A PROBABILISTIC AND EPIDEMIOLOGICAL ASSESSMENT OF HEALTH RISKS ASSOCIATED WITH RECREATIONAL EXPOSURE TO CONTAMINATED SURFACE WATER: A CASE OF THE UMSUNDUZI RIVER

Submitted in fulfillment of the requirements of the Degree of Doctor of Philosophy in Health Sciences in the Faculty of Health Sciences at the Durban University of Technology

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Abstract

Water quality degradation and scarcity have become prominent global concerns. In South Africa, poorly treated effluent from wastewater treatment facilities has contributed to the deterioration of river water quality, often exhibiting elevated levels of microorganisms. This study evaluated the microbial water quality of the uMsunduzi River and established a correlation between exposure to the river and the incidence of diarrheal infections among Dusi Marathon participants. In addition, the study conducted a theoretical assessment of the pre-and post-exposure risk reduction interventions that have the potential to safeguard participants of the canoe race

The study employed a comprehensive approach, which included a water quality analysis using *Escherichia coli, Pseudomonas aeruginosa*, and *Cryptosporidium spp.* as reference pathogens. The detection of *E. coli* and *P. aeruginosa* was conducted using IDEXX Colilert-18 and Pseudolert detection and enumeration kits, and digital droplet Polymerase Chain Reaction (ddPCR) for *Cryptosporidium spp.* An exposure and diarrheal incidence assessment through a prospective survey methodology and a Quantitative Microbial Risk Assessment (QMRA). Data analysis and interpretation for the exposure and incidence assessment were accomplished using the most recent IBM® SPSS® VERSION 27. The probability analysis for the QMRA was conducted using the distribution fitting capability included in the @Risk 7.5 program developed by Palisade Corporation.

The study detected elevated levels of *E. coli, Pseudomonas aeruginosa,* and *Cryptosporidium spp*. in the river water, exceeding acceptable limits set by World Health Organization and Department Water Affairs and Forestry. The exposure diarrheal incidence assessment revealed a significant association between river exposure and diarrheal infections, with a 97% increased risk of developing diarrhea among those who ingested water from the river.

The QMRA indicated a significant risk of infection for populations who depend on the uMsunduzi River for recreational activities, primarily associated with *Cryptosporidium spp.* The conclusions drawn suggest that regular monitoring is crucial to ensure the safety of water for leisure activities such as swimming, boating, and fishing. Monitoring systems may identify possible health threats and apply appropriate mitigation actions

by testing for pathogens, biological contaminants, and chemical pollutants. Recreational bodies of water may act as a reservoir for waterborne infections, offering significant health risks to persons.

This primary data analysis provided a comprehensive overview of the incidence of diarrhea among Duzi Canoe Marathon participants in 2022. We identified participant demographics and exposure assessments, key factors contributed to this health concern. As the Duzi Canoe Marathon continues to attract diverse participants, understanding and addressing health challenges is paramount. This analysis is a valuable resource for future event planning and underscores the commitment to participant welfare.

Keywords: *Cryptosporidium parvum, Escherichia coli (E. coli)*, *Pseudomonas aeruginosa,* Quantitative Microbial Risk Assessment, Epidemiological assessment.

Declaration

I confirm that this thesis is composed of my original work and contains no material previously submitted to the Durban University of Technology or any other institution for academic qualifications. The thesis consists of work I have carried out since the commencement of my PhD studies.

17 February 2026___________________

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Dedication

I dedicate this work to my brother Sandile Shaun Mdluli. To my single greatest advocate, the one person who loved me unconditionally. I am here today because of him. Thank you, big brother, for raising, loving, and protecting me.

May you continue to rest peacefully.

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List of abbreviations

Acronym	**Full Name**
CDC	Centre for disease control
CFU	Colony forming unit.
DdPCR	Digital droplet Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
DUCT	Duzi Umngeni Conservation Trust
DWS	Department of Water and Sanitation
DWAF	Department of Water Affairs and Forestry
EPA	Environmental Protection Agency
GDP	Gross domestic product
GI	Gastrointestinal illness
GPS	Global Positioning System
KZN	KwaZulu-Natal
MUG	4-methyl-umbelliferyl-β-D-glucuronide
NHMRC	National Health Medical Research Council
NICD	National Institute for Communicable Diseases
ORU	Outbreak Response Unit
PCR	Polymerase Chain Reaction
QMRA	Quantitative microbial risk assessment
rRNA	ribosomal RNA
SPSS	Statistical Package for the Social Sciences
TC	Total coliform
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WWTP	Wastewater treatment plant
W_2RAP	Wastewater risk abatement plan

1. CHAPTER ONE

ORIENTATION OF THE STUDY

1.1 BACKGROUND OF THE STUDY

The significance of fresh water as a crucial resource for human survival and the natural ecosystem cannot be overstated. The presence of sufficient quantities of high-quality water resources is a prerequisite for economic progress and ecological preservation (Wu *et al*. 2018: 917). Surface water is a significant potable water source, catering to around two-thirds of the global populace for their water requirements (Ungureanu *et al.* 2020). It encompasses all water bodies situated on the Earth's exterior, such as oceans, lakes, rivers, and estuaries. Surface water serves a multitude of functions, such as potable water supply, agricultural irrigation, livestock watering, industrial applications, hydroelectric power generation, and leisure activities (Ogwu *et al.* 2017).

Despite its significance as a precious commodity, surface water suffers from pollution and overexploitation, which is a major human and environmental concern worldwide (Sithebe 2017: 23). In places with inadequate sanitation and hygiene, surface water is prone to contamination by animal excrement, municipal and industrial waste, and sewage discharges (Singh *et al.* 2016: 2). The quality of freshwater sources is declining globally, and in South Africa, this decline can be attributed to the discharge of urban sewage effluent, overloaded and poorly maintained wastewater systems, industrial pollution, agricultural runoff, as well as urbanization and deforestation. (Gumbo *et al*. 2016: 597 and Adams *et al.* 2019: 86). Pollution of existing water resources is the greatest challenge to sustainable water supply in South Africa (Edokpayi *et al*. 2017: 401). Although both groundwater and surface water can serve as sources of potable water, surface water sources are more vulnerable to contamination from various sources such as agricultural runoff, sewage and wastewater, oil pollution, and radioactive substances (Singh *et al.* 2020).

The phenomenon of water resource degradation has been extensively researched and can arise from natural processes, such as climate change, water-rock interactions, and geological factors, as well as human activities, including agricultural practices and urban waste (Akhtar *et. al.* 2021). Surface water contaminations specifically in rivers and streams are mainly due to urbanization, agriculture, and manufacturing discharge (Sasakova *et al.* 2018). Agricultural activities have been shown to discharge chemical pollutants such as metals, pesticides, fertilizers, nutrients, and salts, which have a significant impact on surface water quality (Akhtar *et. al* 2021). In addition, it has been observed that urban activities such as untreated and partially treated sewage, construction waste, and solid/liquid waste factors have been found to contain hazardous substances that are emitted into the river water (Varol *et al.* 2012). These substances can enter waterways through various means such as runoff from agricultural and urban areas, discharges from industrial plants and wastewater treatment plants, and spills (Akhtar *et al.* 2021).

Water sources can be contaminated by pathogenic microorganisms, such as bacteria, viruses, and parasites, which can cause diseases. These microorganisms can enter water sources through the discharge of human and animal excreta. The pathogens have the potential to induce severe ailments (Alegbeleye *et al.* 2018). Biological pathogens represent a significant category of water contaminants.

