the wastewater treatment plant

Pathogens		**Disease**	**Source**	**References**
	Salmonella	Typhoid fever	Human and animal feces	(Y. Chen et al., 2020;Driscoll, 1986; Department of Water Affairs, Department of Health, Water Research Commission, 2002a)
	Shigella	Dysentery		
	Vibrio cholerae	Cholera		
Enteric viruses	Hepatitis A virus	Hepatitis		
	Rotavirus	Gastroenteritis		
	Adenovirus	Gastrointestinal and respiratory illness		
	Poliovirus	Poliomyelitis		
Protozoan	*Cryptosporidium parvum*	Cryptosporidiosis		
	Giardia lamblia	Giardiasis		

2.3. Factors affecting transportation of microbial contaminants into groundwater

There are several factors and structural geology that contribute to the seepage and hydrologic transport of wastewater bacterial pathogens into groundwater, and their persistence thereof. The major transportation means of microbial contamination to groundwater aquifers are precipitation, soil characteristics, land cover, and slope of the land (Da'ana et al., 2021; Kanyerere et al., 2012; Hurst et al., 1980).

2.3.1. Precipitation

Water is an easy transport medium for bacterial contaminants through absorption and infiltration; heavy storms exacerbate the occurrence and transportation of the microorganisms (Da'ana et al., 2021; Jung et al., 2014; Hurst et al., 1980). Variances in precipitation (the length and intensity of rainfall) and climate change affect groundwater recharge (Dubois et al., 2022; Thomas et al., 2016). A study conducted by Godfrey et al., (2005) on the relationship between rainfall and microbiological contamination in groundwater elucidated that developing countries are experiencing water contamination outbreaks largely in high peak rainy seasons (Rohman, 2018). However, in contrast to this view, a study on the prevalence of diarrheal disease by waterborne pathogens conducted in KwaZulu Natal noted no correlation between the increased rainfall and the incidence of *Cryptosporidium* and *Giardia* (Wang et al., 2023; Jarmey-Swan et al., 2001).

2.3.2. Soil characteristics

Microorganism transportation in the soil is dependent on the variables that affect their carriage to the groundwater aquifers. The major transportation of pathogens includes the movement of sediments which normally carries contaminated waste particles and surface run-offs. In a WWTPs, suspended particles, including microbes can be deposited on the soil surface, acting as a filter, and trapping more contaminants (Crini et al., 2019). During heavy rains, the transportation of microbes through the soil to the aquifers is accelerated by cracks in rocks, root channels, and earthworm channels (Wendel et al., 2022; Bition & Harvey, 1992).

The soil permeability is described as the ability of the soil to transmit any water through seepage-the greater the soil permeability, the more the seepage (Swanson, 2020; Thomas et al. 2016). The rate of water transmission in groundwater is also dependent on the space sizes in the soil. Sediments such as sand and gravel are highly permeable in comparison to clay and silt (Swanson, 2020). Even though the large pores in the soil increase the water transmission rate, it is ineffective in filtering out bacterial contamination (Wendel et al., 2022; Carty & Bourke, 1995). The groundwater whose aquifers are situated under coarse-textured soil is, therefore, more susceptible to microbial contamination than the aquifers under fine soil-which has more ability to screen out most microbes (Seaton et al., 2020; Sindelar, 2015). The deeper the soil filters, the more microbial pathogens in comparison to shallow soils.

Soil saturation also plays a role in the retention of pathogens in groundwater. The microbial contamination is retained in saturated soils than in unsaturated soil (Madumathi et al., 2017; Balkhair, 2016). Studies by Brennan et al., & Banks et al., have indicated that the movement of microbes is increased in saturated soils. Pathogens in saturated soils are transported by mobile water and their pathogenic cells can intermingle with the solid phase resulting in temporary or permanent immobilization of the microorganisms (Michelon et al., 2023; Unc & Goss, 2004). These pathogens are then trapped and released during the rainy seasons, allowing them to migrate and reach aquifers, either by absorption or infiltration and ultimately contaminating the groundwater.

2.3.3. Landcover

The land cover has an important role in groundwater seepage. A denser land cover slows down the water runoff, thereby allowing time for seepage and groundwater recharge (DWAF, 1996a). Land cover can have a negative and positive contribution to the transmission of microbial contamination. Land coverage can negatively affect the microbial quality of water when there is a waste influx in the surrounding area, which promotes the seepage of pathogenic bacteria to groundwater through deep plant roots (Cheng et al., 2022; Hurst et al., 1980). On the larger protozoa of (10 to 15 μm) during water runoffs, the land cover can filter the bacteria and prevent downward movement in the soil column (Wendel et al., 2022; McAllister & Topp, 2012).

2.3.4. Slope of the land

The areas nearby pumping wells are more susceptible to contamination due to the area in relation to the original recharge (Asiwaju-Bello et al., 2020; Singhal et al. 2010). This phenomenon is also applicable to wells that are drilled in the proximity of water recharge bodies such as rivers and dams-the groundwater from the wells draws contaminants from the recharge bodies. The land slope of surrounding wells also affects groundwater contamination in that water on a steep slope runs off quickly and is less likely to be absorbed. Several studies have demonstrated that rivers downstream of cattle farms have high contamination of *Cryptosporidium* and *Giardia* (oo)cysts in comparison to upstream (Ladeia et al., 2022; Ong, Ross & Isaac-Renton,1996).

2.4. Transportation routes of *Cryptosporidium* and *Giardia* (oo)cysts in water, soil, and food

There are numerous routes of *Cryptosporidium* and *Giardia* transmission, direct and indirect routes. The fecal-oral route is the direct route for (oo)cysts, infecting the host after ingestion, and can be directly from humans (anthroponotic) or animals (zoonotic) (Watier-Grillot et al., 2022; Fewtrell & Bartram, 2001). The indirect route involves contact with *Cryptosporidium* and *Giardia*-contaminated water or food sources (Martins et al., 2019; Abeywardena et al., 2015). Several contaminated settings promote the indirect transmission of *Cryptosporidium* and *Giardia* through sewage sludge during agricultural activities (Daniels et al., 2018). Most gastrointestinal outbreaks of *Cryptosporidium* and *Giardia* still indicate water as the main transmission driver behind these outbreaks as indicated in (Table 2).

Table 2. Various waterborne outbreaks of Cryptosporidiosis and Giardiasis

Source	Year	Country	Organism	Infected cases	References
Supply Network (sanitary sewage)	1993	Milwaukee, Wisconsin (US)	cryptosporidiosis	403.000	(Toledo et al., 2017)
Contaminated water (deficiencies in the filtration process)	2004	Norway	giardiasis	1500	(Robertson et al., 2021)
Well water (on the farm)	2011	Michigan, USA	cryptosporidiosis	20	(Centers for Disease Control and Prevention (CDC), 2012)
Tube wells	2012-2013	Puri District, Odisha, India	cryptosporidiosis and giardiasis	65.8% (3,385 children)	(Daniels et al., 2018a)
River (possible discharge from wastewater effluent plant)	2001	Pietermaritzburg, KwaZulu Natal, South Africa	cryptosporidiosis and giardiasis	*Cryptosporidium* (39.3%) in the <1 year age group. *Giardia* 3 to 4-year age group (38.5%)	(Jarmey-Swan, Bailey, and Howgrave-Graham, 2001)

Predominantly, the transportation of wastewater pathogens containing *Cryptosporidium* oocyst and *Giardia* cyst can be transmitted through consumption (drinking), irrigation, food, and swimming (K. A. Hamilton et al., 2018).

2.4.1. Groundwater consumption

Water shortage is a growing concern globally to the point that some countries experiencing water scarcity are reliant on groundwater for potable use (Boretti & Rosa, 2019). There is a misconception in most countries that groundwater is an uncontaminated water source, which does not cause any illness (Li et al., 2021). The shortage of drinking water has been reported, both at global and domestic scales that have reported supply and has been attributed to population increase and climate change. Recently, KwaZulu Natal and some parts of the Eastern Cape experienced extreme flooding that wreaked havoc and left people without water and electricity. The damage to water treatment and power plant stations caused people to start relying on alternative water sources for consumption, including groundwater (Pinto et al., 2022). In 2018, the city of Cape Town underwent a “Day Zero”, where the water level in supply dams had declined to below 10% which compelled the city to turn off the taps in residential areas. This extreme water shortage was due to uncontrollable meteorological factors in the region (Sousa et al., 2018). Such occurrences result in communities relying on borehole water with unknown quality for household uses including consumption.

Contamination of surface and groundwater with fecal pathogens is a concern, especially for the public that uses untreated water without access to water treatment facilities (Daniels et al., 2018b). Very few countries have the luxury of treatment facilities for groundwater. According to the United States Environmental Protection Agency, (USEPA), 97% of the public water system in Michigan uses groundwater (Cabrera Marino, 2017). A study conducted in Michigan for the assessment of the prevalence of *Cryptosporidium* and *Giardia* in water systems indicated that most cases are detected in rural areas where the majority of the community is still dependent on groundwater, which is not treated or monitored for microbial contamination (Dreelin et al., 2014).

An investigation conducted by Daniels et al. (2018) using multivariable modeling in 60 villages in India elucidated that the *Cryptosporidium* and *Giardia* spp. sources are emanating from human fecal loading due to the use of latrines pits, damaged tube wells, and livestock which contaminates the local groundwater. These studies corroborate a study conducted by Jarmy-Swan in KwaZulu Natal on *Cryptosporidium* and *Giardia* endemic occurrences where

the protozoans were identified as causing diarrheal illness in 39% of children <1 year old, and 38.5% of children between 3-4 years of age. The study further indicated the route of protozoan transmission as potable water supply, lack of hygiene and sanitation, and lack of education in rural areas (Jarmey-Swan et al., 2001).

2.4.2. Groundwater use for irrigation

South Africa is a water-scarce country, and this does not only affect household consumption but the farming community's irrigation activities as well (Cabrera Marino, 2017). Most farmers ordinarily depend on untreated water for irrigation with unknown or poor microbial quality from dams, rivers, groundwater, and wells (Malakar et al., 2019; Pachepsky et al., 2011). The irrigation of agricultural produce with water of poor microbiological content presents a risk to public health by transmitting pathogens to the produce. Contamination of the irrigation water generally occurs when rain, the runoffs carrying fecal contamination from a nonpoint source like sludge, and the discharge of effluents from the treatment of wastewater sources end up in rivers and wells. The concern is water with *Giardia* and *Cryptosporidium* (oo)cysts when used for irrigation can have a consequential effect on human health (Domenech et al., 2018).

Cryptosporidium oocysts and *Giardia* cysts have been detected in irrigation water in several African countries (Squire & Ryan, 2017). The processes that contaminate crops with (oo)cysts are spraying of pesticides and herbicides, manure application, and farm workers packing produce with contaminated hands (Malakar et al., 2019; Mota et al. 2009). In a study conducted by Thurston-Enriquez et al. (2002) on the detection of *Cryptosporidium* oocysts and *Giardia* cysts and microsporidia in irrigation waters used for crop production, the researcher noted that 28% of irrigation water samples gave positive results for microsporidia, 60% for *Giardia* cysts, and 36% for *Cryptosporidium* oocysts.

Determining groundwater quality is very important especially if it is utilized for consumption or irrigating food in agricultural fields, more so where some of the agricultural products are eaten uncooked e.g. lettuce (Al-Hashimi, 2021; Davis & Kendall, 2012). Due to the adsorption capacity of vegetable roots and leaves, if such contaminated water is used for

irrigation, pathogenic organisms may accumulate, allowing the microbes to contaminate the vegetables (Gavrilescu, 2021; Alam et al., 2015).

In studies conducted for cryptosporidiosis and giardiasis, very limited cases are related to foodborne outbreaks caused by these protozoans, the reports are mostly linked to water contamination as the route for the *Cryptosporidium* and *Giardia* (oo)cyst transmission (Siwila et al., 2020; Squire & Ryan, 2017). A typical transmission of cryptosporidiosis and giardiasis is from contaminated water to produce that is generally eaten raw (Berrouch et al., 2020; El-Said Said, 2012). The oo(cysts) are usually transmitted by the fecal-oral route, following the ingestion of contaminated food or water. Once the (oo)cyst is in the small intestine, it releases infectious sporozoites for *Cryptosporidium* and trophozoites for *Giardia* causing gastro illness in the host (Fradette et al., 2022). The irrigation of fresh produce by water contaminated with enteric protozoans is a common route for the transmission of the (oo)cyst to food, even though not well-researched (Abidelfatah M. Nasser, 2022; Duhain, 2012).

Cryptosporidium and *Giardia* (oo)cysts are not easy to remove from leafy foods. It has been reported in a study conducted in Greece on the food of plant origin, that simple washing does not remove the (oo)cysts (Sakkas et al., 2020). In a study conducted on *Cryptosporidium and Giardia* spp. on bovines, it was found that the (oo)cysts can penetrate spinach leaves through the stomatal opening (Adeyemo, 2019; Abeywardena et al., 2015). Food contaminated with these pathogenic protozoans, eaten raw can cause illness in humans and animals. A study by Buyukyavuz et al. (2018) indicated that flies can contaminate raw fruits and vegetables with the (oo)cysts. A possible inactivation method of *Cryptosporidium* and *Giardia* (oo)cysts in raw vegetables is blanching (Koutsoumanis et al., 2018).

2.4.3. Swimming activities

Swimming is a well-enjoyed recreational activity globally, even domestically. In other areas, swimming has been noted as potentially presenting some health risks to the public engaging in swimming either due to chemical or microbial pollution (Chalmers et al., 2021). The noted transmission route during swimming is ingesting water or being splashed with recreational water contaminated with the parasites (Omarova et al., 2018; Shields et al., 2008). A study

conducted by Ehsan (2015) in Bangladesh and Belgium on swimming water acknowledges the swimming pools as a crucial transmission route for *Cryptosporidium* and *Giardia.* Most swimming pools use chlorine to disinfect their pools, and studies have shown that *Cryptosporidium* and *Giardia* oo(cysts*)* are resistant to chlorine disinfectants (Adeyemo et al., 2019; Rose et al., 2002).

In Atlanta, Georgia hundred and sixty (160) swimming pools filter backwashes were sampled to examine the presence of *Cryptosporidium* spp. and *Giardia intestinalis* at the end of the swim season (Omarova et al., 2018; Shields et al., 2008). The parasite was detected, even though it was challenging to ascertain the risk of transmission because the study did not measure the viability of the recovered protozoans. Another study was conducted in Beijing, China, for the prevalence of *Cryptosporidium* and *Giardia* in thirty-five (35) swimming pools and the results indicated that 16.7% and 15.0% of samples gave positive results for the abundance of *Cryptosporidium* and *Giardia (*oo)cysts, respectively (Xiao et al., 2017).

2.5. Factors enabling the survival of *Cryptosporidium* Oocyst *and Giardia* cyst in the environment

When *Cryptosporidium* and *Giardia* spp*.* is introduced into the aquatic environment due to microbial contamination caused by seepages, it adapts to survive (Fradette et al., 2022; Grundlingh & De Wet, 2004). The *Cryptosporidium* and *Giardia* (oo)cyst are more resistant to environmental stresses and chemical destruction because their (oo)cyst has a resting stage and is protected by an outer wall which gives them the ability to survive in the harsh and varying environment for a long time (Adeyemo et al., 2019). Once *Cryptosporidium* and *Giardia* spp. are presented to the environment, their viability in an environment is dependent on parameters such as pH, temperature, sunlight, and soil type (Javanmard et al., 2020; Alum et al., 2014).

2.5.1. pH in soil and water

The viability of many bacterial pathogens depends on the pH of the environmental aerosol (Liu et al., 2023; Hurst *et al.*1980). However, few studies have been conducted on the *Cryptosporidium* and *Giardia* (oo)cysts in varying pH in soil and water medium to ascertain

their survival capability (Wang et al., 2023; Barwick et al., 2003). In a study conducted by Robertson et al., (1992), the aluminum sulfate used as a flocculant in water treatment plants for flocculation and clearing turbidity was shown to be inefficient in curbing the persistent (oo)cysts. It was only when high concentrations of lime or ferric sulfate were added that the (oo)cyst population was reduced after prolonged exposure to high pH.

The flocculant in the treatment process enables the small particles to agglomerate onto the large particles and settle to form sludge. However, some of the (oo)cysts remain suspended and the water gets to the next process with only a reduced concentration of (oo)cysts. Chlorine is then added at the following stage of the process to inactivate the microbes. However, it has been indicated that *Cryptosporidium* is resistant to chlorine and chloramines and therefore it is extremely difficult to inactivate. It is only when ozone combined with chlorine dioxide or chlorine has been effective, with a pH of around 6 (Adeyemo et al., 2019; Yuan et al. 2006). In a study by Barwick et al., (2003), the *Cryptosporidium* oocysts appeared lesser in soil with neutral and basic pH, than in acidic media. The remaining sludge in the drying beds with some microbe or (oo)cyst can infiltrate and contaminate groundwater, especially if the drying beds are not lined or have cracks.

2.5.2. Environmental temperature

The concentration of any contaminant in water may vary once removed from its source of origin due to changes in such parameters as temperature (Liu et al., 2023; Alum et al., 2014). Temperature plays a vital role in the protozoan's survival in various environmental conditions-a change in temperature influences its viability. The microorganisms have an optimum temperature at which they can remain viable or be able to function. *Cryptosporidium* and *Giardia* spp. have optimum temperature ranges where they can function and inactivate as detailed in Table 3.

A study by Olson et al. (1999) on the investigation of the viability of *Cryptosporidium* and *Giardia* (oo)cysts in water, soil, and cattle feces was conducted. The outcome of the study monitored at the -4.4 °C to 25°C temperature condition for 7wk, indicated that at a warmer temperature of 25°C, the (oo) cysts are inactivated and survive the cold water of between -4 °C and 4°C for a prolonged period. The temperature effect on the pathogens was further

investigated by Hamilton et al. (2018) the results indicated that *Giardia* is stable at 4°C, *Cryptosporidium* is stable between -4°C and 4°C, thus *Cryptosporidium* and *Giardia* spp. survive longer at lower temperatures.

Table 3. Inactivation of *Cryptosporidium* oocysts and *Giardia* cysts in environmental waters and feces

Genera	Suspending medium	Temperature (°C)	Time (days)	Inactivation (%)	References
Giardia lamblia	River water	12 to 20	3 to 8	90	(Robertson et al., 1992; DeRegnier et al., 1989)
	River water	2 to 5	14 to 143	90	
Cryptosporidium parvum	Human stools	4	178	41 to > 99	
	Cow feces	5 to 10	176	60 to 72	
	Tap water	5 to 10	176	96 to 99	
	River water	5 to 10	176	89 to 99	

2.5.3. Exposure to solar radiation (UV)

Sunlight, which provides ultraviolet (UV) radiation, has an impact on *Cryptosporidium* and *Giardia* (oo)cysts in the terrestrial and aquatic environment (Hamilton et al., 2018). UV light is known for its ability to inactivate microbial pathogens by damaging nucleic acids within the cell (Liu et al., 2023; Hijnen et al., 2006; Sinton et al., 1994). *Cryptosporidium* and *Giardia* (oo)cysts are equipped to resist UV light compared to viruses even though sensitive. The protozoans respond to light by either migrating to the lower darker depth of water or ducking direct exposure to sunlight by hiding in the soil (Sinton et al., 1994). In a study conducted by King et al., (2008) on the assessment of the *Cryptosporidium parvum* inactivation via

radiation in tap and environmental waters, a conclusion was drawn that UV can inactive the *C. parvum* rapidly in water.

2.5.4. Soil type

The clay soil favors the absorption of microbial pathogens by reducing the death rate and protecting their cells whereas on the sandy soil, the survival rate is low (Fongaro et al., 2017). In a study by Peng et al. (2008) where oocysts were stored in dry soil for ten days at 32°C and 15 °C in loamy soil for months respectively; the findings revealed that *Cryptosporidium* (oo)cysts can survive both conditions.

2.6. Indicator parameters and the presence of *Cryptosporidium* and *Giardia spp.* presence in water

2.6.1. Turbidity and Suspended Solids

Turbidity is a measure of dissolved matter in water and the ability of optical characteristics of water that cause the scattering of light i.e. suspended solids (World Health Organization, 2017). The turbidity in water is characterized by muddiness which affects the microbiological quality (DWAF, 1998). If the results of scattered light are high, that denotes high turbidity. The turbidity in water is characterized by muddiness which affects the microbiological quality (DWAF, 1998). Turbidity is often caused by industrial and domestic waste discharge (Yang Liu et al., 2020; Palmer et al., 2000). Highly turbid water has the potential to affect aquatic animals. A study on the occurrence of *Cryptosporidium* and *Giardia* spp. in the Brazilian public water-treatment system showed a correlation between high turbidity and the prevalence of (oo)cysts (Almeida et al., 2015). The discharge of wastewater effluent with high turbidity renders the receiving water unhealthy for human consumption and domestic use, reduces crop yield and ultimately harms marine life, (Rusydi, 2018). *Cryptosporidium* and *Giardia* spp. can evade removal in wastewater by attaching to solid particles. This is partly because water with high turbidity of more than 1 NTU has particles that can shield the microbes from the disinfection process resulting in these microbes being released into the receiving water bodies (Mhlongo et al., 2019). Sente et al. (2016) further established a

correlation between the presence *of Cryptosporidium* spp. with conductivity, pH, and TDS, while the correlation with *Giardia* spp. was on TDS only.

2.6.2. pH

The pH value expresses the acidity or basicity of the medium by having hydroxide (OH^-) ions and hydrogen (H^+) ions balanced in a solution. In a wastewater treatment plant, the extreme levels of pH are an indication of the accumulation of toxic chemicals, particulate matter, and a degree of pollution in the effluent (Khalid et al., 2018). The pH imbalance, either too low or too high in groundwater, affects microbial activity and hinders biological processes (DWAF, 1998). The pH changes are also dependent on the temperature variation, the organic and inorganic ions, and microbiological activity. The low pH level can exacerbate the development of toxic substances influencing species diversity and structural alterations for the species (Spurgeon et al., 2020).

2.6.3. *Escherichia coli* (*E.coli*)

E. coli has been used as an indicator for enteric pathogens. *E. coli* is bacteria that live in the intestines of humans and warm-blooded animals, it is an opportunistic pathogen that can survive in aquatic environments (Z. Chen et al., 2017). *E.coli* can be found in humans, cats dogs, and rodent feces and its occurrence in water is poor maintenance of wastewater treatment systems or spillage of fecal matter from pit latrines leading to microbial contamination (DWAF, 1996a; Abia et al., 2017). The presence of *E.coli* in water is used to evaluate the possibility of fecal contamination (DWAF, 1996c). *Cryptosporidium* and *Giardia* (oo)cysts are detected in the host(s) and water bodies contaminated with feces. The prevalence of *E.coli* in water can, therefore, be used as an indicator for the occurrence of enteric pathogens.

2.7. Detection methods of *Cryptosporidium* and *Giardia* spp. in water

Monitoring the quality of drinking, ground, and recreational water is essential due to the effect that contaminated water has on human health. Consequently, several methods are currently being developed for the detection of *Cryptosporidium* and *Giardia* (oo)cysts around the world as shown in Table 4 In South Africa, there is no standardized methodology

prescribed for *Cryptosporidium* and *Giardia* (oo)cysts in water (Miambo et al., 2019; Grundlingh & De Wet, 2004). In most studies conducted on the prevalence of these protozoans in the country, the US EPA procedure, Method 1623 is often used (Fradette et al., 2022; Dungeni & Momba, 2010). US EPA 1623 includes filtration and extraction of the (oo)cysts from a water sample using immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy for identification. To verify the presence of (oo)cysts, staining carried out using 4', 6-diamidino-2-phenylindole (DAPI) and Fluorescein isothiocyanate FITC is conducted (EPA, 2005).

A standard method ISO 15553:2006 has been developed and published by the UK (Standing Committee of Analyst) and approved by the UK Drinking Water Inspectorate(SAI Global & Iso, 2006). The testing method is almost similar to Method 1623 by US EPA with the only difference being the staining and contrast technique (Fradette et al., 2022). The use of molecular biology (PCR-based detection) to detect *Cryptosporidium* and *Giardia* became popular in the 1990s (Fradette et al., 2022). Isothermal protocols and sequencing-based protocols came into effect to improve the identification and classification of the protozoa in aquatic samples (Sánchez et al., 2018). However, precision is critical when identifying the genetic targets and creating primers to avoid amplifying DNA from another source (Fradette et al., 2022).

Table 4. Advantages and disadvantages of microscopy-based methodology versus molecular biology.

Method	Disadvantage	Advantages	References
(Microscopy) EPA Method 1623 and ISO 15553:2006	Low recovery. Costs associated with sampling large volumes.	The large volume of water during filtration for better concentration of (oo)cysts. The use of the microscope for quantification avoids biases in comparison to PCR. The use of various dyes at the same time gives confidence in the identification.	(Fradette et al., 2022) (EPA, 2005)
(Molecular biology) PCR based detection	High chances of contamination because the test is DNA based. Few laboratories possess the latest equipment required.	Higher presumptive detection. Quicker results compared to microscope based.	(Sánchez et al., 2018)

2.8. Wastewater treatment and *Cryptosporidium* and *Giardia* spp.

2.8.1. Inactivation of *Cryptosporidium* and *Giardia* spp. in a wastewater treatment process

The most important requirement of a safe environment for human beings is a sufficient water supply of acceptable and safe quality (SAHRC, 2000). Technological advancement and an ever-increasing population, especially in urban areas, have led to increasing volumes of wastewater with higher concentrations and diversity of pollutants(Tian et al., 2018; WRC, 2009). Although nature has an inherent capacity to attenuate most of the microbiological pollution, the amount of water and microbes concentrations have intensified to such an extent that without mechanical or chemical intervention, nature cannot cope (Donnenfeld et al., 2018).

Wastewater is water whose quality has been altered due to contamination by human activities with organic and inorganic particles, pathogens, toxins, and pharmaceuticals, to name a few (Crini et al., 2019). The purpose of the wastewater treatment plant is to decontaminate water of pathogenic microorganisms and toxic elements that can cause waterborne illness and to ensure that water is of acceptable quality i.e. clear, clean, and odourless. WWTWs can thus be considered a man-made system by which contamination attenuation mechanisms are developed to relieve the pressure on nature. Pathogens and fecal coliforms (amongst them dangerous species, such as the causative agents of cholera) are carried, fed, and maintained in the effluent (Crini et al., 2019). Viruses carried in the effluent are subsequently ingested by a suitable host. At WWTPs, these organisms are inactivated in the maturation pond and by the addition of chlorine (Yorkshire Environmental Alcontrol UK, 1998). As is well known, surviving organisms can cause outbreaks of dangerous diseases or even epidemics.

The typical wastewater treatment process in South Africa involves screening (removal of solids for disposal), removal of grit (removing any rubbish or clogging substances), oxidation ponds (where waste is treated by UV), primary sedimentation, biological filters, aeration and activated sludge treatment, settling, maturation ponds, wetland polishing treatment, disinfection, sludge thickening, sludge drying beds, and anaerobic digestion (Naidoo, 2013).

The wastewater treatment process followed in most South African WWTPs is not adequate to inactivate the *Cryptosporidium* and *Giardia* spp. Chlorine disinfection is not adequate for the inactivation of the *Cryptosporidium* and *Giardia spp.* from effluent (Adeyemo et al., 2019). The recommended method for the inactivation of these parasites involves ultrafiltration and UV radiation (Water Research Australia, 2016).

The Water Act of 1998 (Act 36 of 1998) requires that water extracted from a river for domestic or industrial uses be purified and discharged to the same river at a quality that does not adversely affect the downstream users. The SANS241:2015 and WHO limits of the study determinants are indicated in Table 5. A heavy responsibility is placed on the shoulders of wastewater treatment plants (WWTP) to ensure that the facility is functional and operated optimally. A constantly functional WWTP ensures that the effluent discharge does not pose a health risk to humans and animals.

Table 5. List of selected parameters analyzed during the study and their limits

Parameter and units	SANS 241:2015	DWAF: Aquatic ecosystem	DWAF: Irrigation	DWAF: Recreational activities	World Health Organization	References
pH	5.0-9.7	5.5-11	6.5-8.4	0-5	6.5 – 8.5	SANS 241:2015, (WHO, 2017; DWAF, 1996).
Electrical conductivity (EC) mSm	0-70	70-150	<6.5	n	n	
Total Suspended Solids (TSS) mg/L	n	<100	n	n	n	
Chloride as Cl (mg/L)	≤300	n	n	n	200-300	
Nitrate as N (mg/L)	0-11	<0.5	0-0.5	n	50	
Total Phosphate as P (mg/L)	n	≤ 0.005	n	n	n	
Free and Saline Ammonia as N (mg/L)	≤1.5	0.025	n	n	n	
E.coli/100 mL	0	1000	0-1000	0-130	0	
Fecal coliform/100 mL	0	1000	0-1000	0-130	0	
Cryptosporidium/10l	0	0	n	n	0	
Giardia/10l	0	0	n	n	0	

n: no determinants or not stipulated

2.8.2. Detection of *Cryptosporidium* and *Giardia* spp. in treated wastewater

The purpose of wastewater treatment is to remove harmful pollutants using a range of methods that are categorized into physical, chemical, and biological processes. Wastewater treatment ensures that the water is within acceptable limits since treated wastewater is discharged into the receiving water bodies(Jr & Orleans, 2020). The untreated pathogenic microorganism has the potential to affect the public if released into receiving water bodies used for domestic purposes and irrigation. *Cryptosporidium* and *Giardia* (oo)cysts have been reported in treated effluent in several countries (Table 6). The wastewater treatment process protocol in most treatment plants is effective in the treatment of other enteric pathogens such as bacteria and viruses except for *Cryptosporidium* and *Giardia* (oo)cysts (Ryu et al., 2021; Ramírez-Castillo et al., 2015).

Several studies have been conducted indicating the ability of *Cryptosporidium* and *Giardia* (oo)cysts to survive the conventional disinfection process used in most WWTPs (Hamilton et al., 2018; de Jong, 2017; Gericke et al., 1995). This is attributed to their (oo)cyst wall anatomy making it easy to escape the physical and chemical barriers in WWTPs (WHO,2006). Due to the (oo)cysts walls, Cryptosporidium and *Giardia* have been proven to survive chloride-based common and cost-effective disinfectants such as those used by the public for pools and households (Adeyemo et al., 2019).

Dungeni & Momba, (2010) conducted a study in four WWTPs (Zeekoegat, Baviaanspoort, Rayton, and Refilwe Water Care Works) in Gauteng on the prevalence of *Cryptosporidium* and *Giardia* and revealed that uncontrolled sewage discharge and improperly maintained WWTPs were identified as key sources of water contamination. In all four WWTPs *Cryptosporidium* and *Giardia* (oo)cysts were detected as indicated in Table 6 Their conclusion was additional water purification procedures are necessary for effectively eliminating these pathogenic microorganisms.

Table 6. Frequency of detection and the concentration of *Cryptosporidium* and *Giardia* in treated Effluent

Country	*Cryptosporidium*	*Giardia*	References
	Tested samples (no. of positive samples)	**Tested samples (no. of positive samples)**	
South Africa, Gauteng province	Four WWTPs (all positive)	Four WWTPs (all positive)	(Dungeni & Momba, 2010)
Spain	108 samples (oocysts 1.38 to 2.6/L)	108 samples (0.6 to 1.7/L cysts)	(Domenech et al., 2018)
Eastern Poland	10 L in municipal WTPs (oocyst detected in eight WTPs (61.5%))	10L in 13 municipal WTPs (cysts in eleven WTPs (84.6%))	(Sroka et al., 2013)
South Africa, Vhembe District	Six sewage treatment plants .155 samples. (59.3%) were positive	-	(Mashau, 2012)
Italy	Four WWTPs (rarely detected)	Four WWTPs (positive for all plants)	(Cacciò et al., 2003)

2.8.3. Wastewater treatment Sludge and transportation of *Cryptosporidium* and *Giardia* spp.

The wastewater treatment sludge, which is a by-product of the treatment plant, has been repurposed for such areas as agriculture as part of cost-saving on waste disposal (Lamastra et al., 2018). However, for the sewage sludge to be used in other operations, waste characterization should be conducted. The typical sludge may contain heavy metals and other contaminants that might be detrimental to the ecosystem (Fijalkowski et al., 2020). A study conducted in three operational WWTPs, in Bekaa valley of Lebanon, evaluated the quality of sludge for agricultural purposes, looking specifically at the pathogenic microorganisms such as *E.coli, Staphylococcus aureus, Salmonella,* Helminth eggs*,* Ascaris, Acinetobacter. Their conclusion was that some pathogenic organisms, mainly *E.coli* could still be detected after treatment and their recommendation was to further treat the sludge before repurposing to reduce the microbial contaminants (Romanos et al., 2019).

An investigation by Rimhanen-Finne et al., (2004) suggested that the *Cryptosporidium* (oo)cysts and *Giardia* cysts are sedimented into raw sludge during flocculation in a wastewater treatment plant process. If the contaminated sludge is used in the agricultural field, the protozoans are transmitted by the zoonotic cycle (Rimhanen-Finne et al., 2004). Martins et al. (2019) conducted a study on monitoring the occurrence of protozoans in raw and treated sewage samples from a wastewater treatment plant in Brazil and reported that water bodies that receive treated wastewater should be assessed for protozoal abundance prior to reuse water in agriculture, human and animal consumption. This is because the protozoans were detected on the effluents from the wastewater treatment plant.

2.9. Health impact of Cryptosporidium and Giardia on groundwater users

Many countries have reported outbreaks of *Giardia* spp. and *Cryptosporidium* spp., the illness involves moderate to severe gastrointestinal illness in humans and animals (Robertson et al., 2020; Carmena et al., 2010). Cryptosporidiosis and giardiasis illness can cause morbidity in healthy people which can be detrimental to immunocompromised people and can often lead to fatality (Utami et al., 2020; Garcia, 2007). The characteristics and

resulting health symptoms of *Giardia* spp. and *Cryptosporidium* spp. are described in Table 7.

Table 7. Characteristics of the intestinal protozoa (*Giardia* and *Cryptosporidium*)

Protozoan	Species	Infectious and environmental stage	Disease	Source	References
Cryptosporidium	parvum	Oocyst	diarrhea, fever, and muscle aches.	cattle, domestic, and wild animal wastes.	(Wolfe, 1992; Garcia, 2007; Rose et al., 2002)
Giardia	lamblia	Cyst	Acute diarrhea	beavers	

Globally, in developed countries (Europe, North America, Australia) there have been reported cases of *Giardia* and *Cryptosporidium* even in areas where water complies with microbiological standards (Ongerth & Karanis, 2018). On the global front, of the waterborne outbreaks reported from 2004 to 2010, 60.3% are related to *Cryptosporidium* spp. (Cho et al., 2013).

Squire & Ryan (2017), in their study of Cryptosporidiosis and Giardiasis outbreaks in Africa, discovered that malnutrition and HIV status also contribute to the prevalence of the epidemiology of these protozoa. The increase of outbreaks is expected to increase in African countries due to climate change and an increase in population which create a shortage in freshwater availability. In South Africa, particularly KwaZulu-Natal, *Cryptosporidium,* and *Giardia* are endemic, because of the rural areas that still depend on river systems for water consumption, the pathogens are transmitted through contaminated water (de Jong, 2017).

2.10. Regulations and monitoring of Cryptosporidium and Giardia in water systems

Monitoring of the *Cryptosporidium* and *Giardia* spp. is conducted worldwide for risk assessment and the investigation of waterborne outbreak trends in countries. However, different countries use various assessment methods and different approaches (DWAF, 1996a). The US EPA has regulated the monitoring of their water system monthly for Cryptosporidium, Giardia, coliforms, turbidity, and a variety of other parameters (EPA, 2001).

In South Africa, the monitoring of *Cryptosporidium* and *Giardia* spp. in all waterbodies is not uniformly conducted. In some water systems where the monitoring is done, it's mostly on the final treated water for drinking (Sigudu et al., 2014). DWAF has not established the standard criteria for monitoring these protozoa in South African domestic water quality guidelines (DWAF, 1996). Without a standard procedure or regulated detection method, it creates a gap.

CHAPTER 3.

RESEARCH METHODOLOGY

3.1. Introduction

This chapter describes the location of the eMbalenhle Township in the Mpumalanga Province of South Africa. It highlights major characteristics in the study area such as climate, soil, vegetation, and water resources. The chapter also describes the research design and methodology that was used to fulfill the objectives of the study, methods for data collection, and analysis as well as ethical considerations for the validity and reliability of the results.

3.2. Study area

3.2.1. Description of the study area

The eMbalenhle WWTW was established in 1988 with a 9.6 ML/d design capacity, providing services to the eMbalenhle community (Govan Mbeki Municipality, 2019). The treatment works location is Latitude: 26°33'.20.19"S, Longitude: 29°04'16.07"E at the lower end of eMbalenhle Township; in Secunda.

The study site is located in South Africa, 150 km east of Johannesburg and 300km southwest of Nelspruit (the capital city of Mpumalanga) under Govan Mbeki Municipality. The Municipality boasts both mining and manufacturing sectors, which include Sasol (the biggest chemicals and energy manufacturer in South Africa), Eskom, Exxaro, and other mines. Govan Mbeki Municipality is one of the seven local municipalities under the jurisdiction of the Gert Sibande District. Figure 1. shows the map of Gert Sibande District Municipality.

3.2.2. Topography and drainage

From the topographical standpoint, the biggest portion of the Gert Sibande District Municipality is situated on the Highveld Grasslands of Mpumalanga (Gert Sibande Municipality, 2015). The study area is relatively flat comprising shales and sandstone. The highest point of the site elevation is 1620 meters above sea level (Coetzee & Kisters, 2016;

Govan Mbeki Municipality, 2014). The eMbalenhle WWTW discharges at Trichardt Spruit, which further contributes to the Water Vaal Dam Catchment Scheme.

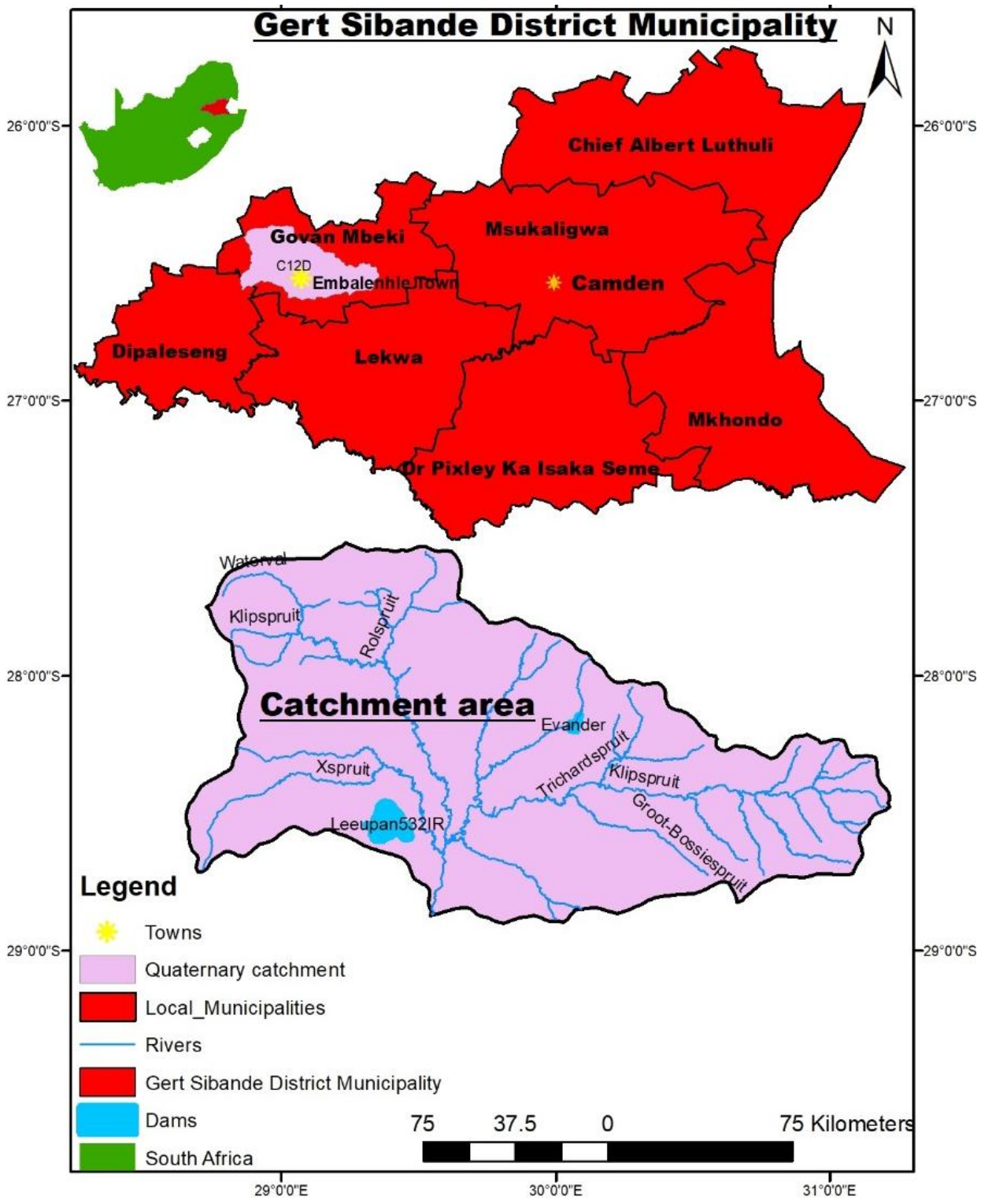

Figure 1. Map of Gert Sibande District Municipality (ArcGIS, 2011)

3.2.3. Climate and Vegetation

Gert Sibande District has dry, sunny summers with an average of 26.5 C and harsh winters coupled with heavy frost. The summer season is experienced in (October - February) and (April –August) the winter season. Govan Mbeki Municipality has a varied climate with an annual average rainfall of 670 mm (Govan Mbeki Municipality, 2014). The rainfall that occurs throughout the summer season is characterized by heavy thunderstorms. Gert Sibande District Municipality is currently the largest agricultural sector in Mpumalanga Province, with a ranging variety of vegetables (Gert Sibande District Municipality, 2017).

3.2.4. Geology and soil

The study area geology comprises sedimentary rocks from Ecca and Karoo Supergroups (Coetzee & Kisters, 2016). The rocks are dominated primarily by sandstones, coal, and dolerite (Govan Mbeki Municipality, 2014).

Table 8. Geological formation in Govan Mbeki Municipality

Geological Formation	ha	%	References
Arenite	134405.04	45.41	(Govan Mbeki Municipality, 2014)
Dolerite	156699.83	52.94	
Granite	38.93	0.01	
Rhyolite	4868.37	1.64	
Total	**296012.18**	**100.00**	

Soils in the study area have been greatly altered over the years due to the historic formation of the Secunda Complex, in the 1970s, consequently resulting in a dolerite and ash mixture as indicated in Table 8 (Govan Mbeki Municipality, 2014).

3.2.5. Water sources and sanitation

Gert Sibande District Municipality like most regions in South Africa has limited water resources. Mpumalanga COGTA reported that the province has 65% surface water, 19%

water transfers, 6% groundwater and 10% industrial process return flows (MP COGTA, 2018). The majority of boreholes used by farming communities are mostly not registered to the Municipality and the quality of water is unknown (Govan Mbeki Municipality, 2014).

According to Mpumalanga COGTA in their Spatial Challenges and Opportunities Report, the water quality issues were elucidated as inadequate maintenance of the sewage system, mining, and agricultural activities (MP COGTA, 2018)**.** These shortfalls inadvertently affect the water that flows to catchment dams which are used for irrigation by farming communities. The poor water quality, not only affect vegetation in the area but perpetuate diseases in animals and human (Gert Sibande District Municipality, 2017).

With the increase in population, approximately 141,741 in eMbalenhle, directly or indirectly accelerates waste generation (Matooane et al., 2011). This consequently puts pressure on the wastewater treatment plant, with the receipt of toxic substances, which in other cases is difficult to eliminate (EPA, 1998). Figure 2. shows the aerial map of the study area, eMbalenhle WWTW. A study done by Green-Drop in 2014 in Mpumalanga province, in all Wastewater Treatment, Works indicated that most plants in the province are having design capacity issues to treat wastewater received from communities, and in other instances, the quality of effluent before discharge to catchments dams is poor (MP COGTA, 2018).

Figure 2. Aerial map of the study area, eMbalenhle Wastewater Treatment Works (Source: Google earth map, 2022)

3.3. Research design

This research followed a quantitative research approach as a fundamental methodology for data collection and analysis. The quantitative research methodology involves numerical data analysis which includes data from experiments and surveys to provide evidence and link to the research questions (Basias & Pollalis, 2018; Apuke,2017). In the quantitative method, numbers are used to prove the theoretical information of the phenomena.

Since the quantitative research approach is based on the cause-and-effect relationship between an independent and dependent variable and making predictions following the method by Apuke, (2017). The independent variable in this study was the pollutants in groundwater caused by the poor quality of the effluent from the Wastewater Treatment Plant seeping through the ground. The dependent variable was the influencing variable which is the borehole water in the plant. To give credible results concerning the determination of the *Cryptosporidium* and *Giardia* spp. in borehole water, laboratory analyses were conducted to quantify protozoans in water. Specific physicochemical parameters were also determined, in previous studies conducted on these protozoans, these parameters were indicators of microbial contamination.

3.4. Methodology

3.4.1. Sample collection

The groundwater samples were collected at the Wastewater Treatment plant using a Micro-Purge sampling procedure by Vail, (2013). The boreholes were fitted with a pump and tap with a hosepipe for sampling convenience (Figure 3). The tap was opened and allowed to run for 5 min before the sample was taken, to purge the stagnant water. The mouth of the hosepipe was disinfected with 70% concentrated alcohol and afterward allowed the pump to run for another 3 min. The borehole sampling and preservation were done following the American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater 21st Edition, Method 2130B (APHA, 2002).

The borehole samples were then collected using sterilized bottles. The sampling for the microbiological analysis (*E. coli*) was done aseptically in a 1 L polypropylene, ensuring the sample was not contaminated by external influences that would contaminate the water sample and give unreliable results. Sampling for *Cryptosporidium* and *Giardia* was collected using 10 L polypropylene plastic containers following the methods by Ongerth & Karanis, (2018); EPA, (2005); and. EPA Method 1623 is an international method for the identification of *Cryptosporidium* and *Giardia* in the aqueous medium. EPA Method 1623 includes filtration of a minimum of 10 L, immunomagnetic separation of the oocysts and cysts, and counting of the protozoans.

Figure 3. Borehole 1 water samples at WWTW eMbalenhle taken to Aquadoc laboratory

For physicochemical analysis such as pH, electrical conductivity (EC), Nitrates as N, Free, and Saline Ammonia as N, Phosphate as P, Chloride as Cl, and total suspended solids (TSS), 1 L HDPE Gamma sterilized plastic bottles were used for sampling. The bottles were filled to the top without leaving space and the cap was immediately replaced. The physicochemical samples were placed in a cooler box filled with ice cubes, to keep the temperature between 2-8 °C and taken to the Aquadoc laboratory for analysis. *Cryptosporidium* and *Giardia* samples were transported after collection at ≤ 20°C, the cooling was only done during the last sampling round which took place when it was hot, ensuring the water does not freeze or exceed 20°C.

3.4.2. Frequency of sampling

The sampling of the boreholes at eMbalenhle WWTW took place from May to November 2022, in four sampling cycles. There were only three boreholes in the sample sites where samples were collected. The total number of samples (3) samples for *Cryptosporidium* and *Giardia*, (3) samples for microanalyses, and (3) physicochemical analyses in duplicates were collected each visit from the locations described in Table 9.

Table 9. Borehole sampling sites description

Description of sampling sites	Research study points	Identification	Condition of Boreholes
eMbalenhle WWTW Located South of the clarifier	Borehole 1	BH 1	The borehole sampling site is paved, next to the compressor room (with a pump). The sampling site has an unpleasant odor.
eMbalenhle WWTW Located North of the clarifier	Borehole 2	BH2	The sampling site is grassy, and the taps are in good condition
eMbalenhle WWTW Located West of the clarifier	Borehole 3	BH3	The sampling site is grassy, and the taps are in good condition

3.5. Analytical Procedures

Table 10. Physicochemical analysis

Parameter and units	Analytical methodology/Instrumentation
pH (pH)	pH meter. South African National Standard. Determination of pH. SANS 5011:2005
Electrical conductivity (mS/m)	Conductivity meter. South African National Standard. Determination of electrical conductivity. SANS 7888:2005.
Total suspended solids	Oven, analytical balance, and filters. TDS dried at 103 - 105ºC. 2540D.APHA, AWWA, WEH.2012.
Nitrate, NO_{3-} (Nitrate/L)	Colorimetric/spectrophotometric. instrument manual.
Total phosphates	4500-P. standard methods for the examination of water and wastewater.
Chloride (Cl/L)	Colorimetric/spectrophotometric. instrument manual.
Ammonia (ammonia/L)	Colorimetric/spectrophotometric. instrument manual.

Table 11. Microbiological analysis

Parameter	Analytical Methodology/Instrumentation
Cryptosporidium spp. and *Giardia* spp.	Method 9711 B.C. 22 nd Ed. 2012. EPA. Method 1623: *Cryptosporidium* and *Giardia* by filtration IMS/FA
E*scherichia coli (E.coli)*	Microbiological analysis of water. South African National Standard. SANS 5221:2006. Ed 4.2.
Fecal coliform	SANS 5221: 2006. SANS 9308 -1: 2004 Microbiological analysis of water. Water quality – Detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method

3.5.1. Microbiological Analysis

3.5.1.1. Isolation and detection of *Cryptosporidium* oocysts and *Giardia* cysts

The isolation of *Cryptosporidium* and *Giardia* was done following the EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. Before groundwater sample filtration, the system was flushed with 2 L reagent water. The 10 L borehole water sample was filtered through a (1-micron membrane filter) Pall EnvirochekTM 1µm HV, using a pump, the oocysts, cysts, and extraneous material were retained on the filter (0.8 µm pore size and 142 mm diameter). Material on the filter was eluted and the eluate was centrifuged (Grant Instrument Ltd, England) at 1100 x g for 16 min, to pellet the oocysts and cysts, and the supernatant fluid was then aspirated. The pellet was then transferred into a flat-side Leighton tube (Dehteq, Kyalami, South Africa) using cleaned pipettes. One (1) mL buffer was added to the samples of 100 µL of *Cryptosporidium* Dynabeads and 100 µL of Giardia Dynabead contained in Leighton tubes. The tubes were then placed on the mixer to rotate for 1 hour at room temperature. Immuno-magnetic separation was then carried out using MPC-1 and

MPC-S (Dehteq, South Africa). The samples were placed on the slide, fixed with methanol, and stained with 50 µL of *Cryptosporidium* FITC and 50 µL Giardia FITC stain. The samples were then placed in an incubator for 30 min at 37°C. The samples were taken out of the incubator, stained with 50 µL DAPI working solution, and left for 2 min at room temperature.

The scanning was done under a fluorescence microscope to identify the *Cryptosporidium* and *Giardia*. (Oo)cysts with apple green, fluorescent cell walls, and spherical were detected and classified as positive for FITC. The UV filter block was used for the DAPI analysis. (Oo)cysts that had been identified using the green filter were then examined under a UV filter for validation. *Cryptosporidium* and *Giardia* were identified as DAPI-positive if they display up to 4 distinctive sky-blue internal nuclei.

The recovery rate of *Cryptosporidium* oocysts was calculated using the below formula:

$$\boldsymbol{Cryptosporidium}\ \textbf{recovery}\ (\%) = \frac{\boldsymbol{Cryptosporidium}\ \textbf{detected}}{\textbf{Number of}\ \boldsymbol{Cryptosporidium}\ \textbf{in Color Seed}} \times \mathbf{100}$$

Equation 3.1

The *Giardia* cysts recovery rate will be calculated using the below formula:

$$\boldsymbol{Giardia}\ \textbf{recovery}\ (\%) = \frac{\boldsymbol{Giardia}\ \textbf{detected}}{\textbf{Number of}\ \boldsymbol{Giardia}\ \textbf{in ColorSeed}} \times \mathbf{100}$$ **Equation 3.2.**

3.5.1.2. *Escherichia coli*

Escherichia coli (E.coli) presence in water was used as an indicator of possible fecal contamination and other waterborne pathogens(Wen et al., 2020; Santo Domingo & Edge, 2010). The analysis of *Escherichia coli (E.coli)* was conducted using the Membrane Filtration Technique for microbiological analysis of water samples as stated by SANS 5221 (2018) and; USEPA, (2006). Briefly, the method involved passing the groundwater sample through a membrane of filter size of 0.45 µm, the membrane was subsequently placed on a medium, (mTEC) Thermotolerant *Escherichia coli* agar (modified membrane) and finally incubated at 35 ± 0.5 °C for approximately 2 ± 0.5 h in an incubator. The targeted species on modified mTEC agar are red or magenta. The membrane was left to attain room temperature, the colonies were then counted

(SABS, 2011). The number of *E.coli* per 100 mL was calculated using Equation 3.3 (USEPA, 2006).

$$\boldsymbol{E.coli/100\ mL} = \frac{\textbf{Number of } \boldsymbol{E.coli} \textbf{ colonies}}{\textbf{The volume of sample filtered (mL)}} \qquad \textbf{Equation 3.3.}$$

The results were reported as *E.coli* CFU per 100 mL of sample.

3.5.1.3. Fecal coliform

The membrane filtration method was used to assess the quantity of fecal coliform per sample according to standard method SANS 9308 -1: 2004 Microbiological analysis of water described in the detection and enumeration of *E. col*i and coliform bacteria. m-Endo agar (Thermo Fisher Scientific, Johannesburg, South Africa) was used for fecal coliforms. The 100 mL dilution of samples was filtered through a membrane filter paper (0.45µm), and the filter was transferred into a petri dish with m-Endo agar, ensuring that no contamination is taking place. The dish was then placed in an incubator for 24 h, at 45 °C. After incubation, the number of colonies was counted and linked directly fecal content of the groundwater. Colony counts for fecal coliform were enumerated as CFU per 100 mL from the agar plates using the formula:

$$\boldsymbol{FC/100\ mL} = \frac{\textbf{No of colonies}}{\boldsymbol{The\ volume\ of\ the\ sample\ filtered\ (mL)}} \qquad \textbf{Equation 3.4.}$$

The results were reported as fecal coliform per 100 mL of sample.

3.5.2. Physicochemical Water Quality Variables

3.5.2.1. pH

pH meter with the readability of 0.01 pH units, HI 9829 Multiparameter Meter (HANNA instruments) with available GPS, logging probe, turbidity, and ion measurements, was used in the laboratory and field. The probe was used to measure the groundwater pH immediately after sample collection to minimize the microbiological decomposition of solids. The buffer solutions were used to calibrate the pH meter before conducting the analysis. The method that was used for pH is the Examination of Water and Waste

Water Method 4500-H B (APHA, 2002). pH imbalance, either too low or too high in groundwater affects microbial activity and hinders biological processes (DWAF, 1998). pH is considered an important parameter in the WWTP groundwater, which determines the viability of most microorganisms (Hurst et al., 1980). The pH measurements indicate the basicity and acidity in water. In the WWTP, the high values of pH signal the presence of ions, which can be chlorine in the treatment process, carbonates, and hydroxide, to mention a few (Wen et al., 2020; Leopold & Freese, 2009).

3.5.2.2. Turbidity

The turbidity was measured using EUTECH Turbidimeter TN-100 (Thermo Fisher Scientific, Johannesburg, South Africa). The intensity of light scattered by the sample being analyzed is compared with the intensity of light scattered by a calibration suspension under the same conditions. If the intensity of scattered light is high, that translates to higher turbidity in the sample. The method is derived from APHA Standard Methods for the Examination of Water and Wastewater. Turbidity was measured as soon as the samples arrived at the lab, to prevent temperature changes that affect the credibility of results and particle flocculation and sedimentation which changes the sample's characteristics.

3.5.2.3. Total suspended solids

Total Suspended Solids (TSS) are part of the total solids in the water sample held by a filter that causes turbidity in water (DWAF, 1998). During TSS analysis, a recorded volume of well-mixed sample will be filtered through a glass fiber filter which was pre-weighed. The residue and filter were oven-dried at 105 ± 5°C and then desiccated before weighing. Suspended solids are part of the total solids in the sample held by a filter. The method is derived from Standard Methods for the Examination of Water and Waste Water, methods 2540 D (APHA, 2002).

$$TSS\ (mg/L) = \frac{(A - B) \times 1000}{sample\ volume\ (mL)}$$

Where: A is the weight of the filter and dried residue

B weight of filter before filtration

3.5.2.4. Electrical conductivity

Conductivity is the degree to which the aqueous solution carries an electric current. The capability relies on the presence of ions, movement, and temperature measurement to mention a few(DWAF, 1998). The conductivity of the sample was measured using Multiparameter Meter (HANNA instruments) in situ, calibration was done before analysis using (CRM) certified reference material. This method is based on APHA Standard Methods for the Examination of Water and Waste Water Method 2510 (APHA, 2002).

3.5.2.5. Inorganic compounds - Phosphate, nitrate, ammonia, and chloride

The inorganic compounds will be determined using the Thermo Scientific Gallery Discrete Analyzer. The method is based on (1) EPA Method 325.2 (2) APHA Standard Methods for the Examination of Water and Waste Water Method 2510 (USEPA, 1983; (APHA, 2002). The sample was mixed with a specific reagent listed in Table 10 to form a complex. The resulting complex was measured spectrophotometrically at a wavelength of 480 nm for chloride, 660 nm for ammonia, 540 nm for nitrate, and 880 nm for phosphate. Prior to analysis, calibration curves were plotted for each of the analytes, and the concentration of each compound was determined utilizing a calibration curve.

(a) Phosphate (PO_4-P)

The orthophosphate anion reacts with ammonium molybdate and antimony potassium tartrate (catalyst) in the presence of the acid media resulting in a 12-molybdophosphoric acid complex. The resulting compound was then reduced with ascorbic acid to form a blue hetero-poly complex. The compound was measured for

absorbance using a spectrophotometer at a wavelength of 880 nm and compared to the phosphate anion concentration using a calibration curve. The unit of measure is mg/L.

b) Nitrate (NO_3-N)

The determination of total oxidized nitrogen and nitrate in groundwater was assessed using the gallery plus discrete analyzer. Nitrate was reduced by hydrazine under alkaline conditions. The resulting total nitrite ions were then reacted with sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride resulting in a highly colored pink azo-dye. The solution was then measured using a spectrophotometer to determine the absorbance at a wavelength of 540 nm. This was then compared to the total organic nitrogen (TON) concentration by means of a calibration curve. The nitrate value was obtained by calculating TON as N. – Nitrite as N.

c) Chloride (Cl)

To determine the concentration of soluble chloride in groundwater, the chloride was reacted with mercury (II) thiocyanate which resulted in the non-ionic compound. That reaction resulted in thiocyanate ions being released which then reacted with an acid solution of iron (III) nitrate to form an iron (III) thiocyanate compound. The resulting complex was measured for absorbance on the spectrophotometer at a wavelength of 480 nm and was compared to the chloride concentration by means of a calibration curve.

d) Ammonia (NH_4-N)

To determine ammonia in groundwater, ammonia was reacted with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitrosprusside at approximately pH 12.6 to form a blue-green indophenol complex. The resulting complex was measured for absorbance using a spectrophotometer at

the wavelength 660 nm and was compared to the ammonia concentration by means of a calibration curve. The unit of measurement is mg/L.

3.6. Statistical analysis of microbial and physicochemical data

The seasonal graph showing different parameters was done to cover all seasons in relation to water quality guidelines. Origin Pro and one-way ANOVA were used to determine the mean variation and comparison for the boreholes and the significant difference between means. The results were compared with SANS241:2015, South African Water Guideline, and WHO(DWAF, 1996c). Correlation analysis was determined using excel to examine the presence of *Cryptosporidium* and *Giardia* and the linear relationship with indicator parameters.