According to Kumar *et al.* (2019: 2), there is a positive correlation between the degree of biological contamination and the likelihood of waterborne infection, as evidenced by multiple reports. The escalation of waterborne illnesses due to inadequate water quality has emerged as a primary cause of morbidity and mortality worldwide, affecting individuals across all age groups Islam *et al*. (2020:15). According to Collins *et al.* (2016: 426), waterborne illnesses represent a significant global health challenge. The primary hazard to human health stems from direct and indirect contact with water that has been contaminated with faeces containing pathogens, as noted by Kombo *et al.* (2021: 2966). Direct exposure to contaminated water pertains to the scenario where an individual encounters water that harbours deleterious substances, such as chemical or biological contaminants (Landrigan *et al*. 2020:1). Waterborne transmission can transpire via actions such as swimming, bathing, or ingestion of inadequately treated or disinfected water. The occurrence of indirect exposure to polluted water refers to the situation where an individual is exposed to pollutants that have infiltrated the environment via water and subsequently impacted the air, food, or surfaces that the individual interacts with (Ismanto, *et al.* (2023: 467).

Waterborne pathogens are responsible for causing illnesses in humans through two primary routes of direct exposure, namely consumption of drinking water and recreational water activities (Islam *et al*. (2020: 15). The ingestion of water contaminated with microorganisms leads to the development of diarrheal diseases and enteropathy, with a higher incidence observed among children under the age of five (Edokpayi *et al*. (2018: 159). Genter *et al*. (2021: 117350) presented sufficient evidence to suggest a significant risk associated with faecal contamination of surface water in developing nations, which can lead to waterborne outbreaks.

According to the World Health Organization (WHO), on a global scale, open water bodies such as lakes, rivers, seas, and lagoons serve as shared recreational resources, offering opportunities for activities such as swimming and various water sports (WHO 2021:1). In low-resource settings, rivers and lagoons are frequently utilized for personal hygiene purposes, in addition to their designated roles as official or unofficial recreational resources (Elmahdy *et al.* 2016:142. It is worth noting that open water sources have the potential to pose a significant health danger to the people who utilize them.

Dale *et al*. (2010) reported that a significant proportion of waterborne outbreaks found in Australia were associated with recreational activities. Defloro-Barker *et al.* (2018) further reported that there is an approximate occurrence of 4 billion instances of surface water recreation annually, resulting in an estimated 90 million instances of disease throughout the United States. Graciaa *et al*. (2018) assert that, untreated outbreaks related to recreational water are predominantly caused by gastrointestinal pathogens, encompassing bacteria, protozoa, and viruses.

A research investigation conducted in the Eastern Cape Province of South Africa revealed the presence of rotaviruses and enteroviruses in the Buffalo River and the dams that receive water from this river. In addition, the presence of enteroviruses, rotaviruses, and adenoviruses was identified in the Umngeni River, located in the KwaZulu-Natal (KZN) Province (Chigor and Okoh, 2012:4017).

The presence of noroviruses has been identified in multiple rivers located in Gauteng (Mans *et al*. 2013:141). Furthermore, a recent study using a Quantitative Microbial Risk Assessment (QMRA) has demonstrated a significant likelihood of Norovirus infection when individuals engage in swimming activities within these rivers that have been contaminated (Van Abel *et al*. 2017:908). These studies emphasize the potential health hazards associated with Noroviruses in open water sources in South Africa.

The National Institute for Communicable Diseases (NICD) in South Africa received a notification from the Outbreak Response Unit (ORU) on 5 January 2017 regarding a significant increase in the incidence of individuals experiencing gastrointestinal symptoms, including diarrhea and/or vomiting, along the coastal region of KZN Province. According to the notification issued by health officials in the KZN Province, a total of 690 individuals sought medical attention for gastrointestinal symptoms at a private hospital located on the south coast of KZN between the dates of 14 December 2016 and 5 January 2017. A total of 600 cases were observed, exceeding the anticipated number of 350 or less cases for the given period (Sekwadi *et al*. 2018: 1318).

Based on the available data, which encompassed regional newspapers and digital publications documenting a discharge of sewage into a proximal lagoon, the investigative team hypothesized that the contamination of the said coastal lagoon may have precipitated the occurrence of the outbreak (Sekwadi *et al*. 2018: 1318). The comprehensive list of waterborne pathogens encompasses a wide array of microorganisms, such as bacteria, viruses, protozoa, and parasitic helminths (Maurya *et al.* 2020:123). The aetiology of prolonged diarrhea can be attributed to microbiological factors such as identifiable bacterial infections caused by entero-aggregative *E. coli* and *Salmonella spp*, as well as parasitic infections caused by *Giardia* and *Cryptosporidium* pathogens (Amuche *et al*. 2022:11).

Kumar *et al.* (2019) revealed that, *E. coli* and other coliforms are principal causative agents of waterborne illnesses. *E. coli*, a bacterial species can synthesize verotoxins, which have been associated with the onset of hemorrhagic colitis, thrombolytic and thrombohemolytic uremia, and other associated symptoms. Efstratiou *et al.* (2017: 14) assert that waterborne protozoa have been identified as a significant factor in numerous global outbreaks. Ahmed *et al.* (2020: 2), further mention that *Cryptosporidium spp.* are among the most frequently reported protozoa in waterborne contexts. The worldwide incidence of gastrointestinal morbidity and mortality is attributed to infections caused by *Cryptosporidium spp.*, as reported by Efstratiou *et al.* (2017: 14). According to Kumbo *et al*. 2021: 2966), *Cryptosporidium spp.* is currently acknowledged as a prevalent source of diarrhea among individuals travelling to developing countries as well as those residing in those regions.

Several South African communities depend on untreated or inadequately treated water from surface sources like rivers and lakes for their water supply. Since 2000, there has been a significant rise in the incidence of waterborne diseases in South Africa, which can be attributed to the lack of or insufficient access to proper sanitation facilities in South African communities. This situation poses a high risk to the health of these communities (Edokpayi *et al*. 2017: 402).

The possibility for waterborne diseases brought on by exposure to pathogens in recreational waters can be assessed by epidemiological studies and quantified using QMRA, as reported by Boehm *et al.* (2013: 441). As demonstrated by Fischer *et al.* (2019), QMRA is a tool utilized for evaluating the potential risk to human health resulting from exposure to pathogens either in water, food or even air and is recognized as a valuable instrument for establishing health-based objectives. The approach, as posited by Bentham *et al.* (2018: 1), has garnered significant recognition as a significant advancement in safeguarding public health. QMRA was developed to evaluate the potential health risks associated with the presence of enteric bacterial and protozoan parasites in water distribution systems. The assessment centres on the ingestion or the faecal route as the predominant means of transmission, as stated by Ramírez-Castillo *et al.* (2015: 322). This approach employs a combination of laboratory and field data to establish an infection threshold. It involves the development of both experimental and meta-analytical data to identify measurable factors that contribute to the extent of disease given an exposure (Fischer *et al*., 2019: 15; Bentham *et al*., 2018: 2). Soller *et al.* (2010), mention that epidemiological studies offer significant insights into the characteristics and scope of health outcomes, the scale of associated risks, as well as how these risks are influenced or linked to levels of faecal contamination and other pollution metrics. Soller *et al*. (2010), further add that, epidemiological investigations typically do not furnish details regarding the precise microbial pathogens that are accountable for disease. However, epidemiological investigations about recreational bathing waters have demonstrated correlative relationships between resultant health effects and diverse microbial indicators or pathogens, as per (Farrel *et al.* 2021: 2). LeWinn *et al.* (2022: 3), proposes that the utilization of questionnaires is imperative for the development of a comprehensive exposure profile in epidemiological investigations. Questionnaires utilized in epidemiological research are used to gather data from participants about their exposure, potential risk factors, and incidence of the targeted disease (Kazi *et.al*., 2012: 4). Questionnaires are a means of obtaining information regarding specific characteristics that are essential for accurate analysis and interpretation of exposure-outcome relationships (LeWinn *et al.* 2022: 3)

Although various studies have been conducted on enteric pathogens present in surface water bodies such as rivers, an inadequate amount of research exists in establishing a direct correlation between exposure to these pathogens in surface water and resultant infections. The objective of the study is to evaluate the microbial pathogens, including bacteria and protozoa, that are recognized as etiological factors in illnesses transmitted through water. The primary objective is to establish a causal relationship between the bacterial and protozoan microbes present in the uMsunduzi River and the potential health risks associated with recreational exposure through a water quality assessment, microbial risk assessment, and infection monitoring.