CHAPTER 4.

RESULTS AND DISCUSSION

4.1. Introduction

This chapter shows the results of the study and discusses the trends that were detected. The results for physicochemical and microbiological test results are reviewed and compared with SANS241:2015, South African Water Guidelines, and WHO (DWAF, 1996; WHO, 2017). The guidelines provide the benchmark of acceptable quality limits for water for compliance, risk, and monitoring purposes. The borehole water analyses were performed to understand the impact that the eMbalenhle WWTW might have on the groundwater quality either due to operation issues or natural attenuation from the plant surroundings. The research data is presented in the form of tables and figures for effective data and results presentation**.**

4.2. Results for physicochemical analyses

4.2.1. pH results

Figure 4 shows the pH mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. The measured pH values from all the boreholes are in the range of 7.0 - 8.1 and are shown in Figure 5 (a). Based on the presented data, the average pH for the boreholes BH1, BH2, and BH2 is (7.325, 7.475, and 7.375) respectively, which are all slightly alkaline with no significant variation between them. According to SANS 241:2015, the acceptable pH limit for irrigation standards should be 5 - 9.7 and 6.5-8.4 (DWAF, 1996). Figure 4 shows the comparison of the pH results for all the boreholes in the wastewater treatment plant with the SANS 241, indicating that all the results were within the permissible limits.

pH is considered an important parameter in the wastewater treatment plant groundwater because it determines the viability of most pathogens (Yushuo Liu et al., 2023; Hurst et al., 1980). The pH measurements indicate the basicity and acidity in water. In the wastewater treatment plant, the high values of pH signal the presence of

ions, which can be chlorine that is used in the treatment process, carbonates, and hydroxide, to mention a few (Leopold & Freese, 2009).

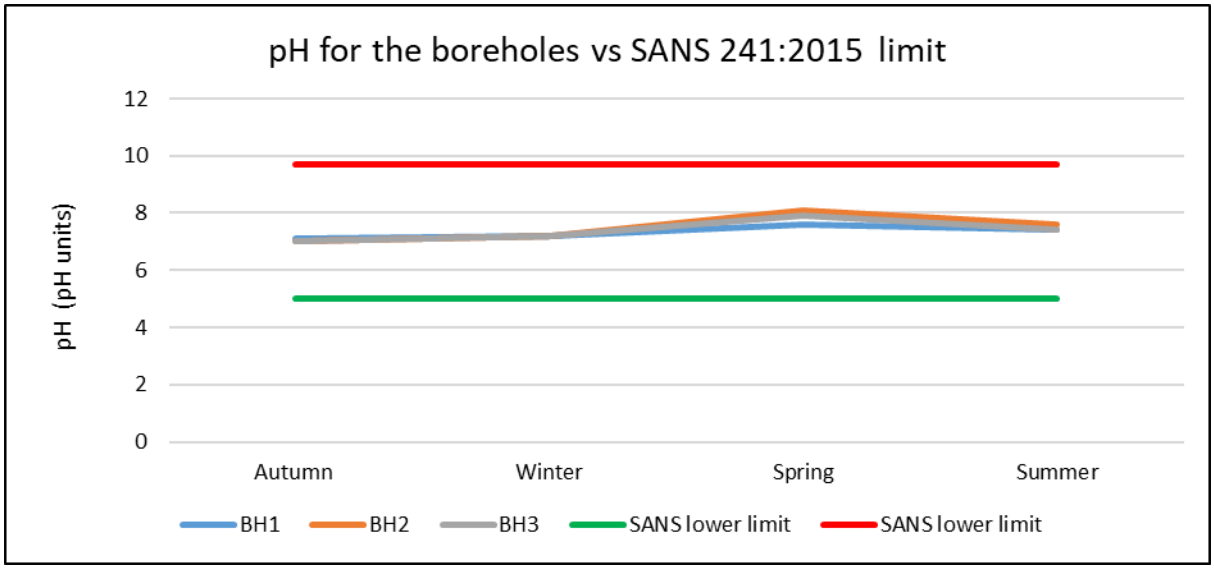

Figure 4.Graphical presentation of boreholes pH values against SANS 241:2015 standard

During the autumn sampling season, the pH range for all three boreholes was in the range of 7.0 - 7, 1 which was slightly alkaline, *Cryptosporidium* and *Giardia* (oo)cysts were identified in the water samples during analyses. This study disagrees with the study conducted by Barwick et al. (2003) on *Cryptosporidium* and *Giardia* spp. which indicated that the (oo)cysts appear less in basic or neutral media but are resistant to acidic media. No major seasonal variations in pH among the sites were observed, except for BH2 where the pH was at 8.1 in spring, making BH2 slightly higher than that of BH1 and BH3. There were no other significant differences detected in seasonal trends for the sampling period. According to Figure 5 (c), the p-value is not less than $\alpha = 0.05$ thus affirming the null hypothesis; there is no statistically significant difference between the pH means of the three boreholes.

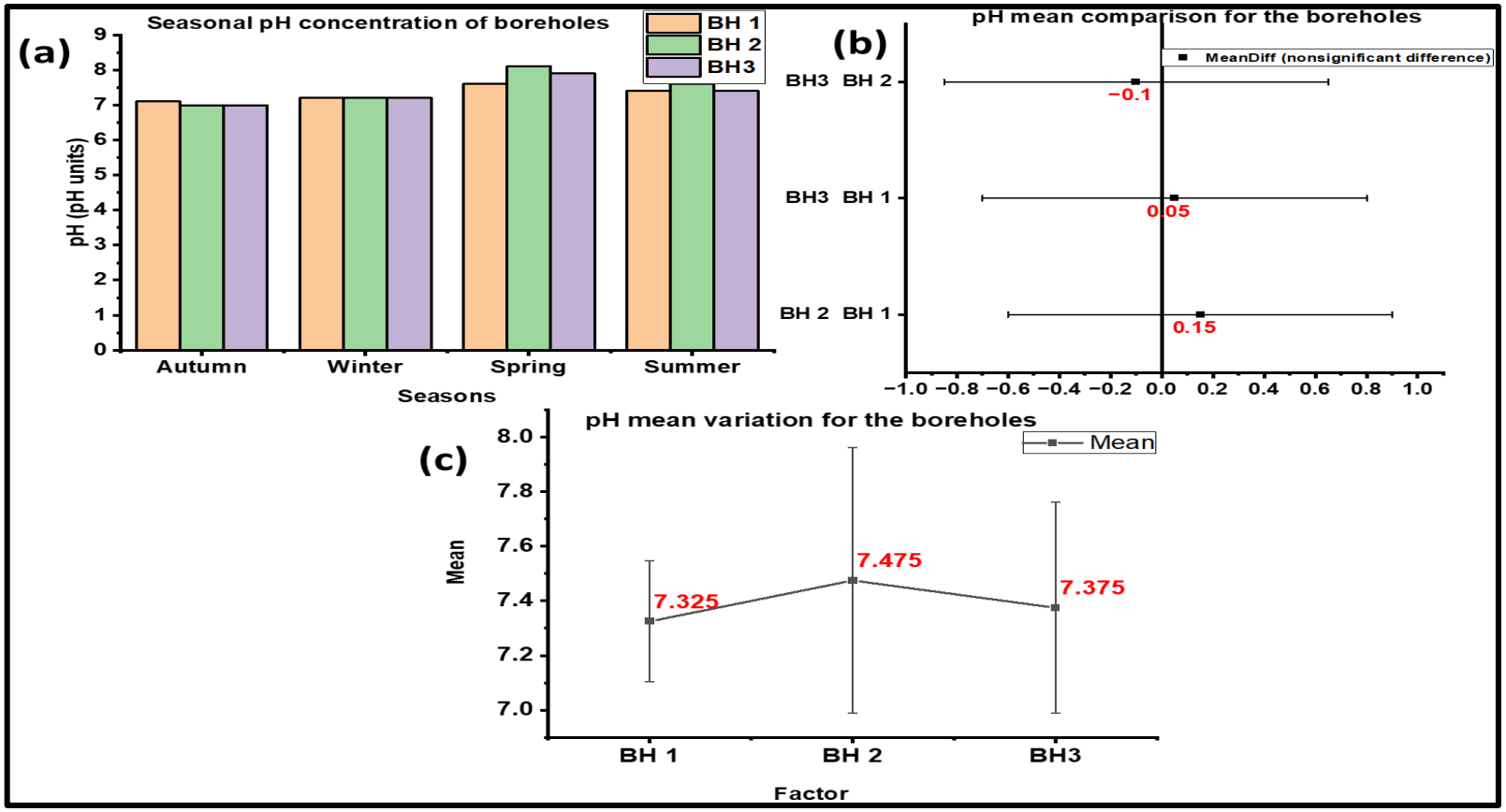

Figure 5. (a) pH values for the borehole sites from autumn to summer, (b) Mean pH variation for the borehole sites from autumn to summer, and (c) Mean pH comparison for the borehole sites from autumn to summer.

4.2.2. Electrical conductivity results

Electrical conductivity (EC) was measured at each of the borehole sampling sites to determine the level of dissolved substances and chemicals in the water. In WWTPs, the high conductivity is indicative of impurities, which can be attributed to a lot of factors (Golnabi et al., 2009). Figure 7 shows the conductivity mean values, mean variation, mean comparison, and standard deviation for the borehole sites from autumn to summer. The measured EC values from all the boreholes ranged from 120 to 129 mS/m and as shown in Figure 7 (a).

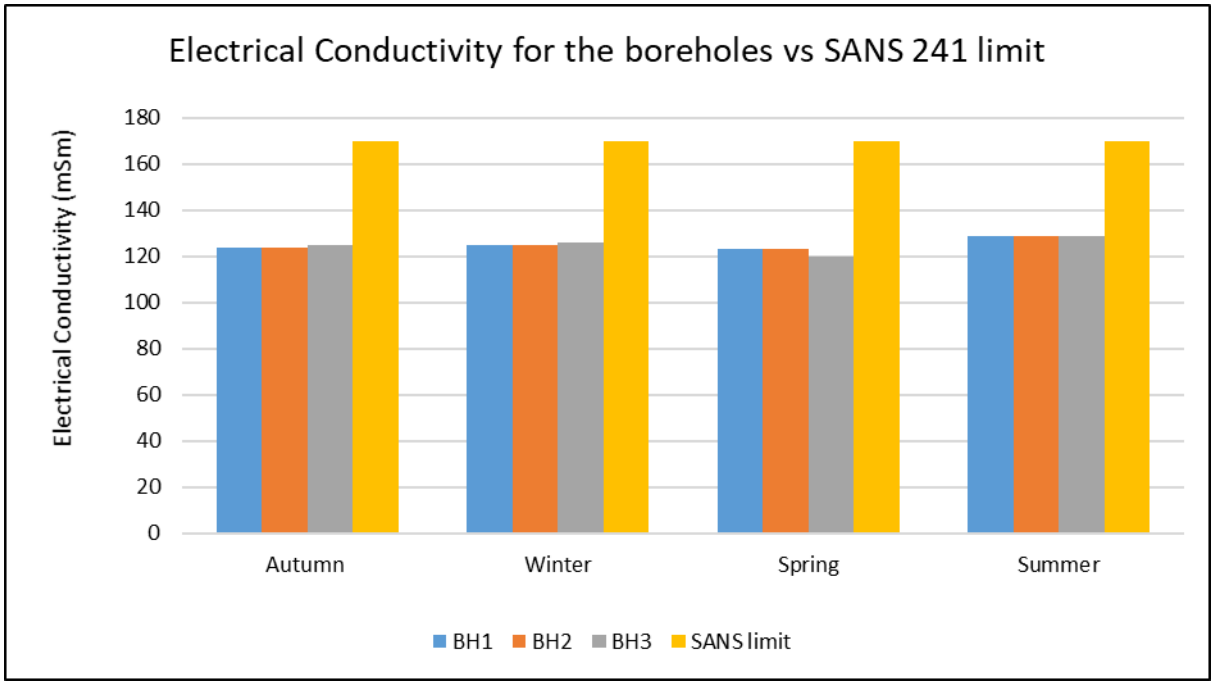

Figure 6. Graphical presentation of boreholes' electrical conductivity against SANS 241:2015 standard

Based on the presented data in Figure 6, the comparison EC results of all the boreholes in the wastewater treatment with the SANS 241, indicates that all the results were within the permissible limits. The conductivity limit as prescribed in SANS 241:2015 should be ≤170 mS/m at 25°C, ≤ 40mS/m for irrigation purposes (DWAF, 1996d)

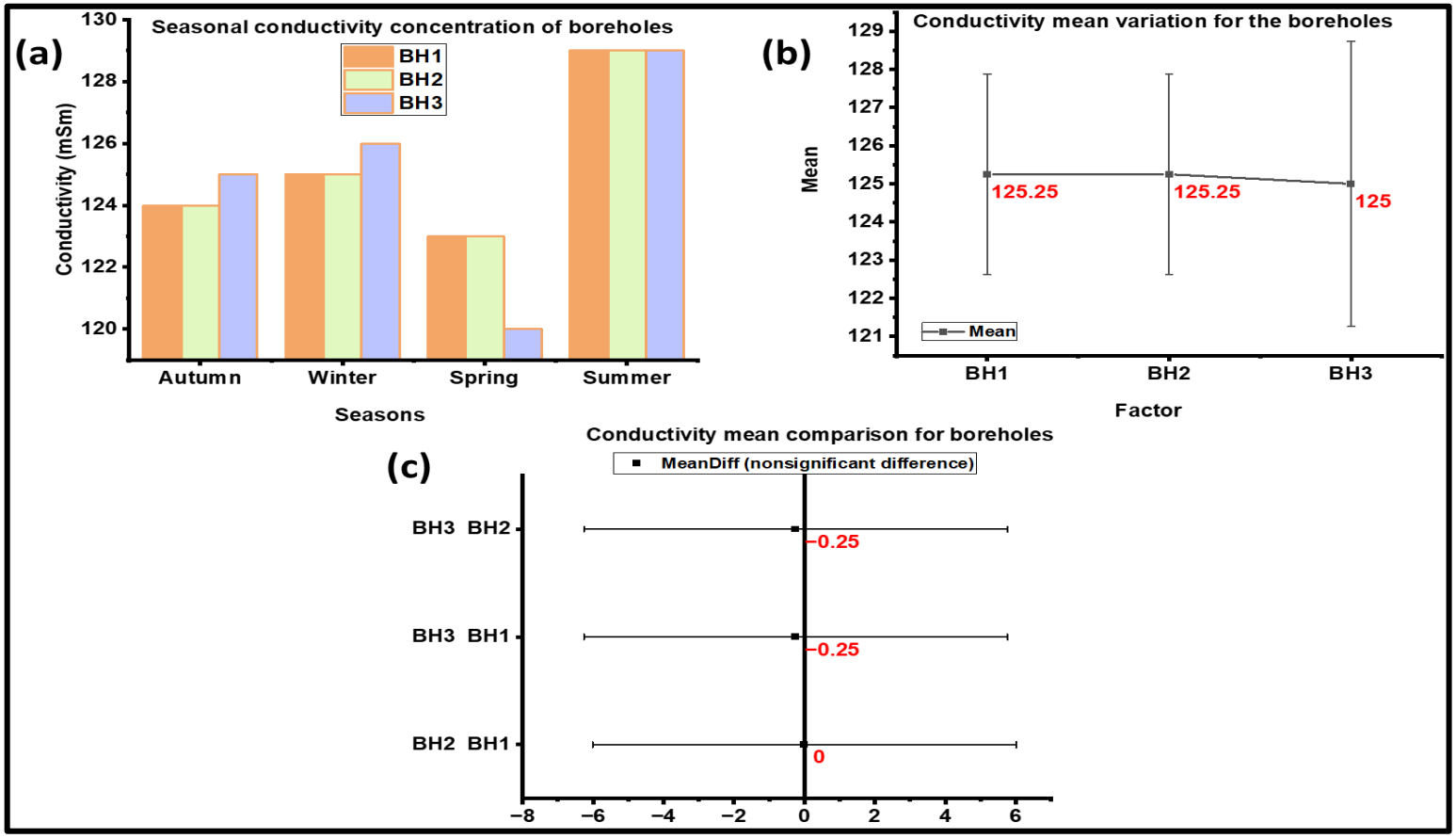

Figure 7. (a) Electrical conductivity values for the borehole sites from autumn to summer, (b) Electrical conductivity mean variation for the borehole sites from autumn to summer, (c) Electrical conductivity mean comparison for the borehole sites from autumn to summer

Even though all the results were within the prescribed SANS 241:2015 limits, the irrigation standard limits were exceeded, and there were variations throughout the sampling season. There was a reduction in all boreholes in the EC during the spring season, with the range of (120 –123 mS/m) which was followed by a slight spike in the summer sampling season, of 129 mS/m, which was the highest in the sampling season. The spike might be partly attributed to rain during the summer season, contaminants in the wastewater treatment plant surrounding area are dissolved, charging the groundwater, and ultimately increasing electrical conductivity. This study agrees with the concept by Joseph et al. (2010) which indicates that during the infiltration process, the amount of dissolved solids increases, resulting in water quality deterioration. According to Figure 7 (c), the p-value is not less than $\alpha = 0.05$, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the conductivity mean of the three boreholes.

4.2.3. Total Suspended Solids results

TSS are the portion of total solids in the sample retained by a filter that causes turbidity in water (DWAF, 1998). Studies have confirmed the correlation between TSS and turbidity; the higher the mass of retained solids, the more turbid the aquatic environment (Hannouche et al. 1996). Huey and Meyer (2010) indicated in their study that turbidity is a strong indicator of TSS as well as other enteric pathogens including *Cryptosporidium* spp. and *Giardia duodenalis.* Based on the presented data in Figure 8, the comparison of all boreholes in wastewater treatment with SA water guidelines indicates some of the boreholes exceeded the permissible limits. The TSS concentration as prescribed by South African water guidelines for the aquatic ecosystem is < 100 mg/L (DWAF, 1996b). Figure 9 shows the TSS mean values, mean variation, and mean comparison for the borehole sites from the autumn to the summer season. According to Figure 9 (c), the p-value is not less than $\alpha = 0.05$, and thus we fail to reject the null hypothesis. This means there is no statistically significant difference between the TSS mean of the three boreholes.

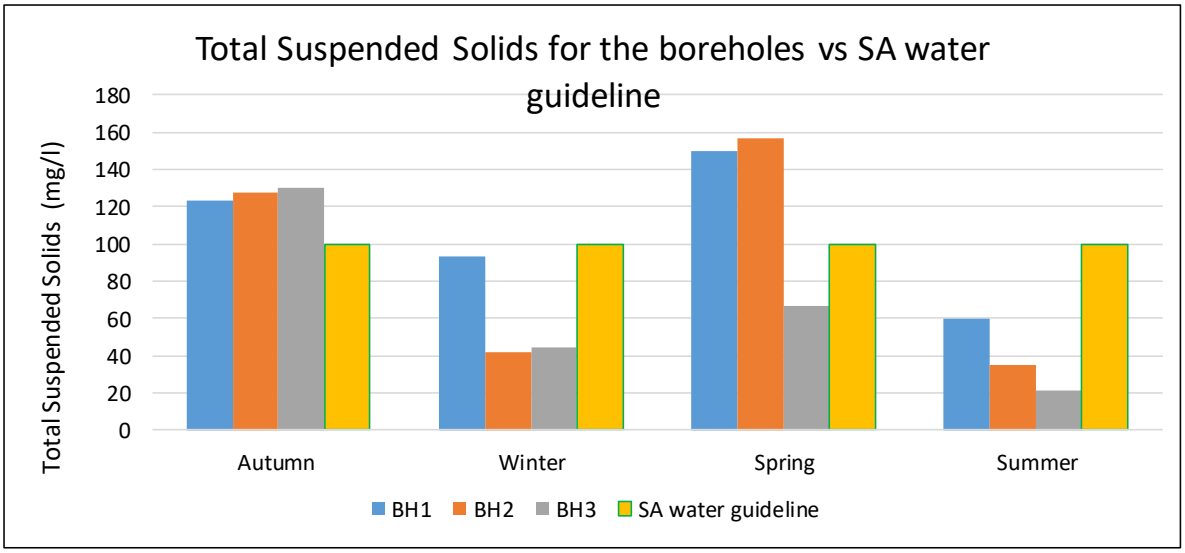

Figure 8. Graphical presentation of boreholes conductivity against SA water guideline

The measured TSS values from all the boreholes ranged from 21 to 157 mg/L and are shown in Figure 9 (a), with fluctuations in sampling seasons. In two sampling seasons (autumn and spring), the total suspended was above 100 mg/L. The autumn sampling season has the highest results, with all the boreholes exceeding the limit, this might be attributed to the rain that was received in autumn while the WWTW was not operational for a while, which resulted in untreated wastewater accumulation, consequently contaminating groundwater. The results are in agreement with the study conducted in Brazil in the public water treatment system, in their findings there was a correlation between the high dissolved substances and turbidity in water with the abundance of *Cryptosporidium* and *Giardia* spp. (Almeida et al., 2015). The winter and summer sampling results were within the SA guideline limits of < 100 mg/L, however, there were variations in the results.

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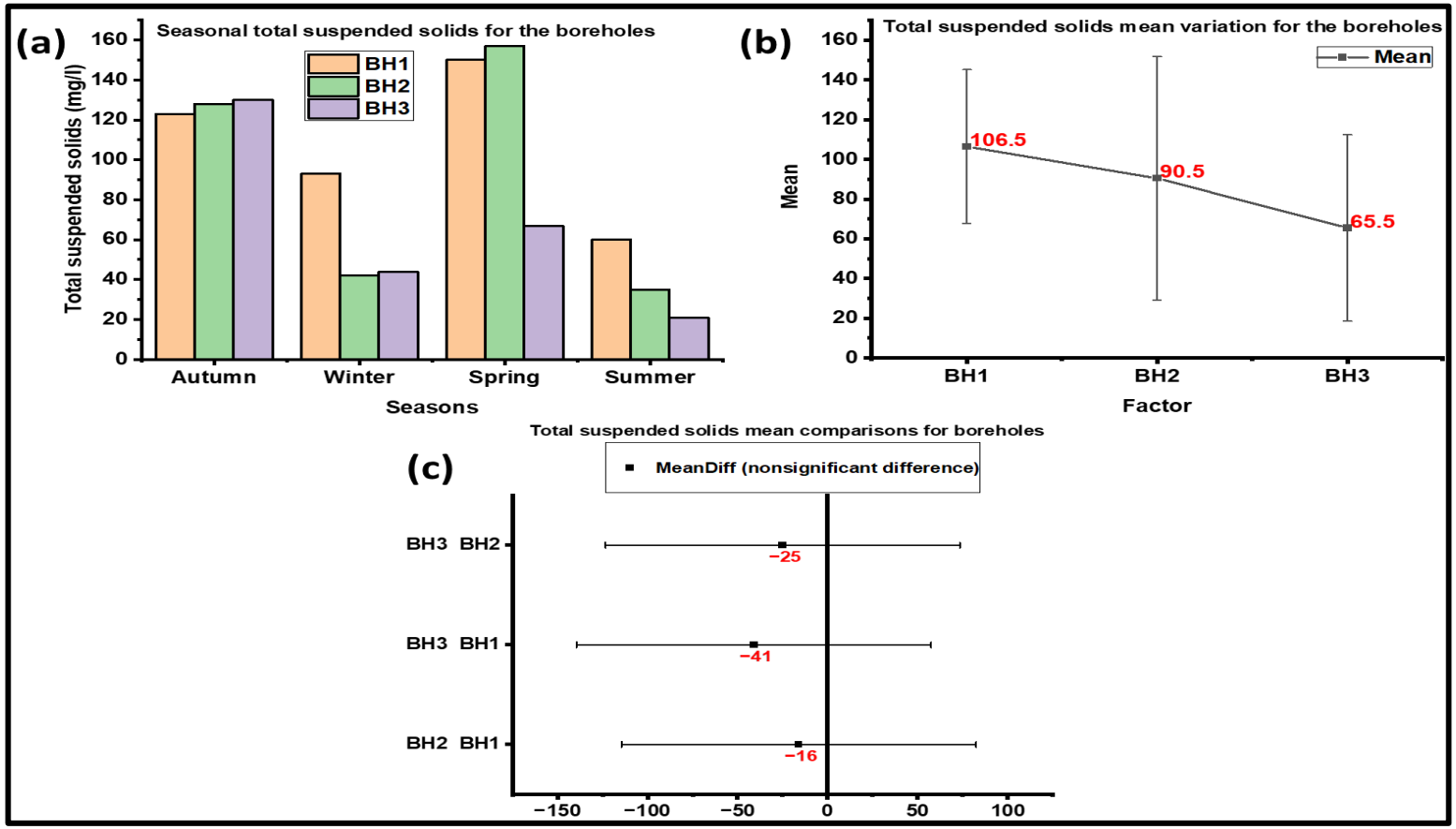


Figure 9. (a) Total suspended solids values for the borehole sites from autumn to summer, (b) Total suspended solids mean variation for the borehole sites from autumn to summer, (c) Total suspended solids mean comparison for the borehole sites from autumn to summer

4.2.4 Chloride as Cl results

Chloride in groundwater can be attributed to several factors in the wastewater treatment plant including, salt deposits from weathering, and the wastewater treatment process (WHO, 2018). Chlorine is naturally present in water however, an increased amount of chloride in the aquatic ecosystem will affect the mortality and reproduction of aquatic species due to increased acidity in water(Adeyemo et al., 2019; Brungs, 1976).

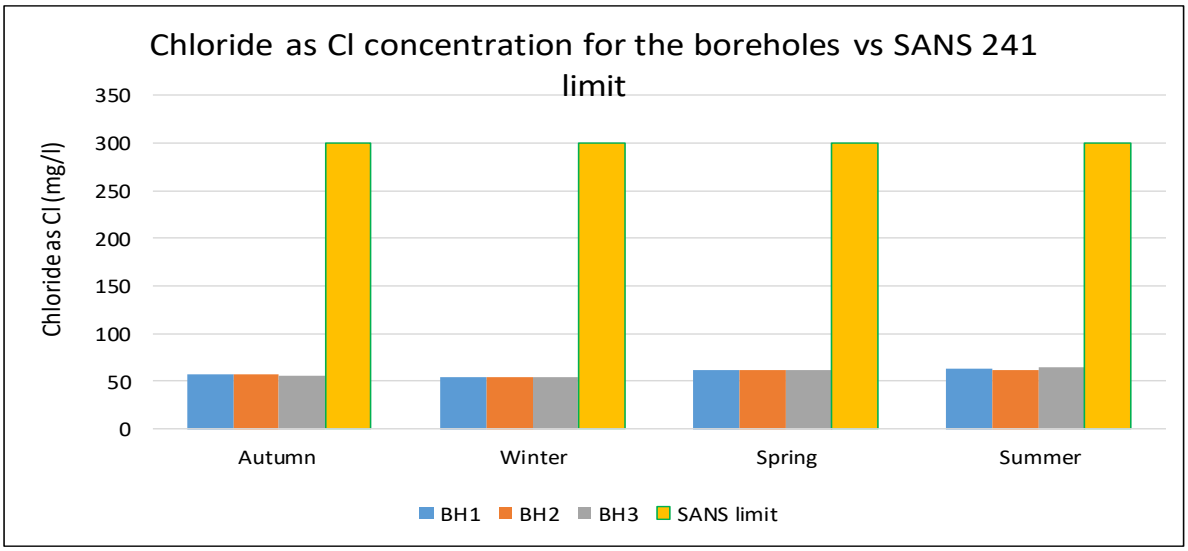


Figure 10. Graphical presentation of borehole's chloride as Cl concentration against SANS 241:2015 standard

Figure 11 shows the Chloride mean values, mean variation, mean comparison, and standard deviation for the borehole sites from autumn to summer. The measured Chloride values from all the boreholes ranged from 54 – 64 mg/L and are shown in Figure 11 (a). Based on the presented data in Figure 10, the comparison of Chloride results of all the boreholes in the wastewater treatment with the SANS 241, indicates that all the results were within the permissible limits. The chloride limit as prescribed in SANS 241:2015 should be 300 mg/L at 25°C**.** According to Figure 11 (c), the p-value is not less than $\alpha = 0.05$, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the conductivity mean of the three boreholes.

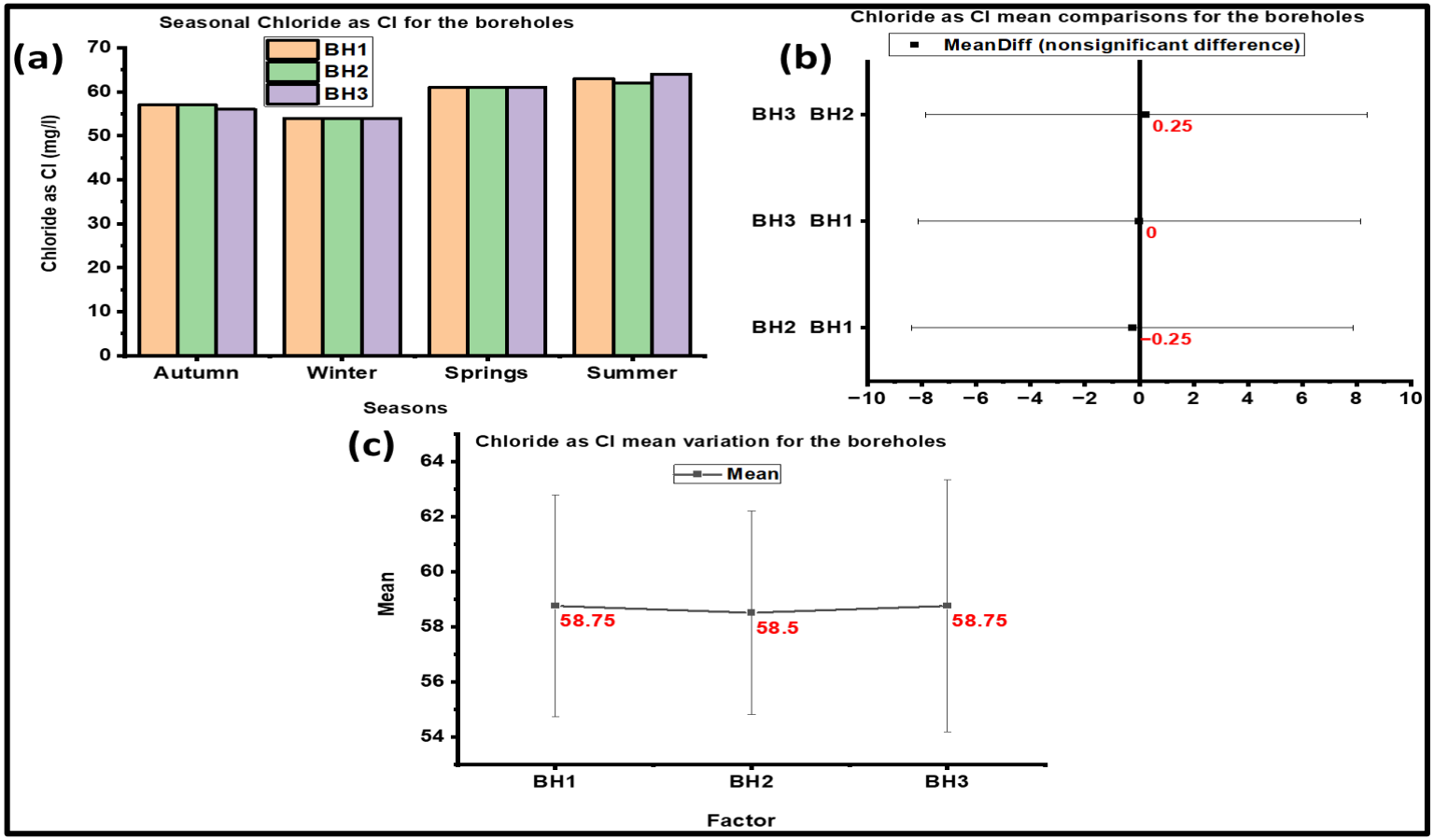


Figure 11. (a) Chloride as Cl values for the borehole sites from autumn to summer, (b) Chloride as Cl mean variation for the borehole sites from autumn to summer, and (c) Chloride as Cl mean comparison for the borehole sites from autumn to summer.

4.2.4. Nitrate as N results

Nitrates in the aquatic ecosystem can be coming from various sources including fertilized soil, wastewater, anthropogenic causes, and septic system (Ward et al., 2018). Even though plants require some nitrate for a better production yield, the excessive amount of nitrates end up building up in the soil and some leach through groundwater, the accumulation of nitrates in plants is harmful to human health

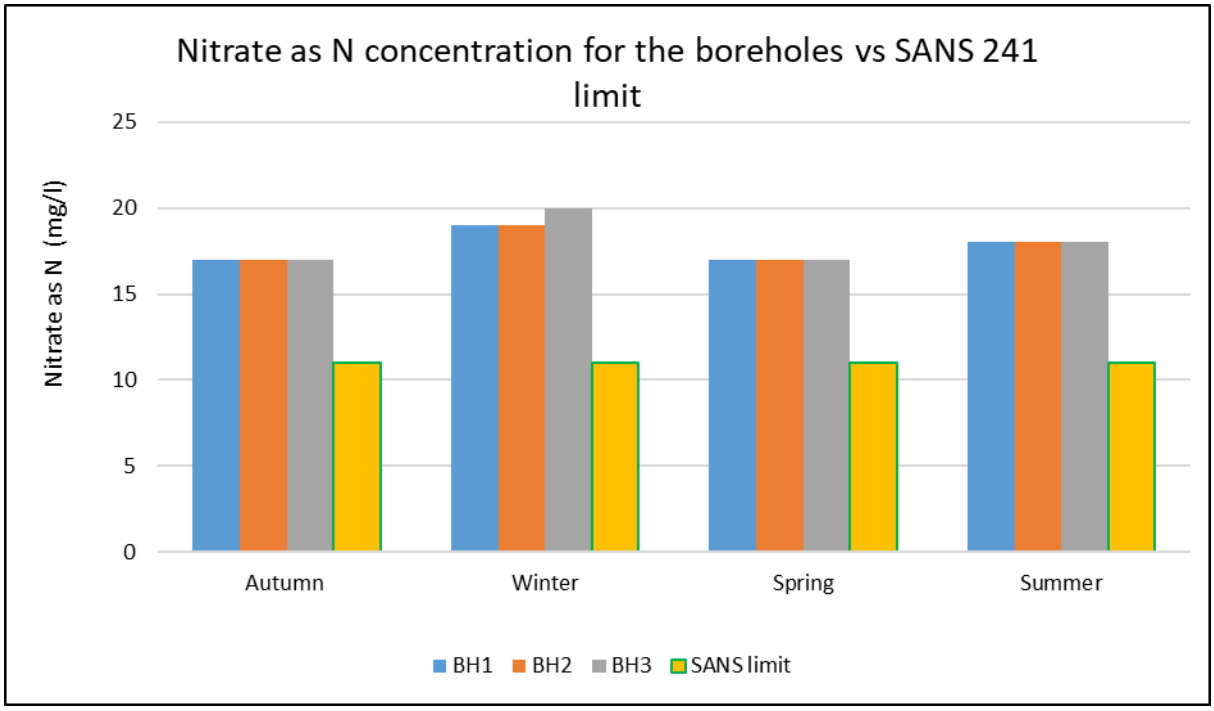


Figure 12. Graphical presentation of boreholes Nitrate as N against SANS 241:2015

Based on the presented data in Figure 12, the comparison of all boreholes in the vicinity of the wastewater treatment plant with SANS241:2015 indicates in all the boreholes nitrate concentration exceeded the permissible limits during all sampling seasons. The nitrate concentration as prescribed by SANS 241 is ≤11 mg/L, 0-100 mg/L for agricultural purposes, without adverse effects on livestock (DWAF, 1996d). According to Figure 13 (c), the p-value is not less than α = 0.05, and we fail to reject the null

hypothesis. This means there is no statistically significant difference between the Nitrate mean of the three boreholes.

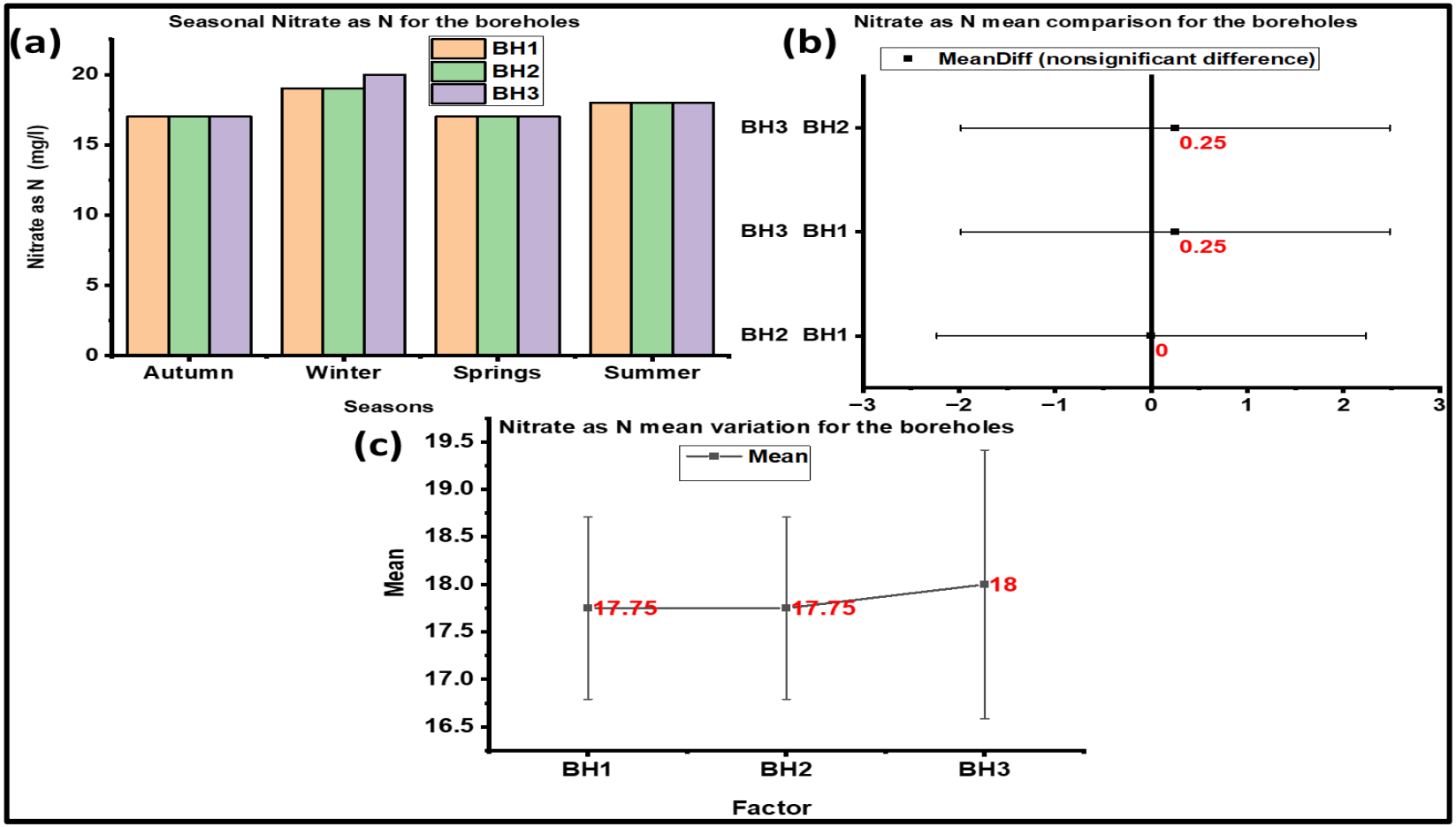


Figure 13. (a) Nitrate as N values for the borehole sites from autumn to summer, (b) Nitrate as N mean variation for the borehole sites from autumn to summer, (c) Nitrate as N mean comparison for the borehole sites from autumn to summer.

Figure 13 shows the nitrate as N mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. The measured Nitrate values from all the boreholes in sampling seasons ranged from 17 – 20 mg/L as shown in Figure 13 (a). In all the boreholes, there was not much fluctuation throughout the sampling season. These elevated nitrates concentrations in the wastewater treatment boreholes might be attributed to wastewater that is not adequately treated consequently infiltration of the groundwater. Berndt, (1990) study in Florida on the distribution of nitrate in groundwater concludes that municipal sewage treatment plant effluent is one of the major sources of high nitrates in groundwater.

4.2.5. Total phosphate as P results

Phosphorus in aquatic ecosystems occurs as a dissolved orthophosphate (PO_4), rarely as phosphorus (P) (Baldwin, 2013; Kotoski,1997). Phosphate is considered a crucial nutrient in the aquatic environment. It originates from surface runoff, wastewater, and fertilizers by attaching itself eventually getting to the water bodies (Correll, 1999). Based on the presented data in Table 12, the comparison of all boreholes in wastewater treatment with SA water guidelines indicates in all the boreholes phosphate concentration exceeded permissible limits during all sampling seasons. The orthophosphate concentration level as prescribed by South African guidelines is recommended to be ≤ 0.005 mg/L in oligotrophic conditions, 0.005-0.025 mg/L in mesotrophic, 0.025-0.250 mg/L and > 0.250 mg/L in hypertrophic conditions, without causing a nuisance to aquatic ecosystem inhabitants (DWAF, 1996b).

Table 12. Phosphate as P concentration of the boreholes against South African Water Guidelines: Aquatic ecosystem

Seasons	BH1	BH2	BH3	SA Water Guidelines
Autumn	0,2	0,2	0,2	0.005
Winter	0,2	0,2	0,2	0.005
Spring	0,2	0,2	0,2	0.005
Summer	0,2	0,2	0,2	0.005

According to Figure 13 (c), the p-value is not less than α = 0.05, thus we fail to reject the null hypothesis. This means there is no statistically significant difference between the Nitrate mean of the three boreholes. Figure 14 shows the Phosphate as P mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. The measured Phosphate values from all the boreholes in sampling seasons was 0.2 mg/L as shown in Figure 14 (a).

In all the boreholes, there were no variations throughout the sampling season. The high concentration of phosphate could indicate that there was contamination from the surrounding resulting in infiltration into the groundwater. These excessive amounts of phosphorus have an impact on the microorganisms in water and facilitate the algal bloom in water bodies due to the eutrophication process (Feng et al., 2023; Carpenter, 2005). Hence, groundwater with elevated levels of phosphate can potentially alter the species and introduce toxic and nuisance species in water bodies (Alexander et al., 2017)

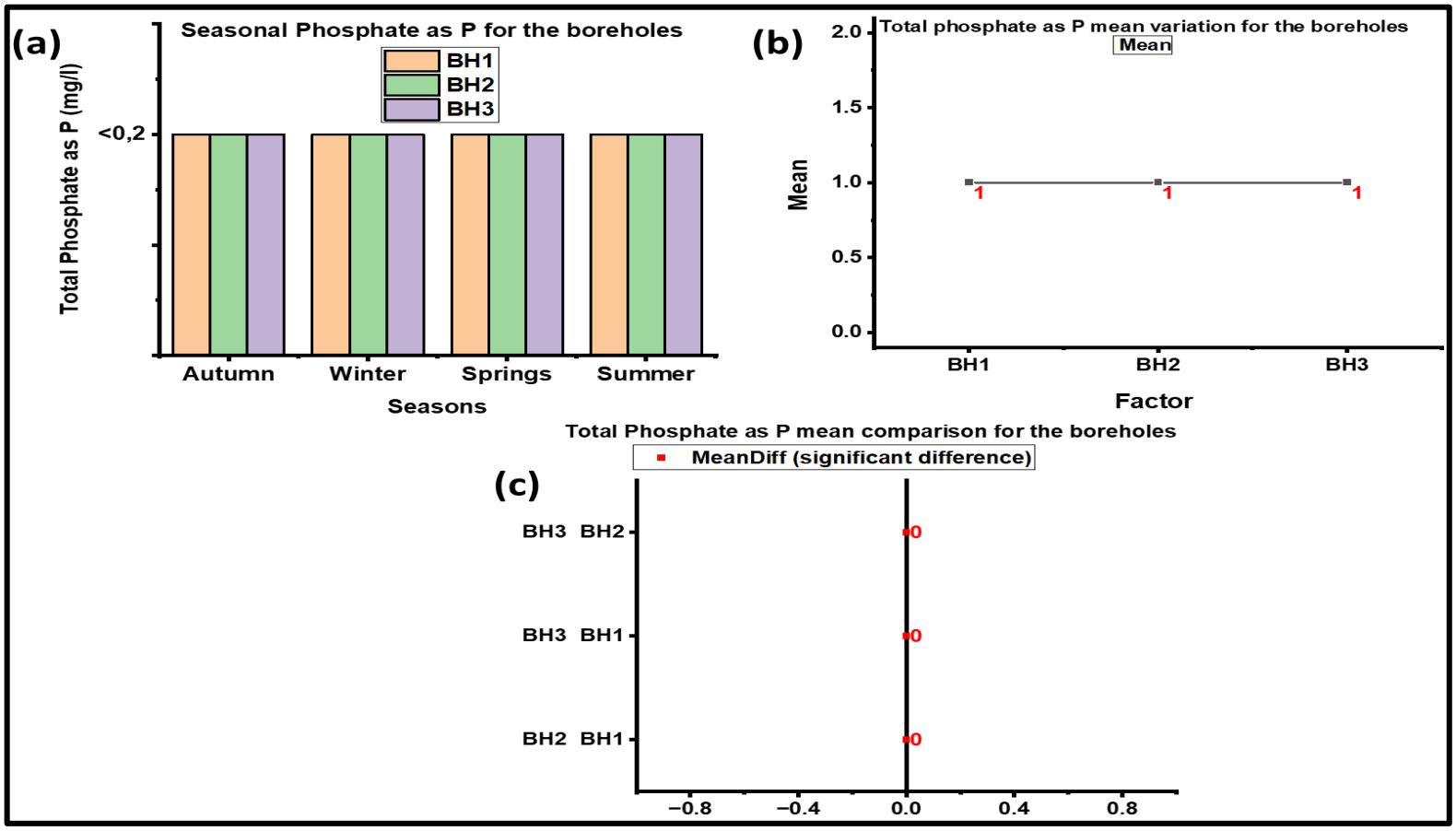


Figure 14. (a) Phosphate as P values for the borehole sites from autumn to summer, (b) Phosphate as P mean variation for the borehole sites from autumn to summer, (c) Phosphate as P mean comparison for the borehole sites from autumn to summer.

4.2.6. Free and saline ammonia results

Ammonia naturally occurs in water bodies as free and saline ammonia which can be converted to nitrite (NO_2) and nitrate (NO_3) by bacteria (WHO, 1996). The ammonia originality in the aquatic environment is diverse, stemming mainly from the decomposition of nitrogen in wastewater from domestic, industrial, and agricultural practices, its presence indicates sewage and animal feed contamination (Corral, 2021; Roch & Maine, 2015; EPA, 1999).

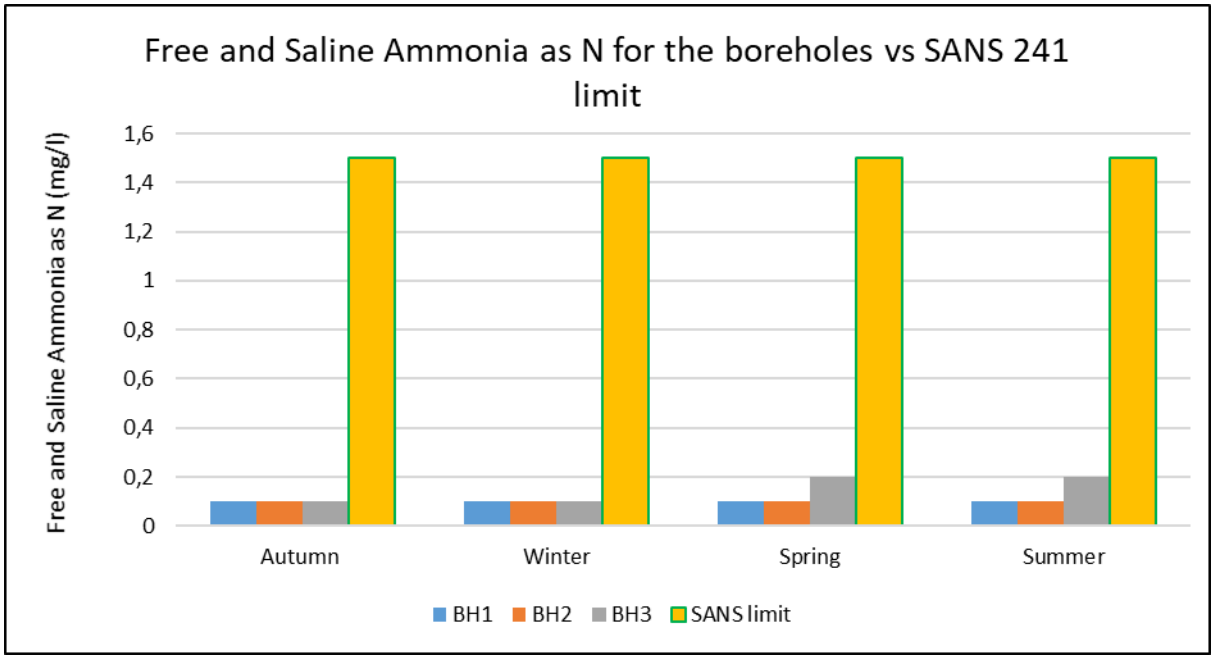


Figure 15. Graphical presentation of boreholes Free and saline ammonia as N against SANS 241:2015

Figure 15 shows the Free and saline ammonia as N mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. The measured free and saline ammonia as N values from all the boreholes in sampling seasons ranged from 0.1 – 0.2 mg/L as shown in Figure 16 (a). In all the boreholes, there was not much fluctuation throughout the sampling season. According to Figure 16 (c), the p-value is not less than $\alpha = 0.05$, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the free and saline ammonia as the N mean of the three boreholes.

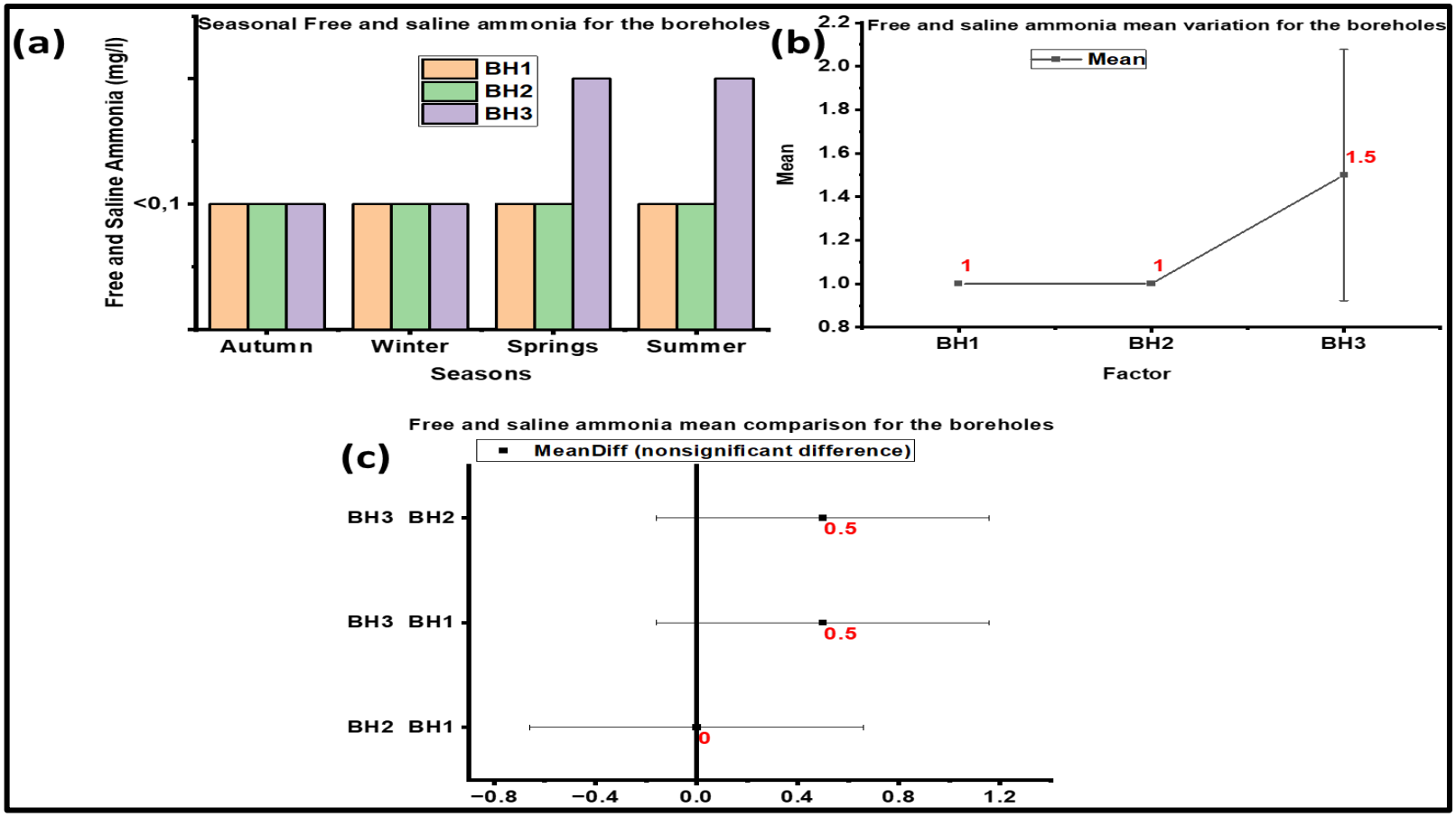


Figure 16. (a) Free and saline ammonia values for the borehole sites from autumn to summer, (b) Free and saline ammonia mean variation for the borehole sites from autumn to summer, (c) Free and saline ammonia mean comparison for the borehole sites from autumn to summer

Based on the presented data in Figure 15, the comparison of all boreholes in wastewater treatment with SANS241:2015 indicates in all the boreholes free and saline ammonia as N concentration is within the permissible limits during all sampling seasons. The free and saline ammonia as N concentration as prescribed by SANS 241 is 1.5 mg/L and 0.025 mg/L according to aquaculture (DWAF, 1996b). Therefore, even though the concentration of 0.1 mg/L reported during the sampling season is within the drinking water level, the limit reported is not fit for the aquatic ecosystem. Excessive levels of ammonia can alter water bodies leading to eutrophication. The high amount of ammonia in groundwater in a wastewater treatment plant can be attributed to poorly treated wastewater effluent leading to salinity. Several studies have been conducted indicating the cause of extreme levels of free and saline ammonia in the aquatic system as sewage leaks increasing fecal contamination and inadequately treated effluent (Roch & Maine, 2015; Dube et al., 2010; WHO, 1996).

4.2.7. Faecal coliforms results

Faecal coliforms was measured at each of the borehole sampling sites to determine the presence of fecal contamination. The high prevalence of these pathogenic bacteria in the borehole water samples is an indication of fecal contamination, which can be attributed to some leakage in the WWTWs (Dey et al., 2022; Leifels et al., 2019).

Based on the presented data in Figure 17, the comparison of fecal coliform results of all the boreholes in the wastewater treatment with the SANS 241 indicates that all the results exceeded the permissible limits. The fecal coliform limit as prescribed in SANS 241:2015 should be 0 cfu/100 mL, 10 cfu/100 mL for operations, 0-150 cfu/100 mL for recreational uses, like swimming, and 10 000/100 mL for irrigation purposes (DWAF, 1996b). Figure 18 shows the fecal coliform mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season.

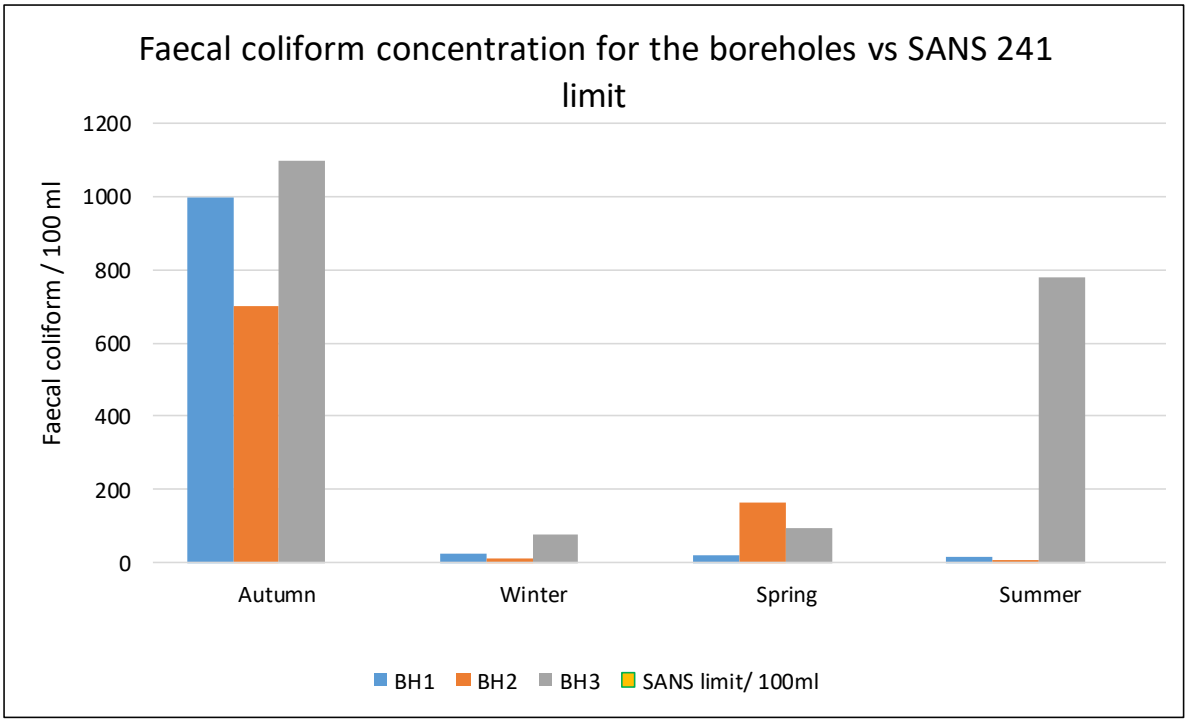


Figure 17. Graphical presentation of borehole's fecal coliform against SANS 241:2015 standard

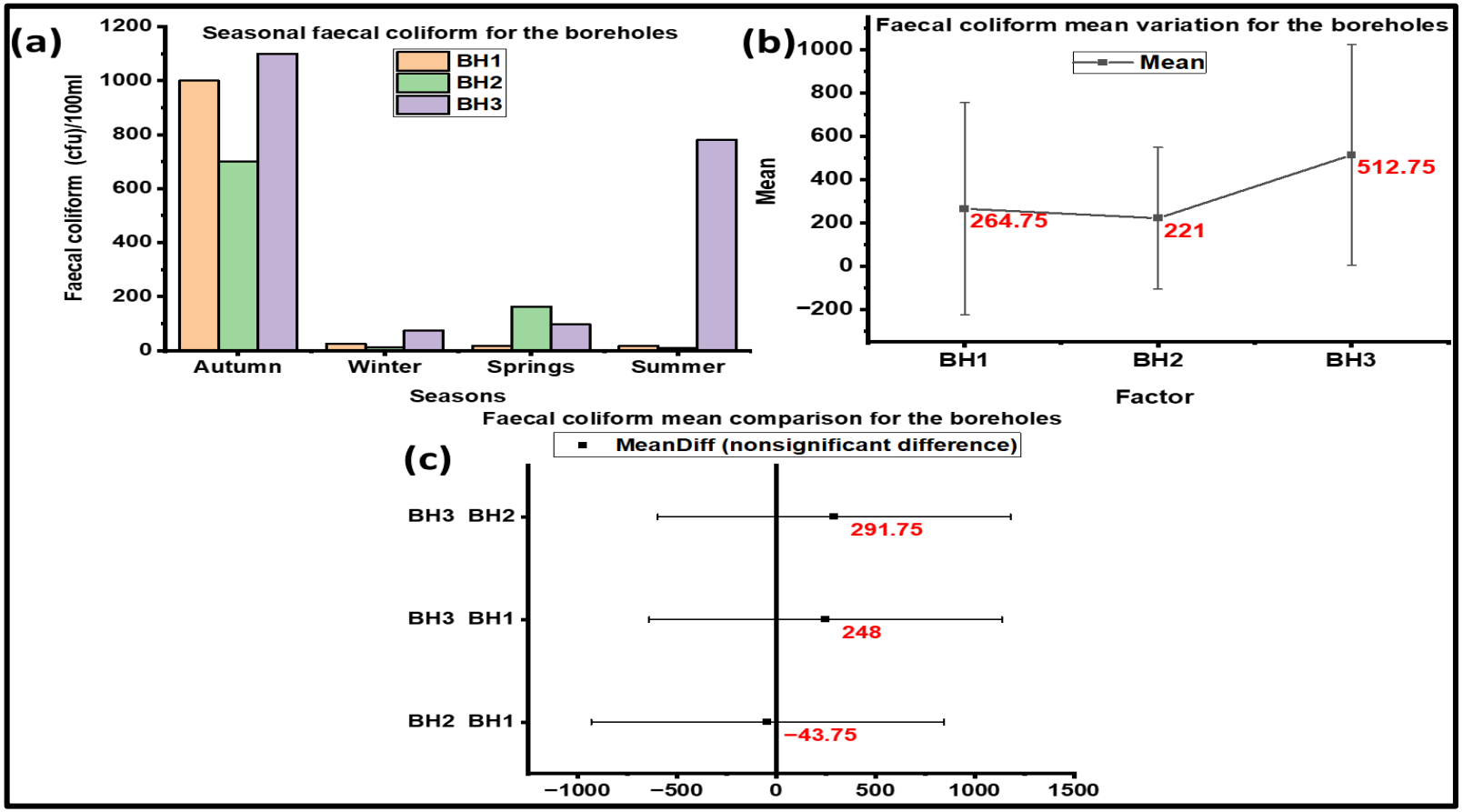


Figure 18. (a) Fecal coliform values for the borehole sites from autumn to summer, (b) Fecal coliform mean variation for the borehole sites from autumn to summer, (c) Fecal coliform mean comparison for the borehole sites from autumn to summer.