1.2 RATIONALE FOR THE STUDY

The uMsunduzi River traverses the midlands of KZN Province and courses through the provincial capital city of Pietermaritzburg. It ultimately converges with the Umngeni River between Nagle and Inanda dams, and ultimately discharges into the Indian Ocean at Durban (Matongo *et al*., 2015: 133, 140). The river traverses regions with significant industrialization, where it receives runoff from both rural communities and the municipality along its path. Additionally, the river flows through agricultural areas, which are a source of organic pollution in the waterway (Adeyinka 2014: 47). As an additional factor, it should be noted that the river is subject to the inflow of treated discharge from a wastewater treatment plant, as well as other sources, resulting in increased levels of organic pollution within the river (Moodley *et al.* 2016: 41).

The river serves as a significant source of potable water for the rural populations inhabiting its vicinity and for the irrigation of agricultural produce and small-scale gardens. Fischer *et al*. (2019) relays that the river is utilized for leisure pursuits, including canoeing and rowing. The Dusi Canoe Marathon is an annual canoeing competition in the region between Pietermaritzburg and Durban. The annual marathon draws between 1600 to 2000 paddlers and takes place along the uMsunduzi River. According to the DUCT (2020), this has been identified as the most significant canoeing event on the African continent. According to Bateman's (2015) study, a survey conducted after the Dusi Canoe Marathon in 2012 revealed that 40% of the participants had reported experiencing chronic diarrhea. Boehm *et al*. (2013: 441) have defined recreational waterborne illness as the sickness contracted by swimmers upon exposure to recreational waters contaminated with pathogens. According to the DUCT (2020), the prevalent waterborne infection among canoeists is commonly referred to as the Dusi Guts, which is typified by the occurrence of diarrhea. According to Dr Oliver's (2020) research conducted at the DUCT, Schistosomiasis has been identified as a prevalent disease among Dusi canoeists.

The study conducted by Bateman (2015) aimed to assess the health status of the participants following their completion of the marathon using a survey. The survey did not effectively assess the potential risks associated with using uMsunduzi River for recreational activities through a microbial risk assessment. According to Gemmell *et al.* (2013: 6551), there is limited knowledge regarding the potential hazards that could be linked to frequent utilization and exposure to the water of uMsunduzi River. The uMsunduzi water quality can pose a significant health risk. This poses a threat to the annual canoe marathon.

Although various studies have reported the presence of enteric pathogens in surface water bodies, particularly rivers, there exists a dearth of research that establishes a correlation between exposure to these pathogens in surface water and subsequent infection, as noted by Rosado-García *et al.* (2017: 1). The combination of epidemiology studies conducted on recreational waters and QMRAs yields significant insights into the scope and severity of health consequences, the scale of hazards, and establishes direct correlations between exposure to waterborne pathogens and resultant infections among individuals (Soller *et al.* 2010: 4736 and Fewtrell *et al.*2015: 85). The present study is centred on the evaluation of the microbial water quality of uMsunduzi River, with a specific emphasis on ascertaining the potential hazards of diarrheal infections and the prevalence of actual infections among recreational users, namely Dusi Canoe Marathon participants. The findings and conclusions of this investigation have the potential to aid in the development of interventions aimed at mitigating the risk of disease outbreaks among rural communities residing along the uMsunduzi a River and Dusi Marathon canoeists.

1.3 AIM OF THE STUDY

This study aimed to establish a correlation between the exposure to uMsunduzi river and the occurrence of pathogenic infections among the Dusi Marathon participants. This was achieved by comprehensively evaluating the water quality, microbial risk, and infection monitoring. The study's final aim was to conduct a theoretical assessment of pre- and post-exposure risk reduction interventions that have the potential to safeguard participants of the canoe race.

1.4 OBJECTIVES OF THE STUDY

The objectives of the study were to:

- Determine the microbial water quality of the uMsunduzi River for a period of three months that is, one month before the commensal of the Dusi Canoe Marathon, during the Dusi Canoe Marathon and one month after the Dusi Canoe Marathon.
- Perform a probabilistic microbial risk assessment for recreational use of the uMsunduzi River.
- Explore the link between exposure to uMsunduzi River and pathogenic infections experienced by the Dusi Marathon participants.
- Develop a risk reduction strategy for recreational use of the uMsunduzi River based on data obtained from the outcomes of objectives 1-3

The framework for this thesis is illustrated in Figure 1.1, which delineates the link between the objectives of this study and the chapters, as well as the objectives and the outputs (publications).

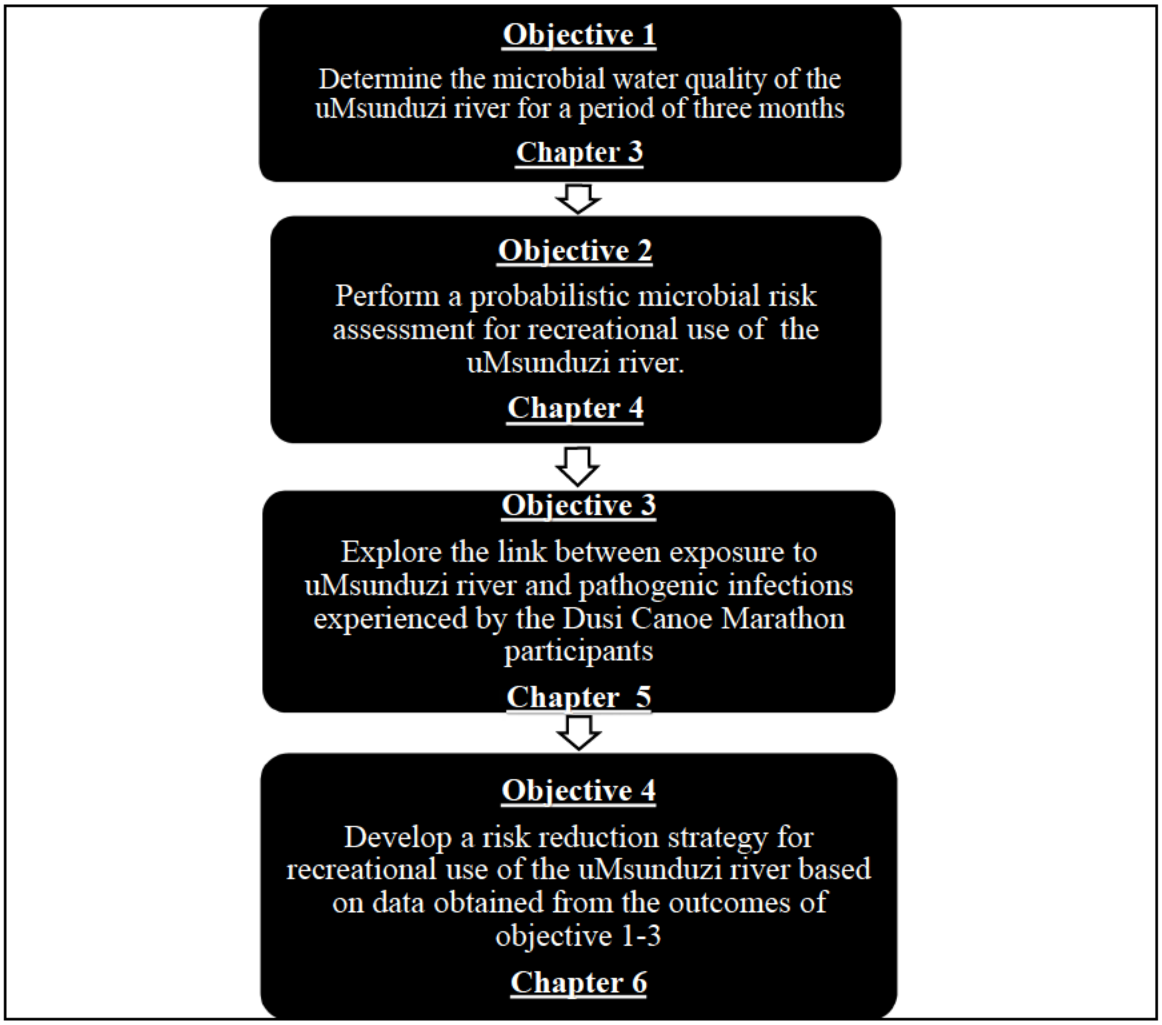

Figure 1.1: Framework of thesis

1.5 SUMMARY OF THE CHAPTER

This chapter presents the context, aim, objectives and rationale for the study. Chapter two will provide an overview of the relevant literature pertaining to the study.

2. CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Water safety and quality are crucial for human development and well-being, and providing access to secure water is one of the most effective means of promoting health and alleviating poverty (WHO 2021). However, inadequate water quality is a global issue, especially in low- and middle-income countries, where over two billion people lack access to safe drinking water (Panhwar *et al.* 2022: 1). One of the primary causes of poor water quality is pollution (Mokarram *et al*. 2020: 123380). The discharge of chemicals, waste, and microorganisms into subsurface groundwater or surface water bodies, such as lakes, streams, and oceans, disrupts the natural functioning of ecosystems and impedes the beneficial use of water (Kumar *et al.* (2019: 2). Industrial activities, agricultural practices, and municipal refuse disposal are examples of common sources of water pollution (Mokarram *et al.,* 2020:123380). Additionally, inadequate water purification facilities contribute to low water quality. In developing nations, the lack of Resafe and clean water causes waterborne diseases that cause illness and mortality (Ferreira *et al.* 2021:146279). Globally, the causes of poor water quality are multifaceted, necessitating a multifaceted strategy to improve water treatment facilities, reduce pollution, and sustainably manage water resources (Irfeey *et al.* 2023: 3105).

The issue of inadequate water quality is a widespread global concern that has a significant impact on millions of individuals across the globe (Saadat *et al*. 2020: 138870). Several factors contribute to this problem, including pollution, water stress or scarcity, and inadequate water treatment facilities (Soller *et al.* 2010: 4736).

Water quality has become a prominent global subject due to the challenges posed by water quality degradation and water scarcity (Sithebe 2017: 7). The release of effluent from wastewater treatment facilities has emerged as a significant contributor to the deterioration of river water quality in South Africa, frequently exhibiting elevated levels of microorganisms. In developing countries like South Africa, contaminated surface water bodies are occasionally used as direct supplies of water for drinking and other household needs, typically without prior treatment (Abia *et al*.2016: 1143). According to Edokpayi *et al.* (2018: 18), there has been a significant surge in the occurrences of waterborne illnesses in South Africa since the year 2000.

The results of research conducted within the Vhembe district of South Africa reveal that the microbial quality of the rivers in the region is unsatisfactory (Traore` *et al.* 2016: 817). This study aimed to examine the impact of human activities on the microbial quality of rivers in the Vhembe District of South Africa. A total of twelve catchments were selected for the collection of river water samples, which were subsequently analysed for the presence of total coliform (TC) and *Eschericha coli (E. coli)* (Traore` *et al.* 2016: 817). According to Traore` *et al.* (2016), the levels of Coliform and E. coli detected surpassed the permissible thresholds set by the South African Department of Water and Sanitation (DWS :1996). Throughout the year, the concentration of total coliforms (TC) remained consistent between the sampling points with values exceeding 2400 most probable number (MPN)/100ml (Traore` *et al.* 2016: 817).

According to the regulations set forth by the South African (DWS 1996), the allowable threshold levels are defined as follows: The permissible levels of microbial contamination in water for various activities have been specified as follows: for full-contact recreation such as swimming, the acceptable limit for total coliforms is 0-5/100 mL and for faecal coliforms, it is 0/100 mL. For intermediate recreation, the limit for faecal coliforms and *E. coli* is 0-130/100 mL and 0-1,000/100 mL respectively, depending on the level of water contact. For irrigation water applied to minimally processed produce, the permissible limit for faecal coliforms is ≤1/100 mL and for *E. coli*, it is ≤1/100 mL. According to Wen *et al.* (2020), the safe levels of coliforms in 100mL sample are nondetectable in several developed countries, such as the United States, the United Kingdom, Canada, and Australia. Invik *et al.* (2017) states that the Revised Total Coliform Rule in the United States sets a maximum contaminant level for *E. coli* and employs *E. coli* and total coliforms as a means to tackle potential faecal contamination that may infiltrate the distribution system.

In a comparable investigation carried out on the Apies River situated in the Gauteng province of South Africa, it was discovered that the microbial populations exhibit a significant bacterial diversity. Abia *et al*. (2017) found that the bacterial diversity in the Apies River was influenced by the presence of informal settlements, which were associated with different land uses and diverse microbial contaminants. The classes *Alphaproteobacteria, Betaproteobacteria*, and *Anaerolineae* were found to be the most frequently documented in the study. The presence of microbial contaminants in rivers poses a significant health risk to communities utilizing them for diverse purposes (Traore` *et al*. 2016: 817).

A study was conducted to assess the physiochemical and microbiological water quality parameters of the Nzhelele river, located in the Limpopo province of South Africa. The findings indicated that the river water was extensively polluted with faecal organisms, rendering it unsuitable for human consumption (Edokpayi *et al*. 2018: 18). The aforementioned studies have demonstrated that the water quality in South Africa exhibits a significant level of microbial contamination. This fact remains unchanged. The need for comprehensive measures to address the water quality challenges affecting communities residing along polluted water sources in South Africa is apparent. There is a persistent need for ongoing research and targeted solutions aimed at addressing the management of inadequate water quality. The utilization of untreated or inadequately treated water from surface resources, such as rivers and lakes, for daily consumption places numerous South African communities at an elevated risk of contracting waterborne diseases. This may result in a significant annual mortality rate among South Africans (Edokpayi *et al*. 2018: 27).

Waterborne illnesses pose a noteworthy public health apprehension in South Africa, particularly in regions with inadequate water and sanitation infrastructure. As per the WHO (2021), waterborne illnesses contribute to roughly 4% of the overall disease burden in

South Africa. Waterborne infections t regularly recorded in South Africa include cholera, typhoid fever, dysentery, hepatitis A, and giardiasis. Waterborne illnesses are primarily caused by drinking water contaminated with pathogenic microorganisms such as bacteria, viruses, and parasites (Edokpayi *et al*. 2018: 27). Several cholera outbreaks were recorded in South Africa in 2018, with the Limpopo province accounting for most cases. The use of contaminated water was found to be related with the occurrence of the outbreaks (Madilonga *et al*. 2021: 6765). In South Africa, the persistence of waterborne diseases poses a considerable public health obstacle, particularly in regions characterized by inadequate water and sanitation facilities (Marutlulle 2021: 16). Ensuring sufficient treatment and monitoring of the water supply is imperative in preventing the dissemination of waterborne illnesses.

2.2 POLLUTION SOURCES

The contamination of surface water by microorganisms can stem from diverse origins. The primary contributors to microbial contamination of surface water on a global scale comprise agricultural runoff, untreated sewage and wastewater, stormwater runoff, and animal waste from feedlots and farms (Rao *et al*., 2022: 100877). Pollutants may be categorized as nonpoint sources, referring to expansive and unconfined regions that introduce a range of pollutants into the water body, or point sources, which are distinct and identifiable sources of pollution, such as effluent from an industrial plant or a municipal sewage system (Adu *et al*. 2018: 1915). Nonpoint source pollution presents a greater challenge and financial burden for mitigation when compared to point source pollution. This is due to its low concentration, numerous sources, and significantly larger water volume (Plunge *et al.* 2022: 107635). Wyckoff (2017) explains that nonpoint source pollution is identified as the primary contributor to water pollution in the United States. The shared characteristic between the two pollution sources is the existence and predominance of pathogenic microbial contaminants.