According to Figure 18 (c), the p-value is not less than α = 0.05, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the conductivity mean of the three boreholes. The measured bacterial counts from all the boreholes ranged from 9 cfu/ 100 mL to 1100 cfu/ 100 mL and are presented in Figure 18 (a), with fluctuations throughout sampling seasons. During the first sampling season in autumn, the plant was not functioning for a couple of weeks due to vandalism, this might have contributed to the highest results for the sampling season due to the accumulation of untreated wastewater, resulting in overflows to the ground.

The results obtained in this study are in line with other studies in waterbodies on the prevalence of fecal coliform, the bacterial counts are exceedingly high after the rainfall seasons (Tornevi et al., 2014; Hill et al., 2006; Shehane et al., 2005). The presence of these bacteria poses a health risk to humans if the groundwater is utilized for consumption or agricultural purposes without treatment. The fecal coliform evaluation aim was used as an indicator of the prevalence of *Cryptosporidium* and *Giardia* spp. in groundwater in this study. In autumn when the excessively high bacterial counts of fecal coliforms were reported, the *Cryptosporidium* and *Giardia* spp. were also confirmed. Literature has confirmed the correlation between fecal coliforms prevalence of *Cryptosporidium* and *Giardia* spp. (Ongerth et al., 2018; Prystajecky et al., 2014;Ratnayaka et al., 2009).

4.2.8. *E. coli*

Figure 19 shows the *E.coli* mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. The measured bacterial counts from all the boreholes ranged from 7 CFU/100 mL to 900 CFU/100 mL and are shown in Figure 20 (a). Based on the presented data, the average bacterial counts for the boreholes BH1, BH2 and BH3 are (206.25 CFU/100 mL, 176.5 CFU/100 mL, and 416.5 CFU/100 mL), with significant variation between them. BH3 had the highest levels with 900 CFU/100 mL for the sampling season. The acceptable limit of *E. coli* in drinking water is 0 CFU/100 mL according to SANS 241:2015, South African water regulation 0 CFU/100 mL for irrigation and 0-130 CFU/100 mL for recreational purposes (DWAF, 1996b). Figure 19 shows the comparison of *E.coli* results of all the

boreholes in the wastewater treatment with the SANS 241, indicating that all the boreholes exceeded the permissible limits.

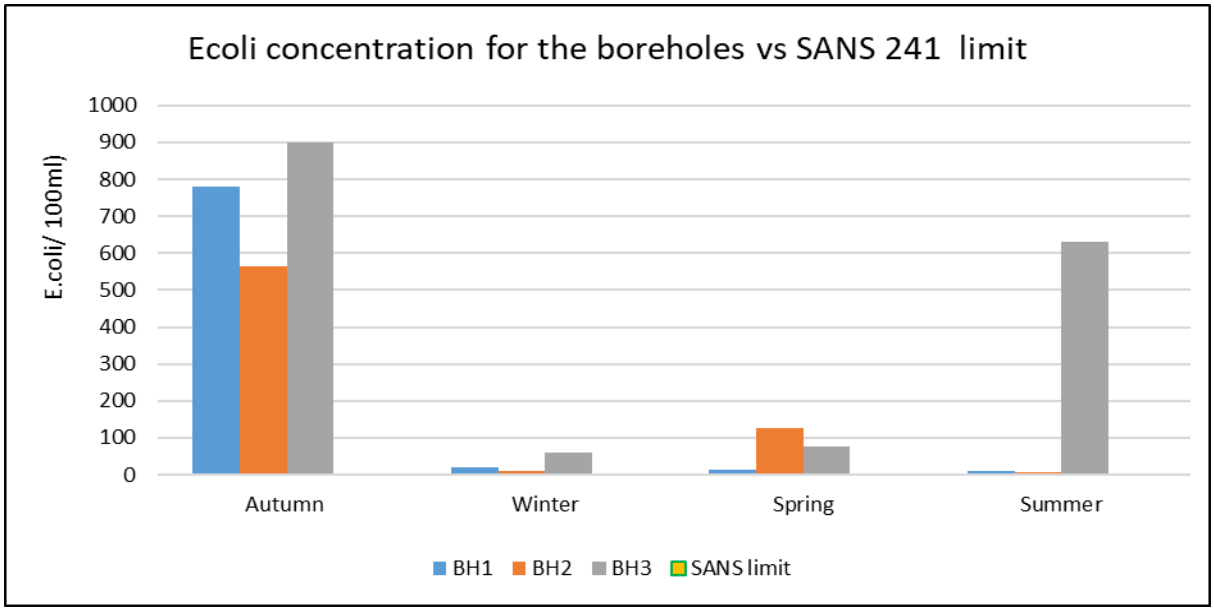


Figure 19. Graphical presentation of borehole's *E.coli* against SANS 241:2015 standard

According to Figure 20 (c), the p-value is not less than α = 0.05, we fail to reject the null hypothesis. This means there is no statistically significant difference between the *E.coli* means of the three boreholes. *E.coli* is considered an important indicator of fecal contamination in aquatic ecosystems, and other waterborne pathogens abundances like *Cryptosporidium* and *Giardia* spp. (Rochelle-Newall et al., 2015; Odonkor & Ampofo, 2013). In the wastewater treatment plant, the high bacterial counts in the groundwater can be attributed to plant operational challenges resulting in overflows in the plant and seepages of wastewater, and leakages amongst other things (Bezuidenhout C, 2013). With shortages of water supply, treated wastewater, borehole water, and water from ponds and rivers are becoming an option, however, if not treated adequately, it can present risks. The prevalence of *E.coli* in drinking water, water used for irrigation, and recreational activities such as swimming presents a health risk to humans and animals exposed (Bonetta et al., 2022; Balkhair, 2016)

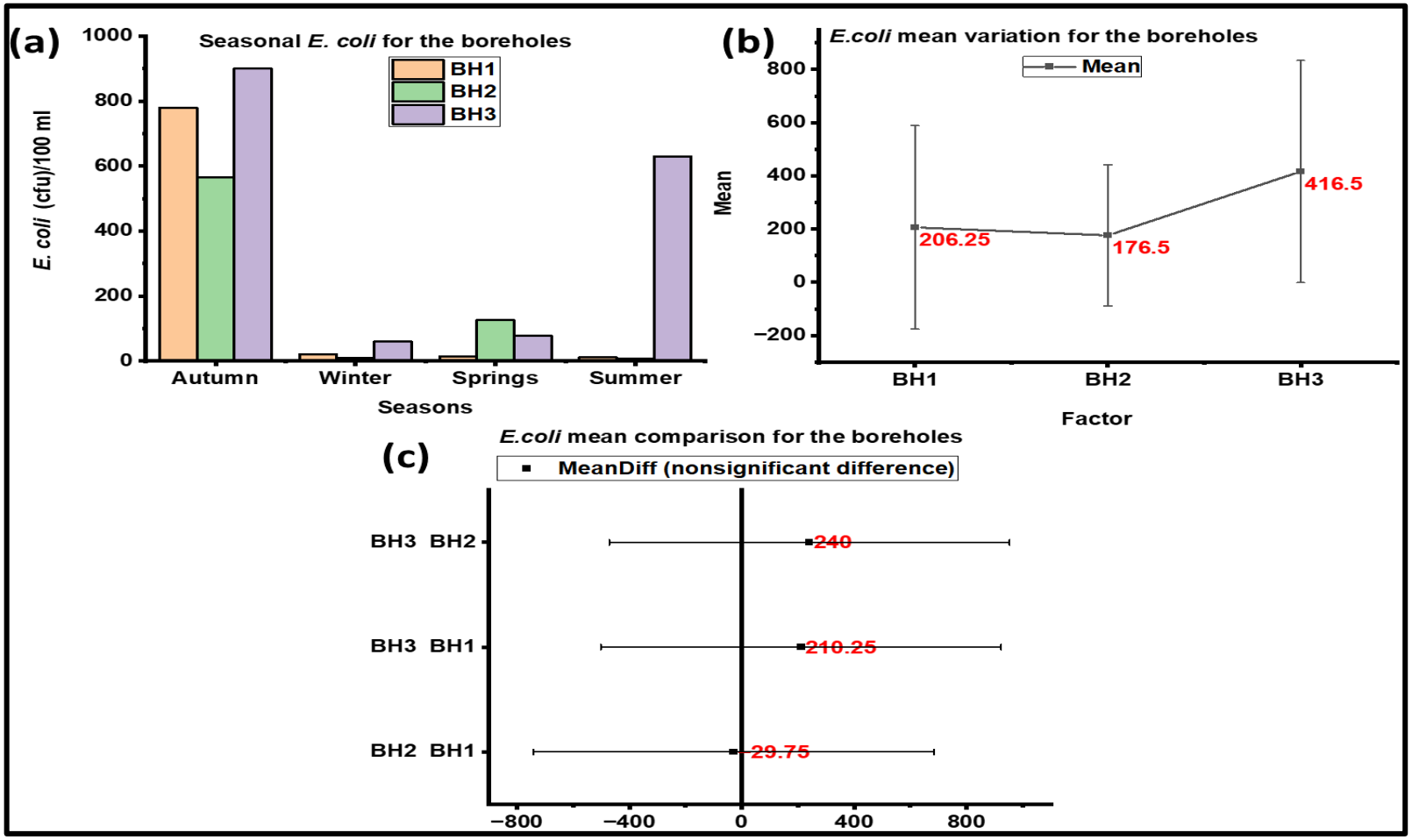


Figure 20. (a) *E. coli* values for the borehole sites from autumn to summer, (b) *E. coli* mean variation for the borehole sites from autumn to summer, (c) *E.coli* mean comparison for the borehole sites from autumn to summer.

4.2.9. *Cryptosporidium* results

Cryptosporidium is an enteric pathogenic protozoan that causes gastrointestinal illness in humans and animals (Lam et al., 2014). It is found mostly in an aquatic environment, which makes it possible for their oocyst to survive (Cho et al., 2013).

Based on the presented data in Figure 21, the comparison of *Cryptosporidium* results of all the boreholes in the wastewater treatment with the SANS 241, indicates that one sampling season results exceeded the permissible limits. The *Cryptosporidium* limit as prescribed in SANS 241:2015 should be 0 cfu/10 l, 0 cfu/10 L for domestic uses (DWAF, 1996c). Figure 22 shows the *Cryptosporidium* mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season.

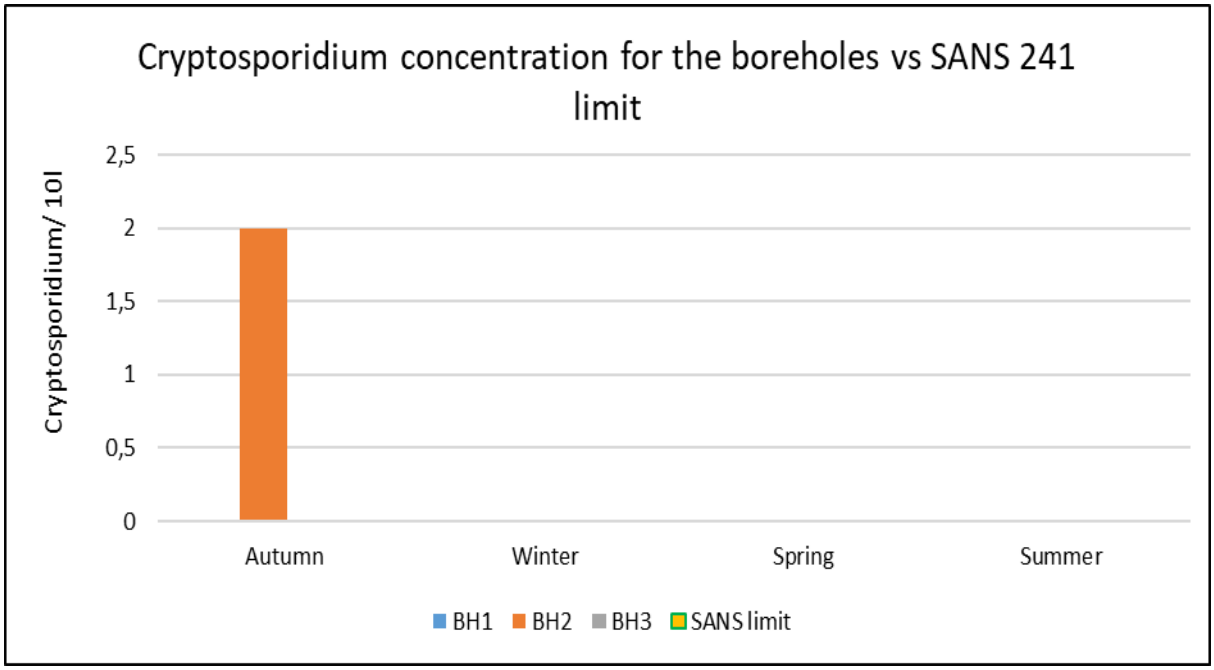


Figure 21. Graphical presentation of borehole's *Cryptosporidium* against SANS 241:2015 standard

The measured protozoan oocysts from all the boreholes were two (2) oocysts, detected in autumn, in BH2 and are presented in Figure 22 (a), with the other boreholes not reporting any *Cryptosporidium* oocysts throughout sampling seasons.

During the first sampling season in autumn, the plant was not functioning for a couple of weeks due to vandalism; this might have contributed to the detection of *Cryptosporidium* in one season only due to the accumulation of untreated wastewater, resulting in overflows to the ground.

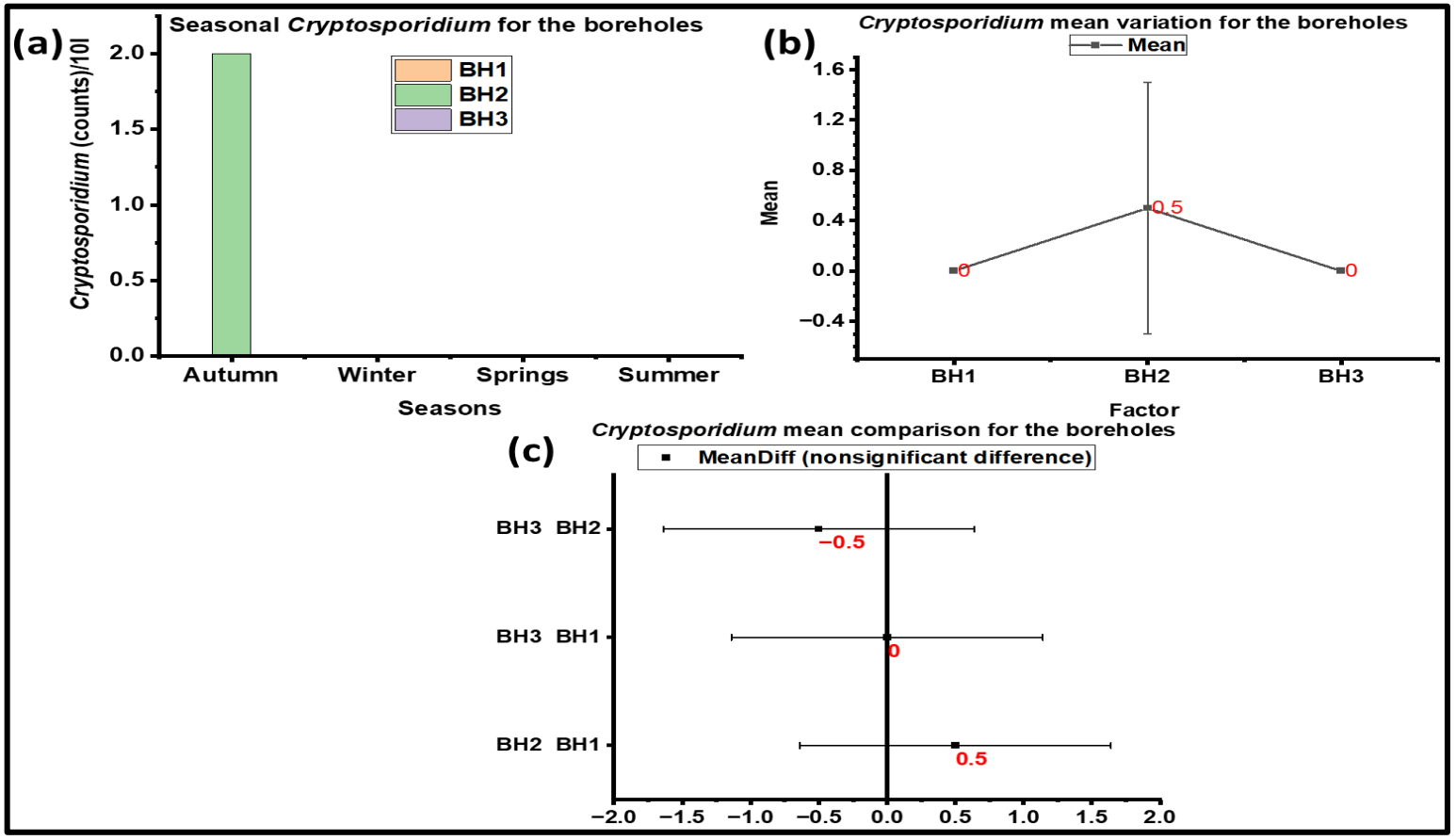


Figure 22. (a) *Cryptosporidium* values for the borehole sites from autumn to summer, (b) *Cryptosporidium* mean variation for the borehole sites from autumn to summer, (c) *Cryptosporidium* mean comparison for the borehole sites from autumn to summer.

The prevalence of the *Cryptosporidium* parasite in the borehole in WWTWs can be attributed to several factors including the ineffectiveness of chlorine to disinfect wastewater off the protozoan parasites to operational issues causing groundwater seepages of untreated water attenuation in the soil as identified in domestic and global studies of cryptosporidium in WWTW (Dungeni et al., 2010; Chique et al., 2020; King et al., 2017). Literature has pointed out that most wastewater treatment plant does not have adequate facilities to disinfect these enteric protozoan pathogens, which consequently end up in the public water supply (Watier-Grillot et al., 2022; Pignata et al., 2019).

The abundance of *Cryptosporidium* spp. in drinking water, water used for irrigation, and recreational activities like swimming used without pre-treatment will result in cryptosporidiosis outbreak, causing gastrointestinal illness in humans and animals (Ongerth et al., 2018; Chique et al., 2020). According to Figure 22 (c), the p-value is not less than $\alpha = 0.05$, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the conductivity mean of the three boreholes.

4.2.10. *Giardia* results

Giardia is primarily transmitted through the fecal-oral route as a 10-12 µm long *Giardia* cyst due to poor hygiene in infrastructure, but most cases are due to ingestion of contaminated water or food (Dixon, 2021). Inadequately treated wastewater used for irrigation may transmit giardia cysts (A. M. Nasser et al., 2012). Giardiasis is caused by the *Giardia* parasite, lamblia which causes gastrointestinal illness (EPA, 2000).

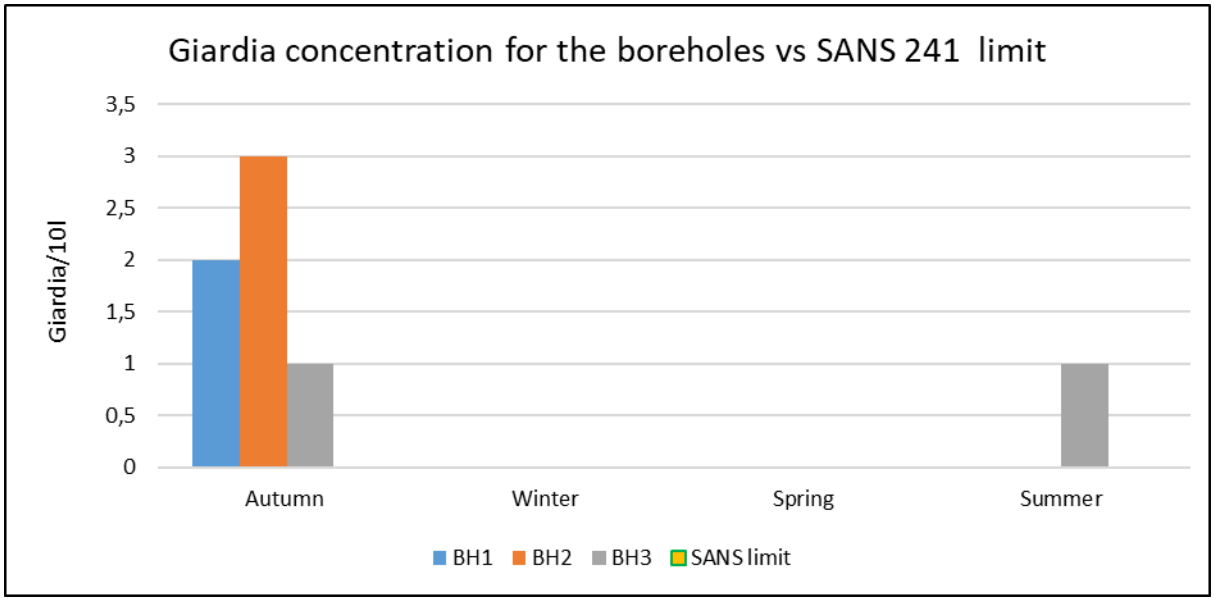


Figure 23. Graphical presentation of borehole's *Giardia* against SANS 241:2015 standard

Based on the presented data in Figure 23, the comparison of all boreholes in wastewater treatment with SANS241 indicates that some of the boreholes exceeded the permissible limits. The *Giardia* concentration limit as prescribed in SANS 241:2015 should be 0 cfu/10 l, 0 cfu/10 l for domestic uses (DWAF, 1996c). Figure 24. shows the *Giardia* mean values, mean variation, and mean comparison for the borehole sites from autumn to the summer season. According to Figure 24. (c), the p-value is not less than $\alpha = 0.05$, and we fail to reject the null hypothesis. This means there is no statistically significant difference between the giardia mean of the three boreholes.

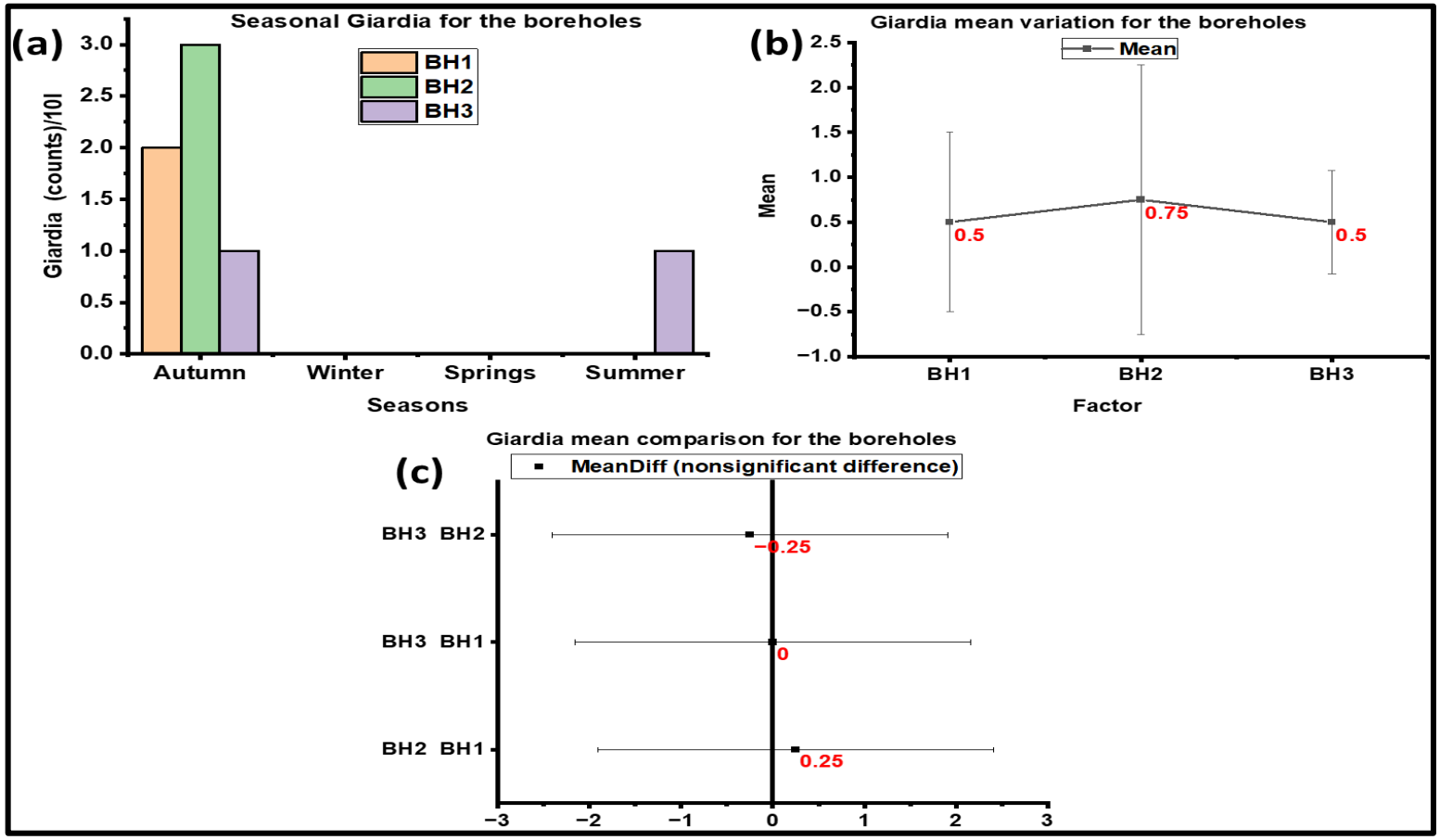


Figure 24. (a) Giardia values for the borehole sites from autumn to summer, (b) Giardia mean variation for the borehole sites from autumn to summer, (c) Giardia mean comparison for the borehole sites from autumn to summer

The measured *Giardia* values from all the boreholes ranged from 1 to 3 cysts and are shown in Figure 24 (a), with fluctuations in sampling seasons. In two sampling seasons (autumn and summer), *Giardia* was detected, in autumn, all boreholes tested positive for *Giardia*, with the highest cysts, and in summer only BH3. The prevalence of these enteric pathogens in groundwater indicates that the water was polluted with fecal matter, which is most likely due to fecal matter accumulating in the ground due to the plant not functioning in autumn or leakage of wastewater (Sroka et al., 2013; Nasser et al., 2012; Dungeni et al., 2010). This study showed consistency with Pitkänen et al. (2015) whose findings indicated that high *Giardia* cysts are detected in groundwater in autumn after the summer rains which might be attributed to more infiltration taking place.

4.2.11. Correlation analysis between protozoans, physicochemical and microbiological parameters

Correlation analysis was determined by looking specifically at the parameters that were selected based on their relationship with *Cryptosporidium* and *Giardia* as indicated in numerous studies on the detection of protozoans in water. The parameters of interest, Total Suspended Solids, *E.Coli*, and fecal Coliforms exceeded the limits during the study.

4.2.11.1. Total Suspended Solids

The trend observed in Figures 25 and 26 reveals that TSS and *Cryptosporidium* in the BH2 borehole had a positive correlation, also TSS showed a positive correlation with *Giardia* in all borehole sampling sites.

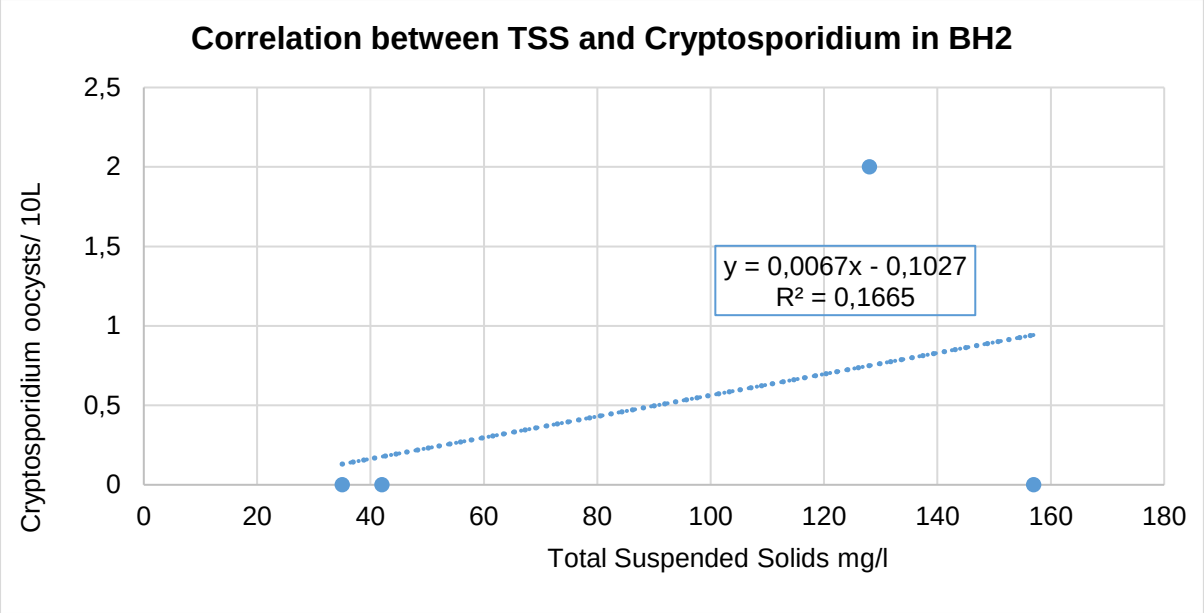


Figure 25. Correlation graph between TSS and *Cryptosporidium*

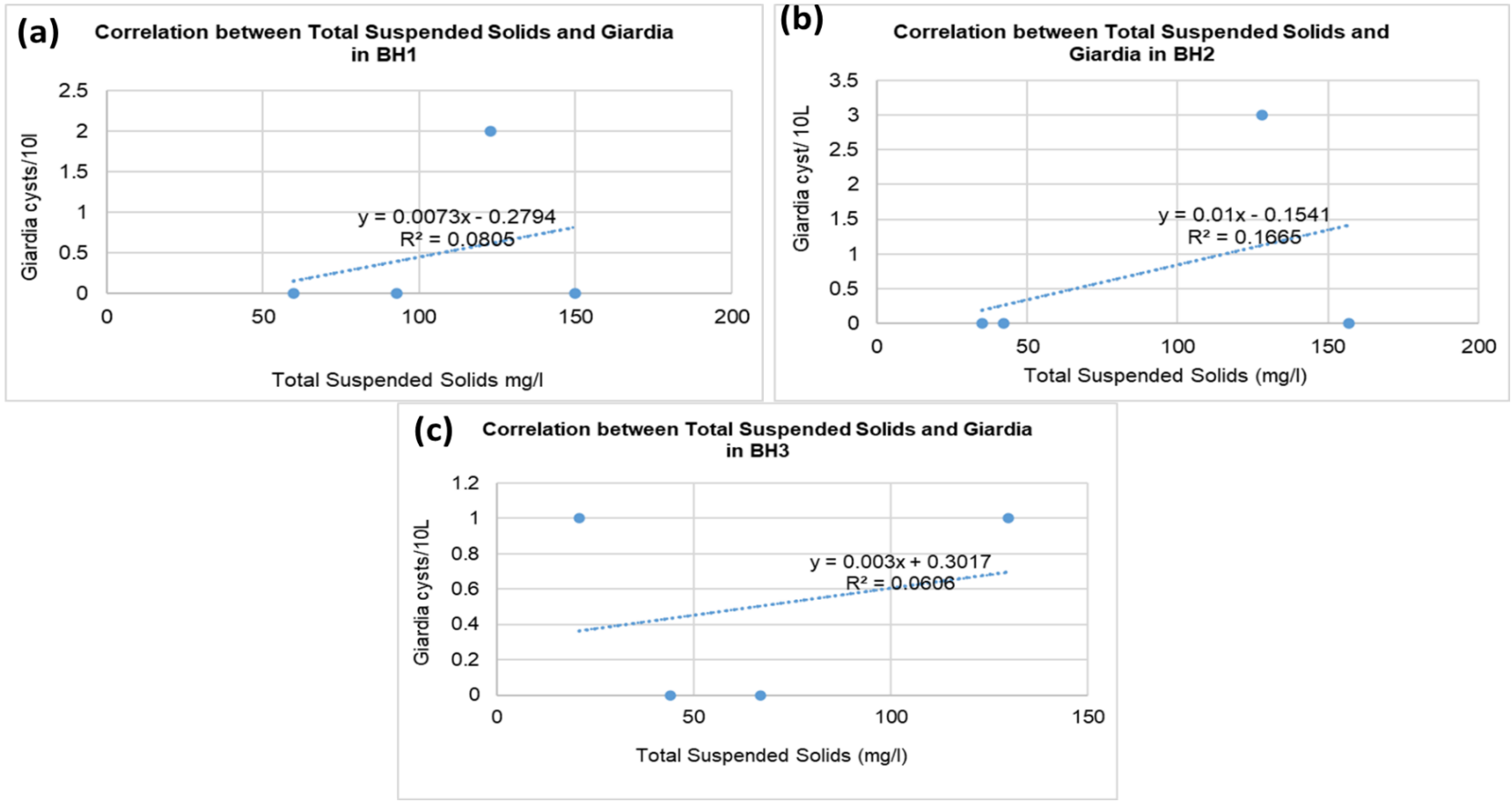


Figure 26. (a) Correlation between total suspended solids and *Giardia* in BH1 (b) Correlation between total suspended solids and *Giardia* in BH2 (c) Correlation between total suspended solids and Giardia in BH3.

4.2.11.2. *E. coli*

The trend observed in Figures 27 and 28 reveals that TSS and *Cryptosporidium* in the BH2 borehole had a positive correlation, also TSS showed a positive correlation with *Giardia* in all borehole sampling sites.

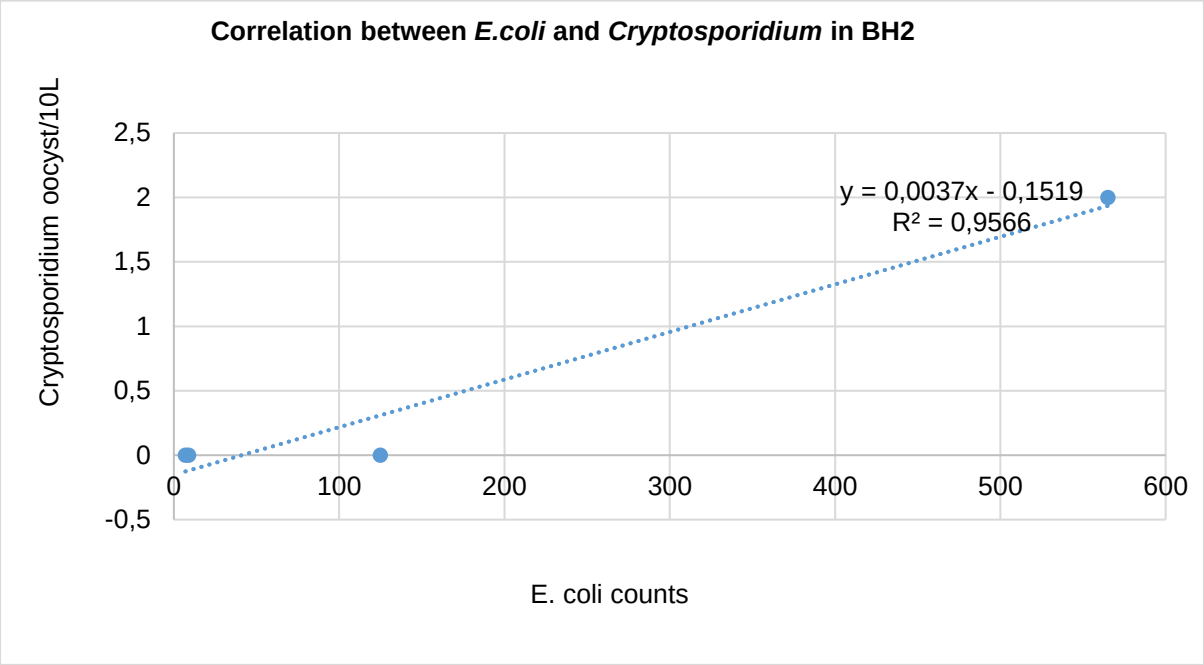


Figure 27. Correlation graph between *E.coli* and *Cryptosporidium*

4.2.11.3. Fecal Coliforms

The trend observed in Figures 29 and 30 reveals that TSS and *Cryptosporidium* in the BH2 borehole had a positive correlation, also TSS showed a positive correlation with *Giardia* in all borehole sampling sites.

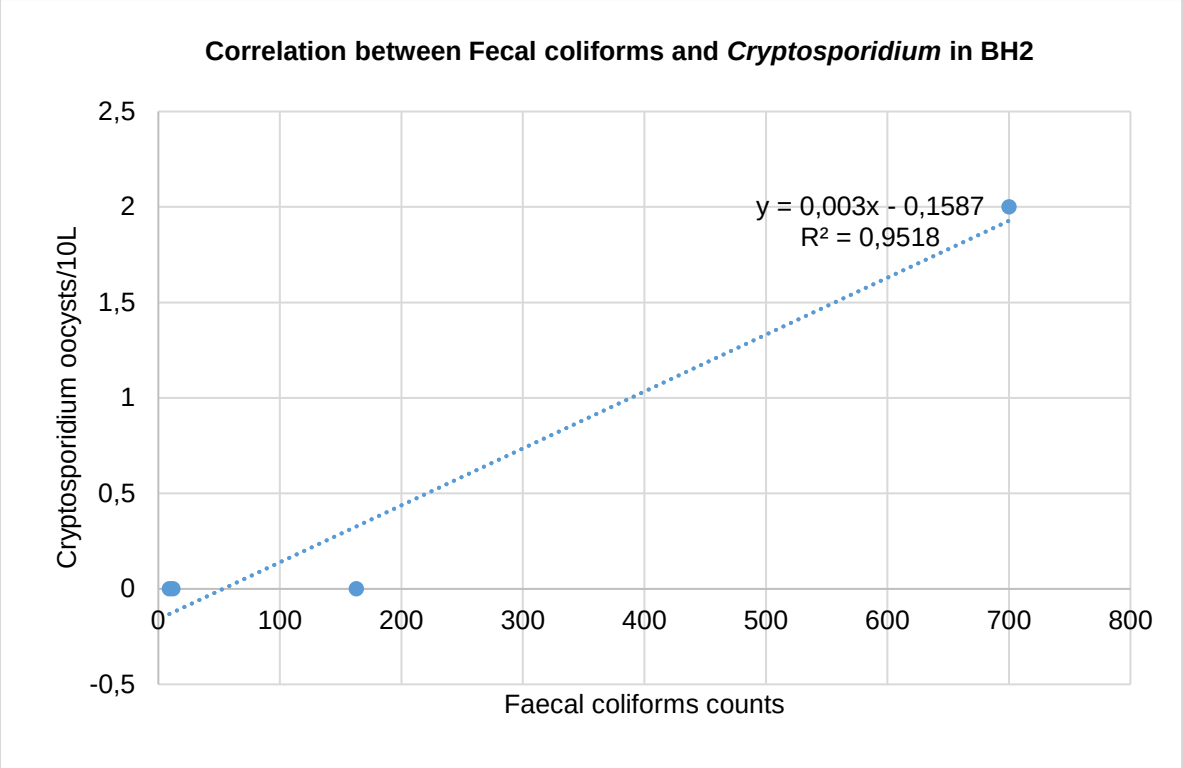


Figure 28. Correlation graph between fecal coliforms and *Cryptosporidium*

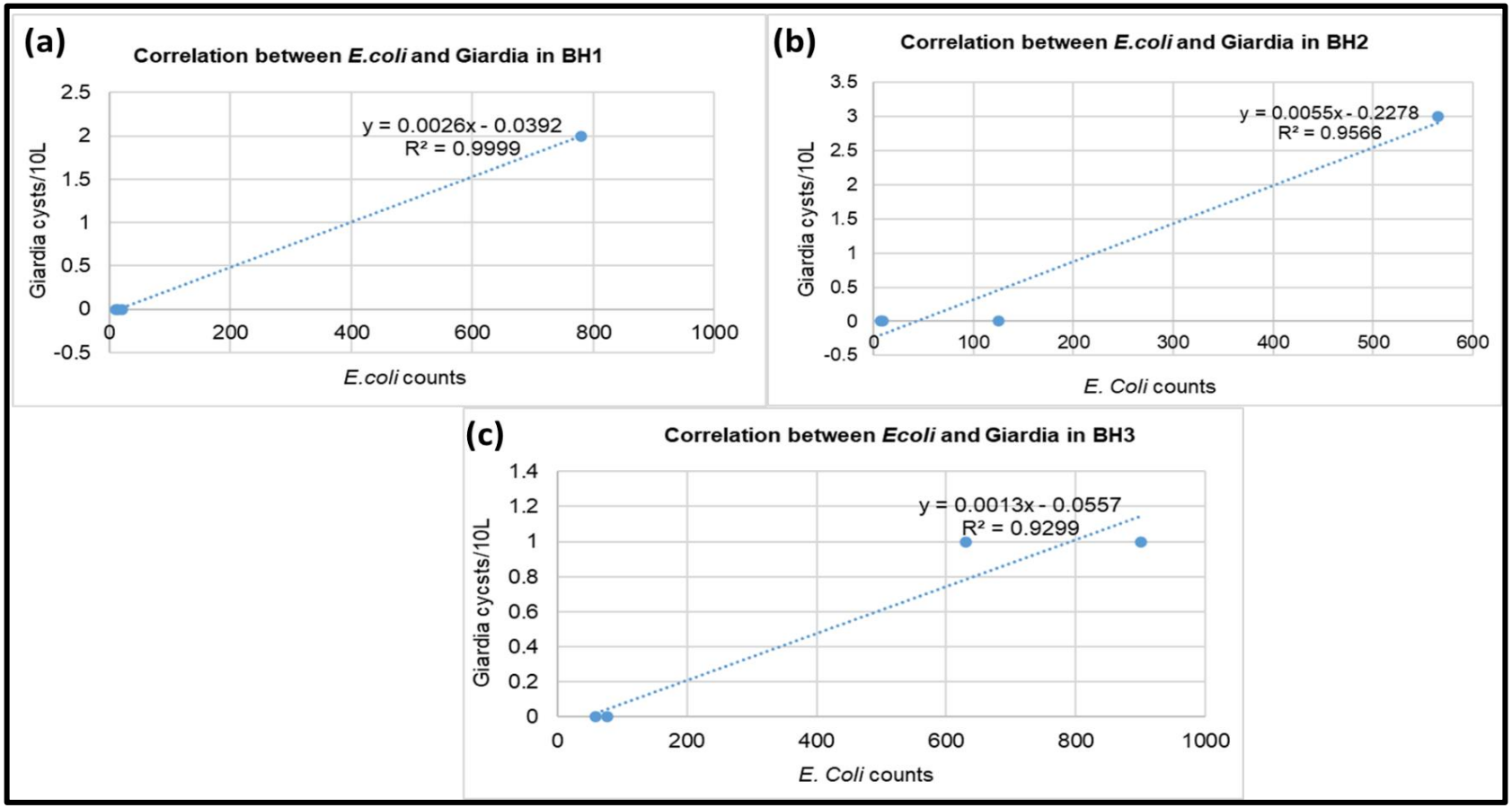


Figure 29. (a) Correlation between *E. coli* and Giardia in BH1 (b) Correlation between *E. coli* and Giardia in BH2 (c) Correlation between *E.coli* and Giardia in BH3.

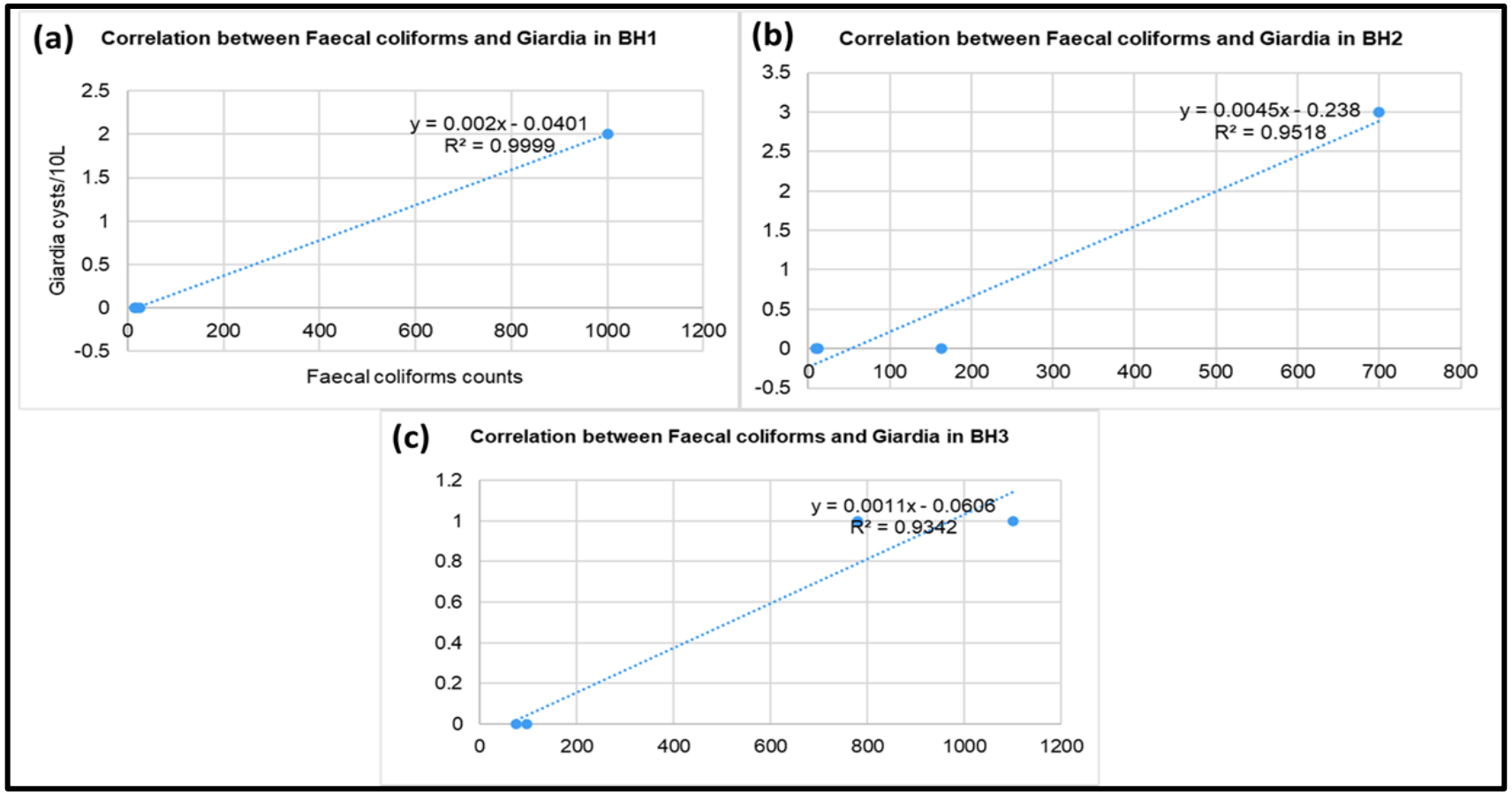


Figure 30. Correlation between fecal coliforms and *Giardia* in BH1 (b) Correlation between fecal coliforms and *Giardia* in BH2 (c) Correlation between fecal coliforms and *Giardia* in BH3.

CHAPTER 5.

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.1. Introduction

This chapter provides the summary, conclusion, and recommendations from the research study. The purpose of the study was to assess the abundance of *Cryptosporidium* and *Giardia* spp. in boreholes water close to the eMbalenhle wastewater treatment plant and associated impacts. To achieve the aim, the study used the three research questions below:

- What are the levels of *Cryptosporidium, Giardia spp.*, and *E. coli* in the groundwater samples?
- What is the concentration of the physicochemical properties in groundwater from the Embalenhle Waste Water Treatment Plant?
- Are both physicochemical properties and microbiological concentration (*Cryptosporidium, Giardia spp*., *and Escherichia coli)* within permissible limits, using drinking water standards by WHO and South African Water Guidelines?

5.2. Summary

There is a growing concern about potable water availability globally, groundwater is becoming a major source of freshwater due to shortages (Boretti & Rosa, 2019). The major contamination source for groundwater is anthropogenic activities which include wastewater treatment, and industrial activities (Li et al., 2021). Inadequately treated wastewater effluent, that is chemically, physically, or microbiologically contaminated has the potential of impacting the aquatic ecosystem, either via groundwater or other receiving bodies (Edokpayi et al., 2017). Enteric pathogens such as *Cryptosporidium* and *Giardia* spp. can use the groundwater as a transmission route ultimately causing potential illnesses to humans and animals (Chique et al., 2020b).

This research project examined the quality of groundwater in the eMbalenhle wastewater treatment plant, comparing the concentration of the examined variables with the South African National Standard for Drinking Water SANS241:2015, South African Water

Guidelines (Irrigation, Recreational Activities, and Aquatic Ecosystem), and WHO. Eleven (11) parameters were examined, (3) samples for *Cryptosporidium* and *Giardia*, (3) samples for microanalyses, and (3) physicochemical analyses, in four sampling cycles, to cover four seasons, from autumn to summer.

Based on the results obtained in comparison with the South African Water guidelines and WHO, the groundwater quality indicates contamination from the WWTWs. The values for pH, electrical conductivity, and chloride in the boreholes were within the SANS241, South African Water Guidelines, and WHO standards. Ammonia was within the SANS241 specification of 1.5 mg/L. The values for TSS, phosphate, and nitrates in all boreholes throughout the sampling seasons exceeded limits. This might be indicative of metal traces and other anthropogenic activities seepages from around the WWTW.

Fecal coliforms, *E.coli*, and TSS were selected as indicator organisms for the prevalence of *Cryptosporidium* and *Giardia* spp. in groundwater. Sente et al. (2016) established that turbid water is an indication of microbiological contamination. In the study conducted on the occurrence of *Cryptosporidium* and *Giardia* spp. in a public water-treatment system in Brazil, there was a correlation between high turbidity and the prevalence of (oo) cysts (Almeida et al., 2015). With TSS exceeding the limit and prevalence of *Cryptosporidium* and *Giardia* spp., fecal coliforms, and *E.coli*, in the study. The findings in this study are in agreement with many studies on the abundance of *Cryptosporidium* and *Giardia* spp. that also showed that *Escherichia coli* and turbidity are important indicators for the abundance of protozoans in water (Farrell et al., 2018; Smith et al., 2008; Hörman et al., 2004).

5.3. Conclusion

Many countries have reported outbreaks of *Giardia* and *Cryptosporidium* spp. Determining groundwater quality is crucial especially if utilized for consumption or irrigating the garden or agricultural fields. South Africa is a water-scarce country, and not only does that affect household consumption but the farming community for irrigation as well (Grewar, 2019); Cabrera Marino, 2017). Most farmers ordinarily depend on untreated water for irrigation with unknown or poor microbial quality from dams, rivers, groundwater, and wells. The WWTPs

discharge the treated water to the receiving bodies like rivers; if the water is inadequately treated and contains contaminants that consequently affect humans and animals.

The abundance of microbial contamination by fecal coliforms, *E.coli*, *Cryptosporidium*, and *Giardia* in eMbalenhle wastewater treatment plant groundwater is of great concern since it indicates fecal contamination. The composition of bacterial seepage varied from sampling point to sampling point throughout the sampling season, which can be attributed to the location of the borehole and the contamination exposure. Undoubtedly, such levels of microbial contamination are due to infiltration of groundwater aquifers stemming from the treatment plant either due to overflow of contaminated wastewater, sewage pipe leaks, or inadequately treated effluent seepages to groundwater.

The overall results obtained are revealing that there is a decline in the groundwater quality in the wastewater treatment plant. The borehole water at the eMbalenhle wastewater treatment plant is not suitable for drinking, aquatic ecosystem, irrigation, and recreation due to the high levels of fecal coliforms, *E.coli*, *Cryptosporidium*, and *Giardia* spp. which can have an adverse impact on humans and animals.

5.4 Recommendations

The groundwater quality is increasingly threatened by WWTPs, leaching contaminants into the aquifers. Based on the findings of this research, the following measures can be implemented to reduce the level of groundwater contamination in the eMbalenhle wastewater treatment plant. Recommendations include the following:

- An improvement in the maintenance and operation of the wastewater treatment plant will reduce microbiological contamination and improve groundwater quality. The plant was not operational for weeks during the sampling seasons which might have contributed to high microbial contamination.
- There needs to be continuous monitoring of the groundwater quality as there are indications of protozoans. The monitoring should include the assessment of enteric pathogens *Cryptosporidium* and *Giardia* on a defined frequency as part of risk management.

- Conduct bioassays on human cells once *Giardia* and *Cryptosporidium* have been isolated and identified to assess how long the human cells die or survive in the cells contaminated with the protozoans.
- The presence of animals around the borehole that can also increase the risk of contamination as animals can carry *Cryptosporidium* and *Giardia* spp. in their feces, which can contaminate the water is worth exploring.
- Boreholes far removed from contaminated sources may be studied as control and compared with boreholes that are located near agricultural areas or sewage treatment plants.
- There is a need to further investigate the boreholes, and occurrence of *Cryptosporidium* and *Giardia* within the study using methods such as PCR method. Method 1623 which was used for the study has a well-documented low recovery rate of the (oo) cyst. The PCR will give a more precise quantification of the amount of a genetic target in the borehole samples.

REFERENCES

Abeywardena, H., Jex, A. R., & Gasser, R. B. (2015). A Perspective on cryptosporidium and giardia, with an emphasis on bovines and recent epidemiological findings. *Advances in Parasitology*, *88*(January), 243–301. https://doi.org/10.1016/bs.apar.2015.02.001

Abia, A. L. K., Schaefer, L., Ubomba-Jaswa, E., & Le Roux, W. (2017). Abundance of pathogenic escherichia coli virulence-associated genes in well and borehole water used for domestic purposes in a peri-urban community of South Africa. *International Journal of Environmental Research and Public Health*, *14*(3). https://doi.org/10.3390/ijerph14030320

Adeyemo, F. E. (2019). *Occurrence and Variations of Cryptosporidium and Giardia in Wastewater Treatment and Receiving River Basins*.

Adeyemo, F. E., Singh, G., Reddy, P., Bux, F., & Stenström, T. A. (2019). Efficiency of chlorine and UV in the inactivation of Cryptosporidium and Giardia in wastewater. *PLoS ONE*, *14*(5), 1–22. https://doi.org/10.1371/journal.pone.0216040

Akhtar, N., Syakir Ishak, M. I., Bhawani, S. A., & Umar, K. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water (Switzerland)*, *13*(19). https://doi.org/10.3390/w13192660

Al-Gheethi, A. A., Efaq, A. N., Bala, J. D., Norli, I., Abdel-Monem, M. O., & Ab. Kadir, M. O. (2018). Removal of pathogenic bacteria from sewage-treated effluent and biosolids for agricultural purposes. *Applied Water Science*, *8*(2), 1–25. https://doi.org/10.1007/s13201-018-0698-6

Al-Hashimi, O. (2021). A Comprehensive Review for Groundwater Contamination and Remediation: Occurrence, Migration and Adsorption Modelling. *Molecules*, *26*, 5913.

Alam, M. S., Feroz, F., Rahman, H., Das, K. K., & Noor, R. (2015). Microbiological contamination sources of freshly cultivated vegetables. *Nutrition and Food Science*, *45*(4), 646–658. https://doi.org/10.1108/NFS-04-2015-0032

Alegbeleye, O., & Sant'Ana, A. S. (2022). Survival and growth behaviour of Listeria monocytogenes in ready-to-eat vegetable salads. *Food Control*, *138*(January), 109023. https://doi.org/10.1016/j.foodcont.2022.109023

Alexander, T. J., Vonlanthen, P., & Seehausen, O. (2017). Does eutrophication-driven evolution change aquatic ecosystems? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712). https://doi.org/10.1098/rstb.2016.0041

Ali Hannouche, Chebbo Ghassan, Gwenaël Ruban, Bruno Tassin, B. J., & Lemaire, C. J. (1996). Relationship between turbidity and total suspended solids concentration within a combined sewer system. *Water Science and Technology*, *64*(12), 2445–2452. https://doi.org/10.2166/wst.2011.779

Almeida, J. C., Martins, F. D. C., Neto, J. M. F., dos Santos, M. M., Garcia, J. L., Navarro, I. T., Kuroda, E. K., & Freire, R. L. (2015). Occurrence of Cryptosporidium spp. and Giardia spp. in a public water-treatment system, Paraná, Southern Brazil. *Revista Brasileira de Parasitologia Veterinaria*, *24*(3), 303–308. https://doi.org/10.1590/S1984-29612015051

Alum, A., Absar, I. M., Asaad, H., Rubino, J. R., & Ijaz, M. K. (2014). Impact of environmental conditions on the survival of cryptosporidium and Giardia on environmental surfaces. *Interdisciplinary Perspectives on Infectious Diseases*, *2014*.

Andersson, Y., & Bohan, P. (2001). Disease surveillance and waterborne outbreaks. Water Quality: Guidelines, Standards and Health. *World Health Organization*, 1–431. www.iwapublishing.com

APHA. (2002). American Public Health Association; American Water Works Association; Water Environment Federation. *Standard Methods for the Examination of Water and Wastewater*.