Nonpoint source pollution encompasses bacteria that originate from animal waste, septic systems, and urban runoff. The aforementioned microorganisms have the potential to induce illnesses in human beings and inflict damage upon aquatic organisms (Damashek *et al.* 2022: 118853). Bacterial microorganisms are frequently encountered as a prevalent form of microbial pollutants, which have the potential to contribute towards nonpoint source pollution of surface water bodies (Walker *et al.* 2019:261).

The successful management and mitigation of pollution necessitates the identification and resolution of the precise sources of pollution within a designated region. It is noteworthy that diverse categories of microorganisms can potentially contribute to the pollution of surface water. The particular microbial pollutants that are implicated may differ based on the geographical location and pollution sources (Jung *et al.* 2014:4292).

2.3 RECREATIONAL UTILIZATION OF SURFACE WATER

Surface waters, such as rivers, lakes, and reservoirs, provide a variety of recreational opportunities, such as swimming, canoeing, fishing, and paddling (DeFlorio-Barker *et al.* 2018: 1). These activities provide opportunities for socialization, stress relief, and mental health enhancement in addition to promoting physical fitness. Moreover, recreational activities in surface waters contribute to local economies by attracting visitors and generating revenue for businesses like hotels, restaurants, and outfitters (Columna *et al.* 2015: 1119).

The value of recreation and leisure activities as a significant sector of the marine economy has been neglected i (Stebbings *et al*. 2020: 103905). It was discovered that in the United Kingdom, leisure sectors account for a significant portion of marine output and provide 26% of marine economy employment, which is more than double that of the largest sector (oil and gas) (Rodwell *et al*. 2014: 251). However, the provision of recreational activities depends in part on environmental factors such as geographical character, water quality, and safety (Stebbings *et al*. 2020: 103905). Furthermore, it is noteworthy that in the United States of America, Swimming, boating, fishing, and sailing are highly favoured water-based leisure pursuits. The aforementioned pursuits are instrumental in bolstering the outdoor recreation economy, which constituted 1.9% of the United States' gross domestic product (GDP) in current dollars during the year 2021 (Bureau of Economic Analysis United States of America 2022).

In recent years, there has been a surge in interest in recreational water sporting events, such as the Dusi Canoe Marathon and Midmar Mile, in South Africa. Both events have gained significant popularity and draw in participants from various regions across the globe (Giampiccoli *et al.* 2015:229).

The Dusi Canoe Marathon is a challenging multi-day competition held in the KZN region of South Africa. The competition spans roughly 120 kilometres and necessitates contestants to traverse through rapids, carry their canoes overland, and basecamp overnight along the river's shores. Notwithstanding its arduous character, the competition has sustained its popularity and consistently draws a substantial cohort of contenders annually (Dusi Canoe Marathon 2024).

The Midmar Mile, is an event that occurs in the KZN Midlands region of South Africa. The aforementioned occasion is a swimming competition spanning a distance of one mile, conducted in the Midmar Dam. It garners participation from individuals belonging to diverse age groups and possessing varying levels of swimming proficiency. The popularity of the event has increased over time, as evidenced by the growing number of participants each year. The 2019 iteration of the event witnessed a remarkable participation of 17,000 individuals, thereby setting a new record (Giampiccoli *et al.* 2015: 229).

There has been a notable surge in the popularity of recreational water sporting events in South Africa, as exemplified by both the Dusi Canoe Marathon and Midmar Mile. The distinctive difficulty and experience presented by these occurrences draw in contenders from various regions of the globe, rendering them an essential spectacle for any appreciator of sports (Giampiccoli *et al.* 2015: 229; Foreman 1997: 2).
According to the research conducted by McKay *et al.* (2019), the economic impact generated by hosting the Dusi Canoe Marathon, the Midmar Mile, and the Comrades

Marathon over a period of five years was found to be approximately equivalent to that of the 2010 FIFA World Cup, which was a one-time event.

The significance of the growing prevalence of water-based recreational activities in various communities should be duly acknowledged. Conducting studies on these communities is imperative to identify potential risks and offer recommendations for mitigating observed risks. Furthermore, to enhance the health and welfare of individuals as well as the environment, we must undertake steps that will aid in the reduction of any potential risks.

2.4 WATERBORNE DISEASE OUTBREAKS ASSOCIATED WITH RECREATIONAL UTILIZATION OF SURFACE WATER

Waterborne illnesses are defined as those that are spread through the consumption of water that has been contaminated (Craun 2018: 5). Waterborne infections can be categorized into four primary groups, as noted by Gleeson *et al*. (1997: 11) and Craun (2018: 6). The groups are:

- <u>Waterborne disease</u>, which occurs when a pathogen is transmitted through the consumption of contaminated water.
- <u>Water-washed diseases</u> refer to infections that are transmitted through the faecal-oral route or through person-to-person contact, which are exacerbated by inadequate access to water for proper hygiene.
- <u>Waterborne infections</u> are caused by pathogenic microorganisms that inhabit aquatic organisms during a certain phase of their life cycle.
- <u>Water-borne illnesses</u>, which are transmitted by insect vectors that propagate in water sources, such as mosquitoes.

For this study, the foci will be on waterborne diseases. The utilization of surface water for recreational purposes is associated with waterborne disease outbreaks, which presents a noteworthy public health concern. Outbreaks of illness can arise due to f microorganisms or chemicals in water, which can result in adverse health effects for individuals who engage in activities such as swimming, paddling, or fishing.

Waterborne diseases are commonly associated with symptoms of diarrhea, which is characterized by frequent bowel movements that can lead to dehydration and potentially fatal outcomes. As per the WHO, diarrheal disease is responsible for approximately 4.1% of the overall daily global burden of disease and results in the demise of 1.8 million individuals annually. Additional projections indicate that the majority (88%) of this burden can be attributed to inadequate access to safe water supply, sanitation, and hygiene, with a particular focus on children residing in developing nations (Ogenyi 2023).

Following Sekwadi *et al.* (2017), there was a surge in the incidence of gastroenteritis cases reported by the healthcare department in 2017 along the KZN coast, South Africa. The outbreak, which occurred over three weeks, was linked to swimming in the lagoon, with over 600 cases recorded. Deflorio-Baker *et al.* (2018) reported a significant surge in the incidence of waterborne illnesses in South Africa since the year 2000. As noted by TuTu *et al.* (2017: 410), there is a substantial body of evidence documenting several occurrences of waterborne illness outbreaks, namely diarrhea, across various regions in South Africa.

Raji *et al.* (2011: 384) conducted a study in Sokoto, Nigeria, which indicated that infectious diarrhea emerged as the primary aetiology of waterborne diseases, accounting for 62.17% of the total reported instances. Pirsaheb (2017) performed a study that revealed that within rural populations in developing countries, the incidence of waterborne diseases resulting from contaminated water was predicted to range from 15% to 20%. Notably, diarrhea emerged as a frequently observed symptom in affected individuals. Hemson (2016) documented a fatal incident that took place in the uKhahlamba district of KZN, South Africa. The incident was attributed to the presence of *E. coli* in the drinking water, leading to severe consequences. The unfortunate incident resulted in the mortality of 78 neonates.

Waterborne diseases are also a significant concern within the United States, as evidenced by estimates suggesting that sole exposure to recreational water leads to an annual occurrence of illnesses ranging from 7.15 million to 50 million (Lee *et al.* 2023: 120244). In the United States, it has been estimated that a significant number of illnesses related to the gastrointestinal, respiratory, ear, eye, and skin systems are attributed to various recreational water activities such as swimming, paddling, boating, and fishing. The annual incidence of these illnesses exceeds 90 million instances. The projected annual cost of these disorders is approximately $2.9 billion, highlighting the global significance of this issue (DeFlorio-Barker *et al.* 2018:7).

Craun (2017: 6) puts forward that, gastrointestinal infections transmitted through water are one of the most prevalent infectious diseases that are currently emerging and re-emerging on a global scale. Despite reports of fewer outbreaks in recent years, data analysis shows that there are still high levels of diarrheal illnesses in vulnerable rural areas (Hemson 2016: 28). Evaluation of the microbial quality of potable water can prevent waterborne disease outbreaks, which is one of the world's greatest challenges (Pirsaheb 2017: 152).

In rural areas of Africa, water is often contaminated with faecal matter due to the runoff from nearby bushes and forests utilized as defecation sites by the local population. Waterborne diseases can arise due to the presence of protozoa, viruses, bacteria, and intestinal parasites (Ogenyi 2023). *E. coli*, *Giardia*, and *Cryptosporidium* are the frequently implicated microorganisms and are often linked to waterborne diseases arising from recreational water use.

The illnesses associated with recreational freshwater activities and their corresponding etiological agents are illustrated in Table 1. This table presents a global overview of the challenges posed by waterborne disease outbreaks related to the recreational use of freshwater bodies. Furthermore, the limited availability of information on this subject highlights the urgent need for regular monitoring of waterborne illness outbreaks associated with recreational activities in freshwater environments. In addition, swimming is the predominant recreational activity that has been documented, which creates a significant gap in the recorded data regarding other leisure activities like canoeing. Moreover, it is worth mentioning that South Africa lacks comprehensive data regarding waterborne disease outbreaks linked to recreational activities in freshwater environments. Similarly, recorded data are scarce regarding the numerous microbes that serve as causative agents. This subsequently motivates for further investigations to document additional causal microbes found in freshwater bodies.

A study was conducted to evaluate the publication output of the top ten nations of drinking and recreational water-related diseases from 1980 to 2015. The findings revealed that the United States of America emerged as the most productive country, with a total of 623 articles, accounting for 27.48% of the overall publications. In terms of institutional productivity, the Environmental Protection Agency (EPA) and Centres for Disease Prevention and Control (CDC) secured the first and second positions, respectively. Notably, six out of the top ten productive institutions were in the United States, while the WHO represented the remaining entity. Finally, it should be noted that countries in Africa and Asia did not rank among the top 10 on the list (Sweileh *et al.* 2016). This may be a contributing factor as to why there are more outbreaks documented in the United States and fewer outbreaks recorded in African nations as illustrated in Table 2.1.

As noted by (Murphy *et al.* 2017), the transmission of pathogens through exposure to contaminated water remains a significant contributor to infections, as evidenced by the numerous reported outbreaks on a global scale. The issue is frequently undervalued when relying exclusively on identified instances of disease outbreaks. The reason for this phenomenon is that the reporting systems often fail to detect most waterborne diseases due to the mild and non-specific nature of gastrointestinal symptoms, which discourages individuals from seeking medical assistance. Typically, the symptoms persist for several days to one week.

Regular monitoring of recreational water quality is crucial in mitigating the incidence of waterborne disease outbreaks. Apart from the surveillance of water quality, educational and outreach endeavours can also contribute to mitigating the likelihood of waterborne ailment epidemics.

Table 2.1: Global outbreaks associated with recreational freshwater activities and associated etiological agents

Aetiological agent	**Year**	**Location**	**No. of Outbreaks**	**No. of cases**	**Illness**	**Reference**
Escherichia coli	2009-2019	USA	19	240	AGI	(Esschert *et al.* 2020)
Cryptosporidium spp.	2009-2019	USA	17	237	AGI	(Esschert *et al.* 2020)
Cryptosporidium SPP. *E. coli*	2011-2012	USA	90	1788	AGI	(Hlavsa *et al.* 2015)
Norovirus	2016-2017	South Africa	1	760	AGI	(Sekwadi et al. 2018).
Cryptosporidium spp.	2017	London	1	338	AGI	(Hall *et al.* 2017)
Total number			128	3363		

* AGI = Acute gastrointestinal illness

2.5 EPIDEMIOLOGICAL ASSESSMENTS

According to Sithebe (2017:28), the evaluation of the hazards linked to exposure to water contaminated with faecal matter can be conducted through either epidemiological investigations or the utilization of QMRA techniques. As stated by Soller *et al.* (2010), epidemiological investigations offer significant insights into the characteristics and scope of health impacts, the scale of hazards, and how these hazards are influenced or linked to degrees of faecal contamination and other pollution metrics. The field of recreational water epidemiology has a history spanning more than six decades, with initial investigations having been carried out in both the United States and the United Kingdom (Fewtrell *et al.,* 2015: 85). According to Viau *et al.* (2011: 3279), initial research indicated a correlation between *enterococci* and *E. coli*, which are frequently employed as bacterial markers of faecal pollution in water, and the incidence of gastroenteritis among individuals who swim.

Studies published between 1990 and 2010 were utilized to produce guidelines and standards aimed at reducing the health risks associated with recreational water exposure. The studies followed three broad protocols: a prospective cohort design, a randomized controlled trial, and a case-control study (Fewtrell *et al.* 2015: 86). Historically and recently, studies have utilized cohort study designs to assess the potential risks linked to recreational water exposure, as noted by Wade *et al.* (2010: 66).

The objective of the cohort study is to ascertain whether the initial exposure status has an impact on the likelihood of developing a subsequent disease. According to Silman *et al.* (2022), there exist two distinct categories of cohort studies, namely the prospective cohort study and the retrospective cohort study. According to Fletcher *et al.* (2022:12), implementation of the cohort approach enables the identification of various disease outcomes resulting from a single exposure.

The prospective approach involves the identification of cohorts based on their current exposure status, followed by a subsequent monitoring period to ascertain any potential onset of disease. The prospective approach offers a significant benefit in that it enables the determination of the exposure being measured, the method of exposure measurement, the timing of exposure status change measurement, and procedures for future identification (Dey *et al*. 2020:57). The study design of prospective cohorts is significant in providing crucial insights into the relationships between exposure and disease, as stated by Kelsy *et al*.(1996: 44). According to Li *et al.* (2021), prospective cohorts in the study of infectious diseases aim to examine the characteristics of the exposure and modes of transmission, as well as to establish the frequency of disease occurrence in the population that has been exposed. The methods utilized in prospective cohort study designs for assessing potential risk factors of interest exhibit variability across different studies, as noted by Kelsy *et al.* (1996:44). Wade *et al*. (2010) conducted an observational prospective cohort study utilizing questionnaires to investigate the relationship between faecal indicator organisms and swimming-related illnesses in recreational water. Questionnaires utilized in epidemiological research are utilized to gather data from participants about their exposure, potential risk factors, and incidence of the targeted disease (Kazi *et.al.* 2012: 4).

In 2010, Wade and peers conducted a study within the United States of America to investigate the correlation between rapid molecular assessments of faecal indicator organisms and instances of swimming-related sickness at coastal beaches (Wade *et al*. 2010). The research utilized a prospective cohort study design. According to Wade *et al.* (2010), the utilization of this particular study design facilitated the establishment of correlations between the mean water quality and the consequent likelihood of disease among the group that was exposed. The study conducted by Sanchez-Nazario *et al.* (2014: 220) involved a prospective cohort analysis of three tropical beaches that were exposed to both point and nonpoint sources of faecal pollution. The study aimed to assess the risk of illness among individuals who swam and those who did not. The findings of this study highlight the need for further epidemiological investigations with larger sample sizes, as well as the use of alternative indicators and techniques in recreational waters.

According to the National Research Council (United States of America) in 2004, epidemiological studies about recreational bathing waters have established significant correlations between associated health effects and diverse microbial indicators or pathogens. Table 2.2 provides a summary of selected epidemiological studies used to estimate the risk of waterborne diseases associated with recreational contact with surface water. The study's results could potentially aid in the development of interventions aimed at mitigating the risk of disease outbreaks among rural communities residing along the uMsunduzi River and Dusi Marathon canoeists.