Apuke, O. D. (2017). Quantitative Research Methods : A Synopsis Approach. *Kuwait Chapter of Arabian Journal of Business and Management Review*, *6*(11), 40–47. https://doi.org/10.12816/0040336

Asiwaju-Bello, Y. A., Olabode, O. F., & Ogunsuyi, M. T. (2020). Pollution potential and causative hydrogeochemical processes in unconfined aquifer systems in a typical urban setting: emphasis on recharge and discharge areas. *Applied Water Science*, *10*(1). https://doi.org/10.1007/s13201-019-1131-5

Baldwin, D. S. (2013). Organic phosphorus in the aquatic environment. *Environmental Chemistry*, *10*(6), 439–454. https://doi.org/10.1071/EN13151

Balkhair, K. S. (2016). Microbial contamination of vegetable crop and soil profile in arid regions under controlled application of domestic wastewater. *Saudi Journal of Biological Sciences*, *23*(1), S83–S92. https://doi.org/10.1016/j.sjbs.2015.10.029

Banks, M. K., Yu, W., & Govindaraju, R. S. (2003). Bacterial Adsorption and Transport in Saturated Soil Columns. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *38*(12), 2749–2758. https://doi.org/10.1081/ESE-120025828

Bartram, J., & Pedley, S. (1996). Water Quality Monitoring- A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. In *United Nations Environment Programme; World Health Organization* (Issue July). https://doi.org/10.1002/ejoc.201200111

Barwick, R. S., Mohammed, H. O., White, M. E., & Bryant, R. B. (2003). Factors associated with the likelihood of Giardia spp. and Cryptosporidium spp. in soil from dairy farms. *Journal of Dairy Science*, *86*(3), 784–791. https://doi.org/10.3168/jds.S0022-0302(03)73660-1

Basias, N., & Pollalis, Y. (2018). Quantitative and Qualitative Research in Business & Technology: Justifying a Suitable Research Methodology. *Review of Integrative Business and Economics Research*, *7*(1), 91–105. http://buscompress.com/journal-home.html

Berndt, M. P. (1990). *Sources and Distribution of Nitrate in Ground Water At a Farmed Field Irrigated With Sewage Treatment-Plant Effluent, Tallahassee, Florida*.

https://pubs.usgs.gov/wri/1990/4006/report.pdf

Berrouch, S., Escotte-binet, S., Amraouza, Y., Flori, P., Villena, I., & Hafid, J. (2020). *fresh vegetables consumed in Marrakech , Morocco*. *20*(4).

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* (Issue 1966). www.wrc.org.za

Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, *3*(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529

Bition, G., & Harvey, R. W. (1992). Transport of pathogens through soils and aquifers. *Environmental Microbiology*, *January 1992*, 103–124.

Bonetta, S., Pignata, C., Gasparro, E., Richiardi, L., Bonetta, S., & Carraro, E. (2022). Impact of wastewater treatment plants on microbiological contamination for evaluating the risks of wastewater reuse. *Environmental Sciences Europe*, *34*(1). https://doi.org/10.1186/s12302-022-00597-0

Boretti, A., & Rosa, L. (2019). Reassessing the projections of the World Water Development Report. *Npj Clean Water*, *2*(1).

Boschi-Pinto, C., Velebit, L., & Shibuya, K. (2008). Estimating child mortality due to diarrhoea in developing countries. *Bulletin of the World Health Organization*, *86*(9), 710–717. https://doi.org/10.2471/BLT.07.050054

Brennan, F. P., O'Flaherty, V., Kramers, G., Grant, J., & Richards, K. G. (2010). Long-term persistence and leaching of escherichia coli in temperate maritime soils. *Applied and Environmental Microbiology*, *76*(5), 1449–1455. https://doi.org/10.1128/AEM.02335-09

Brungs, W. A. (1976). Effects of wastewater and cooling water chlorination on Aquatic life: In *Unpublished report.*

Buyukyavuz, A., Benson, E., Song, J., Ellis, B., Han, I., & Dawson, P. (2018). Escherichia

Coli Transfer to Food by Fruit Flies during Short Time Exposure. *Journal of Food Research*, *7*(4), 131. https://doi.org/10.5539/jfr.v7n4p131

Cabrera Marino, K. M. (2017). Инновационные подходы к обеспечению качества в здравоохраненииNo Title. *Вестник Росздравнадзора*, *6*, 5–9.

Cacciò, S. M., De Giacomo, M., Aulicino, F. A., & Pozio, E. (2003). Giardia cysts in wastewater treatment plants in Italy. *Applied and Environmental Microbiology*, *69*(6), 3393–3398. https://doi.org/10.1128/AEM.69.6.3393-3398.2003

Carmena, D., Campus, H. H., Road, D. C., & Cryptosporidium, K. (2010). *Waterborne transmission of Cryptosporidium and Giardia : detection , surveillance and implications for public health*. *Table 2*, 3–14.

Carpenter, S. R. (2005). Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(29), 10002–10005. https://doi.org/10.1073/pnas.0503959102

Carty, G., & Bourke, N. (1995). Water treatment manuals Filtration. *Environmental Protection Agency*, 80. http://www.epa.ie/pubs/advice/drinkingwater/watertreatmentmanuals-filtration.html#.VlXNs3bhDIU

Centers for Disease Control and Prevention (CDC). (2012). Outbreak of Shiga toxin-producing Escherichia coli O111 infections associated with a correctional facility dairy - Colorado, 2010. In *MMWR. Morbidity and mortality weekly report* (Vol. 61, Issue 9). http://www.ncbi.nlm.nih.gov/pubmed/22398842

Chalmers, R. M., Simmonds, L. P., Wood, M., Luxford, M., Miller, R., & Johnston, R. (2021). Occurrence of cryptosporidium oocysts in leisure pools in the UK, 2017, and modelling of oocyst contamination events. *Water (Switzerland)*, *13*(11), 1–21. https://doi.org/10.3390/w13111503

Chapman, D. (1996). Groundwater Pollution - Developments in Water Science. *Water Quality Assessments - A Guide to Use of Biota, Sediments and Water in Environmental*

Monitoring - Second Edition Edited, *5*(21), 273. http://www.who.int/water_sanitation_health/resourcesquality/wqachapter9.pdf

Chen, Y., Shen, W., Wang, B., Zhao, X., Su, L., Kong, M., Li, H., Zhang, S., & Li, J. (2020). Occurrence and fate of antibiotics, antimicrobial resistance determinants and potential human pathogens in a wastewater treatment plant and their effects on receiving waters in Nanjing, China. *Ecotoxicology and Environmental Safety*, *206*(July). https://doi.org/10.1016/j.ecoenv.2020.111371

Chen, Z., Yu, D., He, S., Ye, H., Zhang, L., Wen, Y., Zhang, W., Shu, L., & Chen, S. (2017). Prevalence of antibiotic-resistant Escherichia coli in drinking water sources in Hangzhou City. *Frontiers in Microbiology*, *8*(JUN), 1–11. https://doi.org/10.3389/fmicb.2017.01133

Cheng, C., Zhang, F., Shi, J., & Kung, H. Te. (2022). What is the relationship between land use and surface water quality? A review and prospects from remote sensing perspective. *Environmental Science and Pollution Research*, *29*(38), 56887–56907. https://doi.org/10.1007/s11356-022-21348-x

Chique, C., Hynds, P. D., Andrade, L., Burke, L., Morris, D., Ryan, M. P., & O'Dwyer, J. (2020). Cryptosporidium spp. in groundwater supplies intended for human consumption – A descriptive review of global prevalence, risk factors and knowledge gaps. *Water Research*, *176*(2020), 115726. https://doi.org/10.1016/j.watres.2020.115726

Cho, E. J., Yang, J. Y., Lee, E. S., Kim, S. C., Cha, S. Y., Kim, S. T., Lee, M. H., Han, S. H., & Park, Y. S. (2013). A waterborne outbreak and detection of Cryptosporidium oocysts in drinking water of an older high-rise apartment complex in Seoul. *Korean Journal of Parasitology*, *51*(4), 461–466. https://doi.org/10.3347/kjp.2013.51.4.461

Coetzee, A., & Kisters, A. (2016). The 3D geometry of regional-scale dolerite saucer complexes and their feeders in the Secunda Complex, Karoo Basin. *Journal of Volcanology and Geothermal Research*, *317*(September 2017), 66–79. https://doi.org/10.1016/j.jvolgeores.2016.04.001

Corral, A. M. (2021). *Treatment and Valorisation of Saline Wastewater Treatment and Valorisation of Saline Wastewater : Principles and Practice*.

Correll, D. L. (1999). Phosphorus: A rate limiting nutrient in surface waters. *Poultry Science*, *78*(5), 674–682. https://doi.org/10.1093/ps/78.5.674

Crini, G., Lichtfouse, E., Crini, G., Lichtfouse, E., & Adsorbents, G. (2019). *Wastewater treatment : an overview To cite this version : HAL Id : hal-02065607 Wastewater Treatment : An Overview*. 1–22.

Da'ana, D. A., Zouari, N., Ashfaq, M. Y., Abu-Dieyeh, M., Khraisheh, M., Hijji, Y. M., & Al-Ghouti, M. A. (2021). Removal of Toxic Elements and Microbial Contaminants from Groundwater Using Low-Cost Treatment Options. *Current Pollution Reports*, *7*(3), 300–324. https://doi.org/10.1007/s40726-021-00187-3

Daniels, M. E., Smith, W. A., & Jenkins, M. W. (2018a). Estimating Cryptosporidium and Giardia disease burdens for children drinking untreated groundwater in a rural population in India. *PLoS Neglected Tropical Diseases*, *12*(1), 1–23.

Daniels, M. E., Smith, W. A., & Jenkins, M. W. (2018b). Estimating Cryptosporidium and Giardia disease burdens for children drinking untreated groundwater in a rural population in India. *PLoS Neglected Tropical Diseases*, *12*(1), 1–23. https://doi.org/10.1371/journal.pntd.0006231

Davis, J., & Kendall, P. (2012). Preventing E. coli from Garden to Plate. *Food and Nutrition Series|Food Safety*, *9*. http://www.ext.colostate.edu/Pubs/foodnut/09369.html

de Jong, A. (2017). *Detection and molecular typing of Cryptosporidium in South African wastewater plants*. *Independen*, 40. http://uu.diva-portal.org/smash/get/diva2:1173869/FULLTEXT01.pdf%0Ahttp://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-338855

Deksne, G. (2022). *Occurrence of Cryptosporidium spp. and Giardia spp. Infection in Humans in Latvia: Evidence of Underdiagnosed and Underreported Cases*. 2–9.

Delahoy, M. J., Wodnik, B., McAliley, L., Penakalapati, G., Swarthout, J., Freeman, M. C., & Levy, K. (2018). Pathogens transmitted in animal feces in low- and middle-income countries. *International Journal of Hygiene and Environmental Health*, *221*(4), 661–676. https://doi.org/10.1016/j.ijheh.2018.03.005

DeRegnier, D. P., Cole, L., Schupp, D. G., & Erlandsen, S. L. (1989). Viability of Giardia cysts suspended in lake, river, and tap water. *Applied and Environmental Microbiology*, *55*(5), 1223–1229. https://doi.org/10.1128/aem.55.5.1223-1229.1989

Dey, U., Sarkar, S., Duttagupta, S., Bhattacharya, A., Das, K., Saha, S., & Mukherjee, A. (2022). Influence of Hydrology and Sanitation on Groundwater Coliform Contamination in Some Parts of Western Bengal Basin: Implication to Safe Drinking Water. *Frontiers in Water*, *4*(June), 1–15. https://doi.org/10.3389/frwa.2022.875624

Dhal, A. K., Panda, C., Yun, S. I. L., & Mahapatra, R. K. (2022). An update on Cryptosporidium biology and therapeutic avenues. *Journal of Parasitic Diseases*, *46*(3), 923–939. https://doi.org/10.1007/s12639-022-01510-5

Division, S. S. (2011). *SANS 5221 : 2011 SOUTH AFRICAN NATIONAL STANDARD Microbiological analysis of water — General test methods*. 7–11.

Dixon, B. R. (2021). Giardia duodenalis in humans and animals – Transmission and disease. *Research in Veterinary Science*, *135*(June 2020), 283–289.

Domenech, E., Amorós, I., Moreno, Y., & Alonso, J. L. (2018). Cryptosporidium and Giardia safety margin increase in leafy green vegetables irrigated with treated wastewater. In *International Journal of Hygiene and Environmental Health* (Vol. 221, Issue 1, pp. 112–119). https://doi.org/10.1016/j.ijheh.2017.10.009

Donnenfeld, Z., Crookes, C., & Hedden, S. (2018). A delicate balance: Water scarcity in South Africa. *Southern Africa Report*, *13*(March), 1–24. https://issafrica.org/research/southern-africa-report/a-delicate-balance-water-scarcity-in-south-africa

Dreelin, E. A., Ives, R. L., Molloy, S., & Rose, J. B. (2014). Cryptosporidium and giardia in

surface water: A case study from Michigan, USA to inform management of rural water systems. *International Journal of Environmental Research and Public Health*, *11*(10), 10480–10503. https://doi.org/10.3390/ijerph111010480

Dube, T., Makaka, C., & Sibanda, Z. (2010). *An Assessment of the Effect of Industrial and Sewage Effluent on Aquatic Invertebrates : A Case Study of A Southern Urban Stream , Zimbabwe*. *June 2014*, 3–8.

Dubois, E., Larocque, M., Gagné, S., & Braun, M. (2022). Climate Change Impacts on Groundwater Recharge in Cold and Humid Climates: Controlling Processes and Thresholds. *Climate*, *10*(1). https://doi.org/10.3390/cli10010006

Duhain, G. L. M. C. (2012). *Occurrence of Cryptosporidium spp. in South African irrigation waters and survival of Cryptosporidium parvum during vegetable processing*. *June*, 1–113. http://repository.up.ac.za/handle/2263/26398

Dungeni, M., & Momba, M. N. B. (2010). The abundance of cryptosporidium and giardia spp. in treated effluents produced by four wastewater treatment plants in the gauteng province of South africa. *Water SA*, *36*(4), 425–432. https://doi.org/10.4314/wsa.v36i4.58413

Dungeni, M., van Der Merwe, R. R., & Momba, M. N. B. (2010). Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water SA*, *36*(5), 607–614. https://doi.org/10.4314/wsa.v36i5.61994

DWAF. (1996a). South African Water Quality Guidelines : Domestic Use (second edition). In *Departmet of Water Affairs and Forestry, Republic of South Africa* (Vol. 1).

DWAF. (1996b). South African water quality guidelines: Aquatic ecosystems. In *South African water quality guidelines* (Vol. 7). http://www.dwa.gov.za/iwqs/wq_guide/edited/Pol_saWQguideFRESH_vol7_Aquaticecosystems.pdf

DWAF. (1996c). South African Water Quality Guidelines: Volume 1 Domestic Use. In

Department of Water Affairs and Forestry (Vol. 1, Issue 2nd ed.).

DWAF. (1996d). *WATER QUALITY GUIDELINES VOLUME 4 AGRICULTURAL USE : IRRIGATION Department of Water Affairs and Forestry*. *4*. http://www.dwa.gov.za/IWQS/wq_guide/edited/Pol_saWQguideFRESHIrrigationvol4.pdf

Ehsan, M. A. (2015). *Water-borne transmission of Cryptosporidium and Giardia in Belgium and Bangladesh*. *January 2015*. http://hdl.handle.net/1854/LU-6536637%0Ahttps://biblio.ugent.be/publication/6536637/file/6569527.pdf%0Ahttps://biblio.ugent.be/publication/6536637

El Said Said, D. (2012). Detection of parasites in commonly consumed raw vegetables. *Alexandria Journal of Medicine*, *48*(4), 345–352. https://doi.org/10.1016/j.ajme.2012.05.005

EPA. (1999). *EPA 1999 Update of Ambient Water Quality Criteria for Ammonia*.

EPA. (2000). *Giardia: Drinking Water Fact Sheet*. *September*, 1–5.

EPA. (2005). Method 1623: *Control*, *December*.

Ezeh, O. K., Agho, K. E., Dibley, M. J., Hall, J., & Page, A. N. (2014). The impact of water and sanitation on childhood mortality in Nigeria: Evidence from demographic and health surveys, 2003–2013. *International Journal of Environmental Research and Public Health*, *11*(9), 9256–9272. https://doi.org/10.3390/ijerph110909256

Feng, W., Wang, T., Zhu, Y., Sun, F., Giesy, J. P., & Wu, F. (2023). Chemical composition, sources, and ecological effect of organic phosphorus in water ecosystems: a review. *Carbon Research*, *2*(1). https://doi.org/10.1007/s44246-023-00038-4

Fewtrell, L., & Bartram, J. (2001). Guidelines , Standards and Health : Assessment of. *IWA Publishing*, 1–431. www.iwapublishing.com

EXCELLENCE ." (2019).

Fongaro, G., García-González, M. C., Hernández, M., Kunz, A., Barardi, C. R. M., & Rodríguez-Lázaro, D. (2017). Different behavior of enteric bacteria and viruses in clay and sandy soils after biofertilization with swine digestate. *Frontiers in Microbiology*, *8*(JAN), 1–9. https://doi.org/10.3389/fmicb.2017.00074

Fradette, M. S., Culley, A. I., & Charette, S. J. (2022). Detection of Cryptosporidium spp. and Giardia spp. in Environmental Water Samples: A Journey into the Past and New Perspectives. *Microorganisms*, *10*(6). https://doi.org/10.3390/microorganisms10061175

Galal-Gorchev, H. (1993). WHO guidelines for drinking-water quality. *Water Supply*, *11*(3–4), 1–16.

Garcia, L. S. (2007). *Www.Myuptodate.Com*.

Gavrilescu, M. (2021). Water, soil, and plants interactions in a threatened environment. *Water (Switzerland)*, *13*(19). https://doi.org/10.3390/w13192746

Gazette, G. (2017). *NATIONAL NORMS AND STANDARDS FOR DOMESTIC WATER AND SANITATION SERVICES Version 3-Final*. *41100*, 82–181.

Genthe, R., & Kfir, R. (1992). *Studies on Microbiological Drinking Water Research Commission*. *469*.

Gericke, M., Bateman, B., Rapholo, F., Mashakana, J., Maharaj, V., & Hhner, C. A. (1995). *Occurrence of Protozoan Parasites in South African Source and Treated Water*. *451*.

Gert Sibande District Municipality. (n.d.). Rural Development Plan Gert Sibande District Municipality Rural Development Plan. *Report*.

Gleick, P. H., & Cooley, H. (2021). Freshwater Scarcity. *Annual Review of Environment and Resources*, *46*, 319–348. https://doi.org/10.1146/annurev-environ-012220-101319

Global, S. A. I., & Iso. (1980). Environment agency. *The Microbiology of Drinking Water*

(2010) - Part 14 - Methods for the Isolation, Identification and Enumeration of Cryptosporidium Oocysts and Giardia Cysts, *6*(2), 95–96. https://doi.org/10.1016/S0378-777X(80)80046-1

Godfrey, S., Timo, F., & Smith, M. (2005). Relationship between rainfall and microbiological contamination of shallow groundwater in Northern Mozambique. *Water SA*, *31*(4), 609–614. https://doi.org/10.4314/wsa.v31i4.5152

Golnabi, H., Matloob, M. R., Bahar, M., & Sharifian, M. (2009). Investigation of electrical conductivity of different water liquids and electrolyte solutions. *Iranian Physical Journal*, *3*(November 2014), 24–28.

Grewar, T. (2019). *South Africa ' s options for mine- impacted water re-use : A review*. *119*, 321–331.

Grundlingh, M., & De Wet, C. M. E. (2004). The search for Cryptosporidium oocysts and Giardia cysts in source water used for purification. *Water SA*, *30*(5), 581–584. https://doi.org/10.4314/wsa.v30i5.5163

Hamilton, K. A., Waso, M., Reyneke, B., Saeidi, N., Levine, A., Lalancette, C., Besner, M., Khan, W., & Ahmed, W. (2018). Cryptosporidium and Giardia in Wastewater and Surface Water Environments . *Journal of Environmental Quality*, *47*(5), 1006–1023. https://doi.org/10.2134/jeq2018.04.0132

Hamilton, P. (2005). Groundwater and surface water: A single resource. *Water Environment and Technology*, *17*(5), 37–41.

Hill, D. D., Owens, W. E., & Tchounwou, P. B. (2006). The impact of rainfall on fecal coliform bacteria in Bayou Dorcheat (North Louisiana). *International Journal of Environmental Research and Public Health*, *3*(1), 114–117. https://doi.org/10.3390/ijerph2006030013

Huey, G. M., & Meyer, M. L. (2010). Turbidity as an indicator of water quality in diverse watersheds of the upper Pecos River Basin. *Water (Switzerland)*, *2*(2), 273–284. https://doi.org/10.3390/w2020273

Hurst, C. J., Gerba, C. P., & Cech, I. (1980). Effects of environmental variables and soil characteristics on virus survival in soil. *Applied and Environmental Microbiology*, *40*(6), 1067–1079. https://doi.org/10.1128/aem.40.6.1067-1079.1980

Jarmey-Swan, C., Bailey, I. W., & Howgrave-Graham, A. R. (2001). Ubiquity of the water-borne pathogens, Cryptosporidium and Giardia, in KwaZulu-Natal populations. *Water SA*, *27*(1), 57–64. https://doi.org/10.4314/wsa.v27i1.5011

Javanmard, E., Mirsamadi, E. S., Olfatifar, M., Ghasemi, E., Saki, F., Mirjalali, H., Zali, M. R., & Karanis, P. (2020). Prevalence of Cryptosporidium and Giardia in vegetables in Iran: a nineteen-years meta-analysis review. *Journal of Environmental Health Science and Engineering*, *18*(2), 1629–1641. https://doi.org/10.1007/s40201-020-00493-w

Joseph, P. V., & Jacob, C. (2010). Physicochemical characteristics of pennar river, a fresh water wetland in Kerala, India. *E-Journal of Chemistry*, *7*(4), 1266–1273. https://doi.org/10.1155/2010/297931

Jr, A. J. E., & Orleans, N. (2020). *Wastewater Treatment & Water Reclamation* (Issue January).

Jung, A. V., Cann, P. Le, Roig, B., Thomas, O., Baurès, E., & Thomas, M. F. (2014). Microbial contamination detection in water resources: Interest of current optical methods, trends and needs in the context of climate change. *International Journal of Environmental Research and Public Health*, *11*(4), 4292–4310. https://doi.org/10.3390/ijerph110404292

Kanyerere, T., Levy, J., Xu, Y., & Saka, J. (2012). Assessment of microbial contamination of groundwater in upper Limphasa River catchment, located in a rural area of northern Malawi. *Water SA*, *38*(4), 581–596.

Khalid, S., Shahid, M., Bibi, I., Shah, A. H., & Niazi, N. K. (2018). *A Review of Environmental Contamination and Health Risk Assessment of Wastewater Use for Crop Irrigation with a Focus on Low and High-Income Countries*. 1–36.

King, B., Fanok, S., Phillips, R., Lau, M., van den Akker, B., & Monis, P. (2017).

Cryptosporidium attenuation across the wastewater treatment train: Recycled water fit for purpose. *Applied and Environmental Microbiology*, *83*(5), 1–18. https://doi.org/10.1128/AEM.03068-16

King, Hoefel, D., Daminato, D. P., Fanok, S., & Monis, P. T. (2008). Solar UV reduces Cryptosporidium parvum oocyst infectivity in environmental waters. *Journal of Applied Microbiology*, *104*(5), 1311–1323. https://doi.org/10.1111/j.1365-2672.2007.03658.x

Kotoski, E. J. (1997). Information on Phosphorus Amounts & Water Quality Environmental Impact : *Spring Harbor Environmental Magnet Middle School*, 1–4.

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cacciò, S., Chalmers, R., … Robertson, L. (2018). Public health risks associated with food-borne parasites. *EFSA Journal*, *16*(12). https://doi.org/10.2903/j.efsa.2018.5495

Kumar, A., Nirpen, L., Ranjan, A., Gulati, K., Thakur, S., & Jindal, T. (2014). Microbial groundwater contamination and effective monitoring system. *Asian Journal of Environenmental Science*, *9*(1), 37–48.

Ladeia, W. A., Martins, F. D. C., de Souza Lima Nino, B., da Cunha Silvério, A., da Silva, A. C., Ossada, R., da Silva, D. A., Garcia, J. L., & Freire, R. L. (2022). High occurrence of viable forms of Cryptosporidium and Giardia in domestic sewage from an agricultural region of Brazil. *Journal of Water and Health*, *20*(9), 1405–1415. https://doi.org/10.2166/wh.2022.127

Lam, S., Sivaramalingam, B., & Gangodawilage, H. (2014). Cryptosporidium outbreaks associated with swimming pools . *Environmental Health Review*, *57*(01), 3–8. https://doi.org/10.5864/d2014-011

Lamastra, L., Suciu, N. A., & Trevisan, M. (2018). Sewage sludge for sustainable agriculture: Contaminants' contents and potential use as fertilizer. *Chemical and Biological Technologies in Agriculture*, *5*(1), 1–6. https://doi.org/10.1186/s40538-018-0122-3

Landrigan, P. J., Stegeman, J. J., Fleming, L. E., Allemand, D., Anderson, D. M., Backer, L. C., Brucker-Davis, F., Chevalier, N., Corra, L., Czerucka, D., Bottein, M. Y. D., Demeneix, B., Depledge, M., Deheyn, D. D., Dorman, C. J., Fénichel, P., Fisher, S., Gaill, F., Galgani, F., … Rampal, P. (2020). Human health and ocean pollution. *Annals of Global Health*, *86*(1), 1–64. https://doi.org/10.5334/aogh.2831

Leifels, M., Sirikanchana, K., & Mongkolsuk, S. (2019). Letter to the Editor RE: High levels of faecal contamination in drinking groundwater and recreational water due to poor sanitation, in the sub-rural neighbourhoods of Kinshasa, Democratic Republic of the Congo by Kayembe et al. 2018. *International Journal of Hygiene and Environmental Health*, *222*(2), 260–261. https://doi.org/10.1016/j.ijheh.2018.05.011

Leopold, P., & Freese, S. D. (2009). Chemistry , Selection and Use. In *Water Science cc* (Issue WRC REPORT NO. TT 405/09).

Li, P., Karunanidhi, D., Subramani, T., & Srinivasamoorthy, K. (2021). Sources and Consequences of Groundwater Contamination. *Archives of Environmental Contamination and Toxicology*, *80*(1), 1–10. https://doi.org/10.1007/s00244-020-00805-z

Liu, Yang, Hou, L., Bian, W., Zhou, B., Liang, D., & Li, J. (2020). Turbidity in combined sewer sewage: An identification of stormwater detention tanks. *International Journal of Environmental Research and Public Health*, *17*(9). https://doi.org/10.3390/ijerph17093053

Liu, Yushuo, Lim, C. K., Shen, Z., Lee, P. K. H., & Nah, T. (2023). Effects of pH and light exposure on the survival of bacteria and their ability to biodegrade organic compounds in clouds: Implications for microbial activity in acidic cloud water. *Atmospheric Chemistry and Physics*, *23*(2), 1731–1747. https://doi.org/10.5194/acp-23-1731-2023

Lyimo, B., Buza, J., & Shahada, F. (2014). A review of the biology and detection methods of Cryptosporidium and Giardia spp in water sources and situation in Tanzania . *World Journal of Water Resource and Environmental Science*, *1*(1), 1–12.

Madumathi, G., Philip, L., & Bhallamudi, S. M. (2017). Transport of E. coli in saturated and unsaturated porous media: effect of physiological state and substrate availability. *Sadhana - Academy Proceedings in Engineering Sciences*, *42*(6), 1007–1024. https://doi.org/10.1007/s12046-017-0650-8

Magana-Arachchi, D. N., & Wanigatunge, R. P. (2020). Ubiquitous waterborne pathogens. *Waterborne Pathogens: Detection and Treatment*, *January*, 15–42. https://doi.org/10.1016/B978-0-12-818783-8.00002-5

Malakar, A., Snow, D. D., & Ray, C. (2019). Irrigation water quality-A contemporary perspective. *Water (Switzerland)*, *11*(7), 1–24. https://doi.org/10.3390/w11071482

Manetu, W. M., & Karanja, A. M. (2021). Waterborne Disease Risk Factors and Intervention Practices: A Review. *OALib*, *08*(05), 1–11. https://doi.org/10.4236/oalib.1107401

Martins, F. D. C., Ladeia, W. A., Toledo, R. dos S., Garcia, J. L., Navarro, I. T., & Freire, R. L. (2019). Surveillance of Giardia and Cryptosporidium in sewage from an urban area in Brazil. *Revista Brasileira de Parasitologia Veterinaria*, *28*(2), 291–297. https://doi.org/10.1590/s1984-29612019037

Mashau, S. (2012). *Distribution of Cryptosporidium Species and Effect of Treatment Process in Wastewater Treatment Plants in the Vhembe District , South Africa*. *1*.

Matooane, M., Oosthuizen, R., & John, J. (2011). Self-reported hypertension in eMbalenhle, Mpumalanga, South Africa: findings from a vulnerability to air pollution assessment. *Southern African Journal of Epidemiology and Infection*, *26*(4), 280–284. https://doi.org/10.1080/10158782.2011.11441468

Mbeki, G., & Municipality, L. (2014). *Govan Mbeki Spatial Development Framework 2014 - 2034*.

McAllister, T. A., & Topp, E. (2012). Role of livestock in microbiological contamination of water: Commonly the blame, but not always the source. *Animal Frontiers*, *2*(2), 17–27. https://doi.org/10.2527/af.2012-0039

McLauchlin, J., Amar, C., Pedraza-Díaz, S., & Nichols, G. L. (2000). Molecular epidemiological analysis of Cryptosporidium spp. in the United kingdom: Results of genotyping Cryptosporidium spp. in 1,705 Fecal samples from humans and 105 fecal samples from livestock animals. *Journal of Clinical Microbiology*, *38*(11), 3984–3990. https://doi.org/10.1128/jcm.38.11.3984-3990.2000

Mhlongo, N. T., Tekere, M., & Sibanda, T. (2019). Prevalence and public health implications of mycotoxigenic fungi in treated drinking water systems. *Journal of Water and Health*, *17*(4), 517–531. https://doi.org/10.2166/wh.2019.122

Miambo, R. D., Laitela, B., Malatji, M. P., Afonso, S. M. d. S., Junior, A. P., Lindh, J., & Mukaratirwa, S. (2019). Prevalence of Giardia and cryptosporidium in young livestock and dogs in magude district of Maputo Province, Mozambique. *Onderstepoort Journal of Veterinary Research*, *86*(1), 1–6. https://doi.org/10.4102/ojvr.v86i1.1709

Michelon, W., Peter, N. R. W., Schneider, T. M., Segalla, D. C., & Viancelli, A. (2023). Enterobacteria Survival, Percolation, and Leaching on Soil Fertilized with Swine Manure. *International Journal of Environmental Research and Public Health*, *20*(7). https://doi.org/10.3390/ijerph20075283

Minimum Requirements for Water Moni T Oring At Waste Management Facilities. (1998).