Table 2.2: Epidemiological studies investigating the association between illness and recreational exposure to surface water

Author /year	**Location**	**Study design**	**Health risk**	**Contamination path- way**	**Water type**	**conclusion**
(Prüss, 1998)	South Africa	Cohort	*Enterococci*/faecal *Streptococci* for both marine and freshwater, and *E coli* for freshwater.	Swimming in polluted fresh water.	Marine	A higher number of indicator bacteria in recreational areas was linked to a higher health risk for swimmers. When comparing swimming in dirty water to swimming in clean water, relative risk (RR) values were often

						significant (usually RR values were between 1.0 and 3.0).
(Wade, 2003)	South Africa	Cohort	*Enterococci* in marine water. E. coli in both fresh and marine environments.	Swimming in polluted fresh water and marine water.	Marine	In fresh water, *E coli* was a better indicator of gastrointestinal illness than enterococci and other bacteria. In both fresh and marine environments, indicators of viral contamination were strong indicators of GI illness.
(Marion *et al.*, 2010)	USA	Cohort	*E.coli* and GI illness.	Wading, playing or swimming in contaminated fresh water.	Fresh water	There is an association between GI symptoms and faecal indicator bacteria
(DeFlorio-Barker *et al.*, 2018).	USA	Prospective cohort	*Shigellosis* *Giardiasis* *Cryptosporidiosis* Intestinal infection due to *E. coli* *Campylobacter* *Norwalk virus* *Leptospirosis*	Swimming, paddling, motor-boating in fresh and marine water.	Fresh water	According to the study, it has been determined that activities such as swimming/wading, paddling, motor-boating, and fishing in surface waters within the United States are responsible for causing more than 90 million instances of gastrointestinal, respiratory, ear, eye, or skin-related disorders. These illnesses can vary in terms of their severity.
(Russo *et al.*, 2020).	USA	Systematic literature review and meta-analysis	*E coli* *Salmonella spp.* *Rotaviruses: Gastrointestinal problems*	Recreational activities as swimming, sports-related contact, minimal contact, and sand contact	Fresh water	This study concludes that the exposures associated to various forms of recreational activities are significant factors in the exposure pathway assessment of illness risks connected with engaging in recreational activities in ambient surface waters.
(Gerdes *et al.*, 2023)	USA	Prospective cohort	Acute otitis externa, *campylobacteriosis, cryptosporidiosis, giardiasis, Legionnaires'* disease *Norovirus* infection, *Pseudomonas* pneumonia, *Pseudomonas* septicemia, *Salmonellosis* (nontyphoidal), Shiga toxin–producing *E coli* infection with an O157 or non-O157 serogroup*shigellosis*and vibriosis caused by *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and other non–*V. cholerae* spp	Swimming in untreated recreational water venues (lakes, rivers, and oceans)	Fresh water and marine	In 2014, a total of 5.61 million (95% credible interval (CrI) 2.97–9.00 million) illnesses were linked to recreational water, Recreational water exposure was responsible for 36% of hospitalizations from waterborne illnesses.

2.6 QUANTITATIVE MICROBIAL RISK ASSESSMENT

The QMRA is a methodology used to evaluate the potential risk of infection or illness from exposure to microorganisms. This assessment is based on the analysis of dose-response relationships between the quantity of pathogens and the corresponding health effects (Haas *et al* 2014). The WHO recognized the QMRA as a legitimate approach to managing the risks associated with recreational water in 2003. Several governments, including Australia, the United States, and New Zealand, have since incorporated QMRA into their guidelines.

This information is supported by sources such as (NHMRC (2008), USEPA (2012), and MfE (2003)) (Leonard *et al*., 2021).

According to current international guidelines, the utilization of QMRA is recommended as a risk assessment methodology to manage microbial water quality (WHO, 2016). QMRA is a useful tool in risk analysis as it enables the prediction of the likelihood of illness, particularly in cases where the contribution of water to the disease is not easily measurable through epidemiological studies or public health surveillance (Latchmore *et al.,* 2023). QMRA is being utilized more frequently in recreational surface water environments to assess the risk posed by waterborne pathogens (Ryan *et al*., 2022: 118659). Recent studies have examined *Rotavirus*, *Norovirus*, *Adenovirus*, *Cryptosporidium* (Deere *et al*., 2022: 1543-1557), *Giardia* (Mustafa *et al.,* 2020: 115351), and *E. coli* (Williams *et al*., 2020: 33). Due to their prevalence and potential health consequences, these pathogens are frequently employed in QMRA for recreational surface water (WHO, 2018: 15). Additionally, QMRA serves to pinpoint high-risk populations and regions, prioritize strategies for mitigation, and assess the efficacy of implemented interventions (Fewtrell *et al.,* 2020: 4172). QMRA serves as an important method for assessing risks associated with waterborne pathogens; however, it presents several limitations. Methods for microbial quantification exhibit constraints in sensitivity and accuracy (Huang *et al.,* 2020: 105924).

Methods for microbial quantification are essential in QMRA; however, their previous limitations affected the accuracy of risk evaluations. Culture-based methods, including plate counting, resulted in an underestimation of pathogen concentrations due to incomplete recovery (Garcia *et al*. 2020: 12). Molecular techniques such as qPCR can identify non-viable pathogens, potentially leading to an overestimation of risk (Kim *et al.,* 2020: 345). Droplet digital PCR (ddPCR) provides superior absolute quantification for aquatic pathogens, but its use in QMRA is constrained by a number of important drawbacks. A key worry is the method's inability to differentiate between viable and non-viable organisms, as it identifies all target nucleic acids, thereby overestimating actual infectious risk (WRC 2024: 12). In addition to the technique's narrower dynamic range compared to traditional qPCR, environmental water matrices frequently contain complex substances that cause rain ambiguous droplets that make it difficult to set an accurate threshold and require significant sample dilution for high-concentration outbreaks (Abad *et al*. 2020: 10). Additionally, ddPCR requires more intense workflows and specialized training, and its labour and material costs are almost double those of qPCR (Yang *et al.* 2014: 11996). The small reaction volumes used can lead to dead volume losses, which is particularly problematic when attempting to quantify rare pathogens in large environmental samples (Zheng *et al.* 2020: 3). Microscopic techniques, such as fluorescence microscopy, are often labour-intensive and necessitate specialized expertise (Singh *et al*. 2020: 567). Further constraints to QMRA involve the variability in pathogen concentrations and the uncertainty in exposure assessment, which can impede accurate risk estimation (Heida *et al*., 2024: 121858). Moreover, insufficient data on pathogen prevalence and the complex structure of water systems may further constrain the use of QMRA (Chen *et al*. 2021: 116285; Xu *et al.* 2020: 115964).

The limitations that were found showed that in order to increase the dependability of QMRA, microbial quantification methods needed to be continuously improved and validated. Global progress in QMRA and waterborne illness prevention has therefore subsequently shifted toward proactive, real-time, molecular-based approaches as opposed to reactive ones. The use of QMRA to help climate change adaptation in water management, the integration of AI for predictive modelling, and the combination of QMRA with sophisticated molecular techniques to manage high-risk, low-dose infections are some of the major developments for 2025–2026 (Brower *et al.* 2018: 293, Khodaparast *et al.* 2024: 3 and Izah *et al.* 2025:).

In order to guarantee the efficacy of tests for use in both developed and developing nations, the WHO advised that molecular diagnostic tests for waterborne illnesses meet the ASSURED criteria, which stand for Affordable, Sensitive, Specific, User-friendly, Robust, Rapid and Deliverable (land *et al.* 2019: 46).