Mota, A., Mena, K. D., Soto-Beltran, M. A., Tarwater, P. M., & Cháidez, C. (2009). Risk assessment of Cryptosporidium and Giardia in water irrigating fresh produce in Mexico. *Journal of Food Protection*, *72*(10), 2184–2188. https://doi.org/10.4315/0362-028X-72.10.2184

Naidoo, J. (2013). *Assessment of the Impact of Wastewater Treatment Plant*. *December*.

Nasser, A. M., Vaizel-Ohayon, D., Aharoni, A., & Revhun, M. (2012). Prevalence and fate of giardia cysts in wastewater treatment plants. *Journal of Applied Microbiology*, *113*(3), 477–484.

Nasser, Abidelfatah M. (2022). Transmission of Cryptosporidium by Fresh Vegetables. *Journal of Food Protection*, *85*(12), 1737–1744. https://doi.org/10.4315/JFP-22-152

Odiyo, J. O., Mathoni, M. M., & Makungo, R. (2020). Health risks and potential sources of contamination of groundwater used by public schools in vhuronga 1, Limpopo Province, South Africa. *International Journal of Environmental Research and Public Health*, *17*(18), 1–15. https://doi.org/10.3390/ijerph17186912

Odonkor, S. T., & Ampofo, J. K. (2013). Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, *4*(1), 2.

Olson, M. E., Goh, J., Phillips, M., Guselle, N., & McAllister, T. A. (1999). Giardia Cyst and Cryptosporidium Oocyst Survival in Water, Soil, and Cattle Feces. *Journal of Environmental Quality*, *28*(6), 1991–1996. https://doi.org/10.2134/jeq1999.00472425002800060040x

Omarova, A., Tussupova, K., Berndtsson, R., Kalishev, M., & Sharapatova, K. (2018). Protozoan parasites in drinking water: A system approach for improved water, sanitation and hygiene in developing countries. *International Journal of Environmental Research and Public Health*, *15*(3), 1–18. https://doi.org/10.3390/ijerph15030495

Ongerth, J. E., & Karanis, P. (2018). Cryptosporidium & Giardia in Water—Key Features and Basic Principles for Monitoring & Data Analysis. *Proceedings*, *2*(11), 691. https://doi.org/10.3390/proceedings2110691

Ongerth, J. E., Plutzer, J., & Karanis, P. (2018). *Cryptosporidium and Giardia—Levels and Distribution in Surface Water throughout Europe*. 690. https://doi.org/10.3390/proceedings2110690

Pachepsky, Y., Shelton, D. R., McLain, J. E. T., Patel, J., & Mandrell, R. E. (2011). Irrigation Waters as a Source of Pathogenic Microorganisms in Produce. A Review. In *Advances in Agronomy* (1st ed., Vol. 113). Elsevier Inc. https://doi.org/10.1016/B978-0-12-386473-4.00007-5

Palmer, T., Berold, R., & Muller, N. (2000). Integrated Water Resources Management : From Research to Implementation. In *Water* (Vol. 25, Issue 1).

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination

of water resources by pathogenic bacteria. *AMB Express*, *4*(1), 1–16. https://doi.org/10.1186/s13568-014-0051-x

Peng, X., Murphy, T., & Holden, N. M. (2008). Evaluation of the effect of temperature on the die-off rate for Cryptosporidium parvum oocysts in water, soils, and feces. *Applied and Environmental Microbiology*, *74*(23), 7101–7107. https://doi.org/10.1128/AEM.01442-08

Pignata, C., Bonetta, S., Bonetta, S., Cacciò, S. M., Sannella, A. R., Gilli, G., & Carraro, E. (2019). Cryptosporidium oocyst contamination in drinking water: A case study in Italy. *International Journal of Environmental Research and Public Health*, *16*(11), 1–10.

Pinto, I., Zachariah, M., Wolski, P., Landman, S., Phakula, V., Maluleke, W., Bopape, M.-J., Engelbrecht, C., Jack, C., Mcclure, A., Bonnet, R., Vautard, R., Philip, S., Kew, S., Heinrich, D., Vahlberg, M., Singh, R., Arrighi, J., Thalheimer, L., … Dipura, R. (2022). *Climate change exacerbated rainfall causing devastating flooding in Eastern South Africa*. 5–59. https://apps.who.int/iris/bitstream/handle/10665/353573/OEW17-1824042022.pdf

Pitkänen, T., Juselius, T., Isomäki, E., Miettinen, I. T., Valve, M., Kivimäki, A. L., Lahti, K., & Hänninen, M. L. (2015). Drinking water quality and occurrence of Giardia in finnish small groundwater supplies. *Resources*, *4*(3), 637–654.

Proposals, S. (2018). *Mpumalanga Spatial Development Framework*. *September*.

Prystajecky, N., Huck, P. M., Schreier, H., & Isaac-Renton, J. L. (2014). Assessment of Giardia and Cryptosporidium spp. as a microbial source tracking tool for surface water: Application in a mixed-use watershed. *Applied and Environmental Microbiology*, *80*(8), 2328–2336. https://doi.org/10.1128/AEM.02037-13

Putignani, L., & Menichella, D. (2010). Global distribution, public health and clinical impact of the protozoan pathogen cryptosporidium. *Interdisciplinary Perspectives on Infectious Diseases*, *2010*. https://doi.org/10.1155/2010/753512

Ramírez-Castillo, F. Y., Loera-Muro, A., Jacques, M., Garneau, P., Avelar-González, F. J.,

Harel, J., & Guerrero-Barrera, A. L. (2015). Waterborne pathogens: Detection methods and challenges. *Pathogens*, *4*(2), 307–334. https://doi.org/10.3390/pathogens4020307

Ratnayaka, D. D., Brandt, M. J., & Johnson, K. M. (2009). Coliform bacteria content of water pumped from the wells at Big Rock Mesa as part of the mitigation program continues to increase. *Water Supply*, 195–266.

Release, S. (2021). *Mid-year population estimates*. *July*.

Rimhanen-Finne, R., Vuorinen, A., Marmo, S., Malmberg, S., & Hänninen, M. L. (2004). Comparative analysis of Cryptosporidium, Giardia and indicator bacteria during sewage sludge hygienization in various composting processes. *Letters in Applied Microbiology*, *38*(4), 301–305. https://doi.org/10.1111/j.1472-765X.2004.01487.x

Robertson, L. J., Campbell, A. T., & Smith, H. V. (1992). Survival of Cryptosporidium parvum oocysts under various environmental pressures. *Applied and Environmental Microbiology*, *58*(11), 3494–3500. https://doi.org/10.1128/aem.58.11.3494-3500.1992

Robertson, Lucy J., Johansen, Ø. H., Kifleyohannes, T., Efunshile, A. M., & Terefe, G. (2020). Cryptosporidium Infections in Africa—How Important Is Zoonotic Transmission? A Review of the Evidence. *Frontiers in Veterinary Science*, *7*(October), 1–25. https://doi.org/10.3389/fvets.2020.575881

Robertson, Lucy J., Jore, S., Lund, V., & Grahek-Ogden, D. (2021). Risk assessment of parasites in Norwegian drinking water: opportunities and challenges. *Food and Waterborne Parasitology*, *22*, 1–8. https://doi.org/10.1016/j.fawpar.2021.e00112

Roch, N., & Maine, E. (2015). *ANALYSIS OF AMMONIA REMOVAL FROM WASTEWATER MARKET : By PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF*.

Rochelle-Newall, E., Nguyen, T. M. H., Le, T. P. Q., Sengtaheuanghoung, O., & Ribolzi, O. (2015). A short review of fecal indicator bacteria in tropical aquatic ecosystems: Knowledge gaps and future directions. *Frontiers in Microbiology*, *6*(MAR), 1–15.

Rohman, H. (2018). Spatial Patterns of Pulmonary Tuberculosis Analysing Rainfall Patterns

in Visual Formation. *International Journal of Public Health Science (IJPHS)*, *7*(1), 13. https://doi.org/10.11591/ijphs.v7i1.11376

Rose, J. B., Huffman, D. E., & Gennaccaro, A. (2002). Risk and control of waterborne cryptosporidiosis. *FEMS Microbiology Reviews*, *26*(2), 113–123. https://doi.org/10.1016/S0168-6445(02)00090-6

Rusydi, A. F. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. *IOP Conference Series: Earth and Environmental Science*, *118*(1). https://doi.org/10.1088/1755-1315/118/1/012019

Ryu, H., Addor, Y., Brinkman, N. E., Ware, M. W., Boczek, L., Hoelle, J., Mistry, J. H., Keely, S. P., & Villegas, E. N. (2021). Understanding microbial loads in wastewater treatment works as source water for water reuse. *Water (Switzerland)*, *13*(11), 1–15. https://doi.org/10.3390/w13111452

SAHRC. (2000). The Right to Sufficient Water. *3rd Economic and Social Rights Report*, *1999*, 297–322. http://www.rdsn.org.za/documents/archive/water_sanitation.html.

Sakkas, H., Economou, V., Bozidis, P., Gousia, P., Papadopoulou, C., & Karanis, P. (2020). Detection of Cryptosporidium and Giardia in foods of plant origin in North-Western Greece. *Journal of Water and Health*, *18*(4), 574–578. https://doi.org/10.2166/WH.2020.037

Sánchez, C., López, M. C., Galeano, L. A., Qvarnstrom, Y., Houghton, K., & Ramírez, J. D. (2018). *Molecular detection and genotyping of pathogenic protozoan parasites in raw and treated water samples from southwest Colombia*. 1–11.

Santo Domingo, J. W., & Edge, T. a. (2010). Identification of primary sources of faecal pollution. In *Safe Management of Shellfish and Harvest Waters*.

Sayato, Y. (1989). WHO Guidelines for Drinking-Water Quality. *Eisei Kagaku*, *35*(5), 307–312. https://doi.org/10.1248/jhs1956.35.307

Seaton, F. M., George, P. B. L., Lebron, I., Jones, D. L., Creer, S., & Robinson, D. A. (2020).

Soil textural heterogeneity impacts bacterial but not fungal diversity. *Soil Biology and Biochemistry*, *144*(March). https://doi.org/10.1016/j.soilbio.2020.107766

Sente, C., Erume, J., Naigaga, I., Mulindwa, J., Ochwo, S., Magambo, P. K., Namara, B. G., Kato, C. D., Sebyatika, G., Muwonge, K., & Ocaido, M. (2016). Prevalence of pathogenic free-living amoeba and other protozoa in natural and communal piped tap water from Queen Elizabeth protected area, Uganda. *Infectious Diseases of Poverty*, *5*(1), 1–14. https://doi.org/10.1186/s40249-016-0162-5

Sharma, S., & Bhattacharya, A. (2017). Drinking water contamination and treatment techniques. *Applied Water Science*, *7*(3), 1043–1067. https://doi.org/10.1007/s13201-016-0455-7

Shehane, S. D., Harwood, V. J., Whitlock, J. E., & Rose, J. B. (2005). The influence of rainfall on the incidence of microbial faecal indicators and the dominant sources of faecal pollution in a Florida river. *Journal of Applied Microbiology*, *98*(5), 1127–1136. https://doi.org/10.1111/j.1365-2672.2005.02554.x

Shields, J. M., Gleim, E. R., & Beach, M. J. (2008). Prevalence of Cryptosporidium spp. and Giardia intestinalis in swimming pools, Atlanta, Georgia. *Emerging Infectious Diseases*, *14*(6), 948–950. https://doi.org/10.3201/eid1406.071495

Sibande, G., & Municipality, D. (2015). *Gert Sibande District Municipality*. 0–234.

Sigudu, M. V., du Preez, H. H., & Retief, F. (2014). Application of a basic monitoring strategy for Cryptosporidium and Giardia in drinking water. *Water SA*, *40*(2), 297–312. https://doi.org/10.4314/wsa.v40i2.12

Sindelar, M. (2015). Soils Clean and Capture Water. *Soil Science Society of America*, *April*. https://www.soils.org/files/sssa/iys/april-soils-overview.pdf

Singhal, B. B. S., Gupta, R. P., Singhal, B. B. S., & Gupta, R. P. (2010). Groundwater Contamination. *Applied Hydrogeology of Fractured Rocks*, 221–236. https://doi.org/10.1007/978-90-481-8799-7_12

Siwila, J., Mwaba, F., Chidumayo, N., & Mubanga, C. (2020). Food and waterborne protozoan parasites: The African perspective. *Food and Waterborne Parasitology*, *20*. https://doi.org/10.1016/j.fawpar.2020.e00088

Sousa, P. M., Blamey, R. C., Reason, C. J. C., Ramos, A. M., & Trigo, R. M. (2018). The "Day Zero" Cape Town drought and the poleward migration of moisture corridors. *Environmental Research Letters*, *13*(12). https://doi.org/10.1088/1748-9326/aaebc7

Spurgeon, D., Lahive, E., Robinson, A., Short, S., & Kille, P. (2020). *Species Sensitivity to Toxic Substances : Evolution , Ecology and Applications*. *8*(December), 1–25. https://doi.org/10.3389/fenvs.2020.588380

Squire, S. A., & Ryan, U. (2017). Cryptosporidium and Giardia in Africa: current and future challenges. *Parasites and Vectors*, *10*(1), 1–32. https://doi.org/10.1186/s13071-017-2111-y

Sroka, J., Stojecki, K., Zdybel, J., Karamon, J., Cencek, T., & Dutkiewicz, J. (2013). Occurrence of Cryptosporidium oocysts and Giardia cysts in effluent from sewage treatment plant from eastern Poland. *Annals of Agricultural and Environmental Medicine : AAEM*, . *1*(1), 57–62.

States, U. (1998). *Wastewater Treatment Works... The Basics*. *May*.

States, U. (2001). Cryptosporidium : Drinking Water Health Advisory. *Environmental Protection*, *March*, 1–31.

Swanson, R. G. (2020). Porosity and Permeability. *Sample Examination Manual*, 16–18. https://doi.org/10.1306/mth1413c5

Thomas, B. F., Behrangi, A., & Famiglietti, J. S. (2016). Precipitation intensity effects on groundwater recharge in the southwestern United States. *Water (Switzerland)*, *8*(3), 12–17. https://doi.org/10.3390/w8030090

Thomson, S., Hamilton, C. A., Hope, J. C., Katzer, F., Mabbott, N. A., Morrison, L. J., & Innes, E. A. (2017). Bovine cryptosporidiosis: impact, host-parasite interaction and

control strategies. *Veterinary Research*, *48*(1), 42. https://doi.org/10.1186/s13567-017-0447-0

Thurston-Enriquez, J. A., Watt, P., Dowd, S. E., Enriquez, R., Pepper, I. L., & Gerba, C. P. (2002). Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. *Journal of Food Protection*, *65*(2), 378–382. https://doi.org/10.4315/0362-028X-65.2.378

Tian, Y., Ding, J., Zhu, D., & Morris, N. (2018). The effect of the urban wastewater treatment ratio on agricultural water productivity: based on provincial data of China in 2004–2010. *Applied Water Science*, *8*(5), 1–10. https://doi.org/10.1007/s13201-018-0788-5

Toledo, R. D. S., Martins, F. D. C., & Freire, R. L. (2017). Waterborne Giardia and Cryptosporidium: Contamination of human drinking water by sewage and cattle feces. *Semina:Ciencias Agrarias*, *38*(5), 3395–3415. https://doi.org/10.5433/1679-0359.2017v38n5p3395

Tornevi, A., Bergstedt, O., & Forsberg, B. (2014). Precipitation effects on microbial pollution in a river: Lag structures and seasonal effect modification. *PLoS ONE*, *9*(5). https://doi.org/10.1371/journal.pone.0098546

Toze, S. (1997). Microbial Pathogens in Wastewater: Literature Review for Urban Water Systems. *Technical Report*, *1*, 1–103. https://publications.csiro.au/rpr/download?pid=procite:3effd8f9-a923-4ca9-a42a-9e6ce63ec1a6&dsid=DS1

Tram, N. T., Phuc, P. D., Phi, N. H., Trang, L. T., Nga, T. T., Ha, H. T. T., Cam, P. D., Canh, T. Q., & Karanis, P. (2022). Cryptosporidium and Giardia in Biogas Wastewater: Management of Manure Livestock and Hygiene Aspects Using Influent, Effluent, Sewage Canal Samples, Vegetable, and Soil Samples. *Pathogens*, *11*(2). https://doi.org/10.3390/pathogens11020174

Tredoux, G., Cavé, L., & Engelbrecht, P. (2004). Groundwater pollution: Are we monitoring appropriate parameters? *Water SA*, *30*(5), 662–667.

https://doi.org/10.4314/wsa.v30i5.5180

Unc, A., & Goss, M. J. (2004). Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, *25*(1), 1–18. https://doi.org/10.1016/j.apsoil.2003.08.007

UNEP, Rucevska, & Ieva. (2008). Vital Water Graphics: An Overview of the State of the World's Fresh and Marine Waters. *Vital Water Graphics*, A world of salt. https://wedocs.unep.org/handle/20.500.11822/20624%0Ahttp://www.unep.org/dewa/vitalwater/article5.html

USEPA. (1983). Methods for Chemical Analysis of Water and Wastes. EPA/600/4-79/020. *Environmental Protection*, *March*, 491.

USEPA. (2006). in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC). *Standard Methods*, *July*.

Utami, W. S., Murhandarwati, E. H., Artama, W. T., & Kusnanto, H. (2020). Cryptosporidium Infection Increases the Risk for Chronic Diarrhea Among People Living With HIV in Southeast Asia: A Systematic Review and Meta-Analysis. *Asia-Pacific Journal of Public Health*, *32*(1), 8–18. https://doi.org/10.1177/1010539519895422

Vahdat-Aboueshagh, H., Tsai, F. T. C., Bhatta, D., & Paudel, K. P. (2021). Irrigation-Intensive Groundwater Modeling of Complex Aquifer Systems Through Integration of Big Geological Data. *Frontiers in Water*, *3*(April), 1–16. https://doi.org/10.3389/frwa.2021.623476

Vail, J. (2013). Operating Procedure Groundwater sampling. *Science and Ecosystem Support Division*, 1–33.

Wang, X., Wang, X., & Cao, J. (2023). Environmental Factors Associated with Cryptosporidium and Giardia. *Pathogens*, *12*(3), 420. https://doi.org/10.3390/pathogens12030420

Ward, M. H., Id, R. R. J., Brender, J. D., Kok, T. M. De, Weyer, P. J., Nolan, B. T., Villanueva,

C. M., & Breda, S. G. Van. (2018). *Drinking Water Nitrate and Human Health : An Updated Review*. 1–31. https://doi.org/10.3390/ijerph15071557

Water Research Australia. (2016). *Fact Sheet Removal and inactivation of Cryptosporidium in the environment*. *July*, 4.

Watier-Grillot, S., Costa, D., Petit, C., Razakandrainibe, R., Larréché, S., Tong, C., Demont, G., Billetorte, D., Mouly, D., Fontan, D., Velut, G., Corre, A. Le, Beauvir, J. C., Mérens, A., Favennec, L., & de Santiid, V. P. (2022). Cryptosporidiosis outbreaks linked to the public water supply in a military camp, France. *PLoS Neglected Tropical Diseases*, *16*(9), 1–13. https://doi.org/10.1371/JOURNAL.PNTD.0010776

Wen, X., Chen, F., Lin, Y., Zhu, H., Yuan, F., Kuang, D., Jia, Z., & Yuan, Z. (2020). Microbial indicators and their use for monitoring drinkingwater quality-A review. *Sustainability (Switzerland)*, *12*(6), 1–14. https://doi.org/10.3390/su12062249

Wendel, A. S., Bauke, S. L., & Amelung, W. (2022). *Root - rhizosphere - soil interactions in biopores*. 253–277.

WHO. (1996). *Ammonia in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality*. *2*.

WHO. (2007). *Physical removal processes: sedimentation and filtration*. 21–50. http://www.who.int/water_sanitation_health/dwq/WSH02.07_5.pdf

WHO. (2017). Guidelines for Driking water-Quality. In *Proceedings of the Royal Society of Medicine* (Vol. 55).

WHO. (2018). *Sodium and Chloride in Drinking Water Wells*. *401*.

Willocks, L., Crampin, A., Milne, L., Seng, C., Susman, M., Gair, R., Moulsdale, M., Shafi, S., Wall, R., Wiggins, R., & Lightfoot, N. (1998). A large outbreak of cryptosporidiosis associated with a public water supply from a deep chalk borehole. Outbreak Investigation Team. *Communicable Disease and Public Health / PHLS*, *1*(4), 239–243.

Wolfe, M. S. (1992). *Giardiasis*. *5*(1), 93–100.

World Health Organization. (2017). WATER QUALITY AND HEALTH - REVIEW OF TURBIDITY: Information for regulators and water suppliers. *Who/Fwc/Wsh/17.01*, 10. https://www.who.int/water_sanitation_health/publications/turbidity-information-200217.pdf%0Ahttp://www.who.int/water_sanitation_health/publications/turbidity-information-200217.pdf

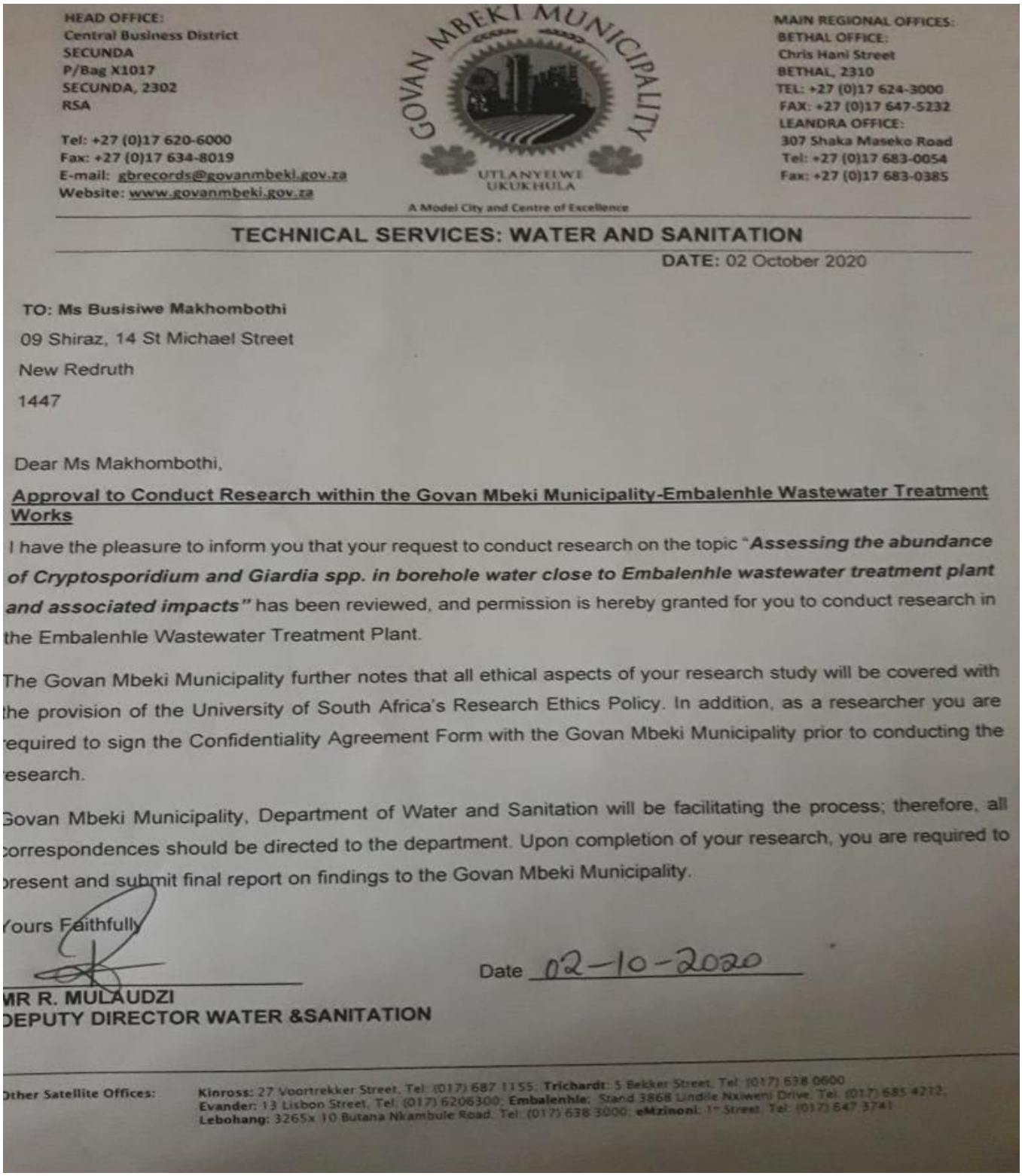
WRC (Water Research Commission). (2009). *Guidelines for the Utilisation and Disposal of Wastewater Sludge: Volume 3 Requirements for the on-site and off-site disposal of sludge* (Vol. 3).

Xiao, S., Yin, P., Zhang, Y., & Hu, S. (2017). Occurrence of cryptosporidium and giardia and the relationship between protozoa and water quality indicators in swimming pools. *Korean Journal of Parasitology*, *55*(2), 129–136. https://doi.org/10.3347/kjp.2017.55.2.129

Yorkshire Environmental Alcontrol UK. (1998). *Review of Disinfection and Associated Studies on Cryptosporidium Report commissioned by the Department of the Environment , Transport and the Regions (DETR), managed by the Drinking Water Inspectorate (DWI), from*. 1–56. https://webarchive.nationalarchives.gov.uk/20120905033617/http://dwi.defra.gov.uk/research/completed-research/reports/dwi0805.pdf

Yuan, J. S., Reed, A., Chen, F., Stewart, C. N., Smits, T. H. M., Devenoges, C., Szynalski, K., Maillard, J., Holliger, C., Woolsey, I. D., Blomstrand, B., Øines, Ø., Enemark, H. L., Ramakers, C., Ruijter, J. M., Lekanne Deprez, R. H., Moorman, A. F. M., Paulos, S., Saugar, J. M., … Quality, D. W. (2006). Multicentric evaluation of a new real-time PCR assay for quantification of Cryptosporidium spp. and identification of Cryptosporidium parvum and Cryptosporidium hominis. *Journal of Clinical Microbiology*, *51*(4), 1–17. https://doi.org/10.1128/aem.61.11.3849-3855.1995

Appendix A-Approval Letter-Govan Mbeki Municipality



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TECHNICAL SERVICES: WATER AND SANITATION

DATE: 02 October 2020

TO: Ms Busisiwe Makhombothi
09 Shiraz, 14 St Michael Street
New Redruth
1447

Dear Ms Makhombothi,

Approval to Conduct Research within the Govan Mbeki Municipality-Embalenhle Wastewater Treatment Works

I have the pleasure to inform you that your request to conduct research on the topic "***Assessing the abundance of Cryptosporidium and Giardia spp. in borehole water close to Embalenhle wastewater treatment plant and associated impacts"*** has been reviewed, and permission is hereby granted for you to conduct research in the Embalenhle Wastewater Treatment Plant.

The Govan Mbeki Municipality further notes that all ethical aspects of your research study will be covered with the provision of the University of South Africa's Research Ethics Policy. In addition, as a researcher you are required to sign the Confidentiality Agreement Form with the Govan Mbeki Municipality prior to conducting the research.

Govan Mbeki Municipality, Department of Water and Sanitation will be facilitating the process; therefore, all correspondences should be directed to the department. Upon completion of your research, you are required to present and submit final report on findings to the Govan Mbeki Municipality.

Yours Faithfully

Date 02-10-2020

MR R. MULAUDZI
DEPUTY DIRECTOR WATER &SANITATION

Other Satellite Offices: **Kinross:** 27 Voortrekker Street, Tel: (017) 687 1155; **Trichardt:** 5 Bekker Street, Tel: (017) 638 0600
Evander: 13 Lisbon Street, Tel: (017) 6206300; **Embalenhle:** Stand 3868 Lindile Nxiweni Drive, Tel: (017) 685 4212;
Lebohang: 3265x 10 Butana Nkambule Road, Tel: (017) 638 3000; **eMzinoni:** 1st Street, Tel: (017) 647 3741

Appendix B-Submitted letter to Municipality

09 Shiraz, 14 St Michael

New Redruth

1447

10 August 2020

Att: The Manager: Water and Sanitation Department

Govan Mbeki Municipality

Horwood Street

CBD Secunda

2302

Dear Sir/Madam

Re: Request to Collect Research water samples from eMbalenhle Wastewater Treatment Works borehole

I am studying for a Masters Environmental Science degree at the University of South Africa (UNISA). I am asking for permission to collect research water samples at the eMbalenhle Wastewater Treatment Works borehole starting from January 2021 to December 2021.

The results of the water quality will be analyzed and reported as part of the thesis. The title of the thesis "Assessing the abundance of *Cryptosporidium and Giardia spp..* in borehole water close to eMbalenhle wastewater treatment plant and associated impacts". I am committed to respecting and protecting the

privacy of data collected and analyzed and to the ethical use of information. The results will be treated with the highest level of confidentiality. Photographs taken will not include people or private properties where consent and confidentiality will be required. There are no foreseeable risks in participating in this research. This complies with the provision of the UNISA CAES Research Ethics Policy.

The study will add to the in-depth knowledge on the water quality of eMbalenhle Wastewater Treatment Works. It will also increase awareness to protect the water resources for the future generation, by implementing measures to prevent pollution.

Should you have any concerns and questions about the study you may contact my Supervisor: Thandazile Mhlongo eshongtn@unisa.ac.za.

Yours Sincerely

Busisiwe Makhombothi