Recent attempts to create novel nucleic acid amplification assays have helped to create isothermal amplification methods that satisfy the ASSURED requirements for molecular diagnostics and can be used as point-of-application testing. Loop-mediated isothermal amplification (LAMP), helicase-dependent amplification, recombinase polymerase amplification (RPA), rolling circle amplification (RCA), cross-priming amplification, and nucleic acid sequence-based amplification (NASBA) are some of the more recent assays (Vincent *et al.* 2004: 795, Piepenburg *et al.* 2006: 204, Xu *et al.* 2012: 246, Compton 1991: 91,andNotomi*etal.*2000:3).

The LAMP approach, the most often used of these tests, operates at a single temperature and is so reliable that samples do not require DNA extraction ensuring that a field technician can easily conduct the process (Khodaparast *et al.* 2022: 2641).

The application of LAMP, particularly in QMRA frameworks, is a developing field in South Africa. Although qPCR or culture-based techniques continue to be employed by many South African QMRAs, new research shows that LAMP is a useful technique for supplying the high-sensitivity input data needed for risk modelling (Martzy *et al.* 2017: 62 and Hedrich *et al.* 2016: 2038).

In the United Kingdom and the United States, LAMP assays are primarily applied as rapid, field-deployable alternatives to laboratory-based qPCR for detecting high-priority waterborne pathogens such as *Cryptosporidium spp.*, *Legionella*, and human faecal indicators (Wong *et al.*, 2018; Martzy *et al.*, 2019). While standard QMRA in these regions historically relied on qPCR for high-precision quantification, LAMP is increasingly integrated into point-of-care environmental monitoring to provide real-time hazard identification in complex matrices like wastewater and groundwater (Burch *et al.*, 2021; Foo *et al.*, 2020). Studies in the USA particularly in statewide groundwater assessments, highlight that although LAMP may be less sensitive than nested PCR or qPCR, its superior specificity and resistance to environmental inhibitors make it a more robust tool for immediate risk characterization in the field (Burch *et al.*, 2021; Foo *et al.*, 2020). In England and broader European contexts, LAMP has been successfully benchmarked against USEPA approved methods for the rapid detection of human faecal contamination, showing 100% sensitivity and specificity compared to traditional qPCR assays (Martzy *et al.*, 2019; Wong *et al.*, 2018).

In South Africa, the LAMP assay has emerged as a high-efficacy tool for detecting waterborne pathogens like *Schistosoma haematobium*, *Cryptosporidium spp.*, and *Vibrio cholerae*, offering sensitivities up to 100-fold greater than conventional PCR in resource-limited rural settings (Water Research Commission(WRC), 2019). Studies in provinces such as Limpopo and Mpumalanga have utilised LAMP to identify active transmission foci that traditional microscopy often misses, providing critical high-sensitivity data for QMRA and the development of risk-based toolboxes (Archer *et al.*, 2022; WRC, 2019). By facilitating rapid, on-site pathogen screening in under 60 minutes without the need for sophisticated laboratory infrastructure, LAMP supports municipal safety planning and addresses the high microbiological risk reported in 46% of the country's water supply systems (DWS, 2023; WRC, 2021).

QMRA methodology employs numerical simulations to investigate various scenarios and forecast potential risks to human health (Federigi *et al* 2019). Therefore, it is plausible to utilize it in combination with or as a substitute for epidemiological findings as a significant structure for the management of risks. The QMRA process involves four connected steps as illustrated in Table 2.3: Risk categorization, exposure assessment, dose-response assessment, and hazard identification (Sithebe 2017: 88).

Table 2.3: Steps involved in QMRA (Amoah 2018)

Step	**Aim**
1. Hazard identification	To describe acute and chronic human health effects (toxicity, carcinogenicity, mutagenicity, developmental toxicity, reproductive toxicity and neurotoxicity) associated with any particular hazard, including pathogens.
2. Exposure assessment	To determine the size and nature of the population exposed and the route, amount and duration of the exposure.
3. Dose–response	To characterize the relationship between various doses administered and the assessment incidence of the health effect.

4. Risk characterization	To integrate the information from exposure, dose–response and hazard identification steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty.

2.7 SUMMARY OF THE CHAPTER

Research studies, using interdisciplinary and transdisciplinary approaches to investigate and enhance a deeper understanding of these complexities and linkages among land use, surface water quality, and water-related infectious diseases, are crucial in developing integrated measures for sustainable water quality monitoring and diseases prevention. This study aims to establish a correlation between the exposure to uMsunduzi River and the occurrence of pathogenic infections among the Dusi Marathon participants. This will be achieved by comprehensively evaluating the water quality, microbial risk, and infection monitoring. In the next section, Chapter three provides an in-depth analysis of the water quality. Furthermore, chapter three correlates with the first objective of this study.

3. CHAPTER THREE

MICROBIAL WATER QUALITY: A PRIMARY ANALYSIS OF THE WATER QUALITY OF UMSUNDUZI RIVER

3.1 INTRODUCTION

Water quality is a crucial aspect of environmental health, with microbial contamination posing significant risks to human well-being. Surface waters, such as rivers, lakes, and reservoirs, provide a variety of recreational opportunities, such as swimming, canoeing, fishing, and paddling (DeFlorio-Barker *et al.* 2018: 1). In recent years, there has been a notable surge in the popularity of recreational water sporting events in South Africa (Giampiccoli *et al.* 2015: 229). These activities provide opportunities for socialization, stress relief, and mental health enhancement in addition to promoting physical fitness. Moreover, recreational activities in surface waters contribute to local economies by attracting visitors and generating revenue for businesses like hotels, restaurants, and outfitters (Columna *et al.* 2015: 1119). However, the provision of recreational activities depends in part on environmental factors such as geographical character, water quality, and safety (Stebbings *et al.* 2020: 103905).

Water quality has become a prominent global subject due to the challenges posed by water quality degradation and water scarcity (Sithebe 2017: 7). The release of effluent from wastewater treatment facilities has emerged as a significant contributor to the deterioration of river water quality in South Africa, frequently exhibiting elevated levels of microorganisms. According to Kumar *et al.* (2019: 2), there is a positive correlation between the degree of biological contamination and the likelihood of waterborne infection, as evidenced by multiple reports.

Waterborne transmission can transpire via actions such as swimming, bathing, or ingestion of inadequately treated or disinfected water. Dale *et al.* (2010) reported that a significant proportion of waterborne outbreaks found in Australia were associated with recreational activities. Deloro-Barker *et al.* (2018) further reported that there is an approximate occurrence of 4 billion instances of surface water recreation annually, resulting in an estimated 90 million instances of disease throughout the United States. Graciaa *et al.* (2014) assert that, untreated outbreaks related to recreational water are predominantly caused by gastrointestinal pathogens, encompassing bacteria, protozoa, and viruses. Therefore, it is crucial to continually monitor and assess the water sources used for recreational activities.

The objective of this chapter was to establish the microbiological water quality of the uMsunduzi River month , before the Dusi canoe marathon, during the Dusi canoe marathon, and after the Dusi canoe marathon. Microorganisms were used as primary indicators to assess water quality for this study. The pathogens included for analysis were *E. coli, Pseudomonas aeruginosa,* and *Cryptosporidium* spp. as depicted in Table 3.1 below.

Escherichia coli, also known as *E. coli*, is a bacterial species that is frequently detected in the gastrointestinal tract of both humans and animals (Odonkor *et al.* 2013: 5). Although certain types of *E. coli* are benign, there exist strains that can induce sickness in humans, resulting in symptoms such as diarrhea, abdominal discomfort, and elevated body temperature (Aminian *et al*. 2023: 235) *E. coli* is frequently employed as a faecal contamination indicator in surface water, given that its existence implies that the water has been polluted with animal or human excreta (Khan *et al.* 2020: 235).

The genus *Pseudomonas aeruginosa* comprises of rod-shaped, gram-negative bacteria that have the potential to contribute to nonpoint source pollution of surface water (Wilson *et al.* 2020). *Pseudomonas aeruginosa* encompasses a diverse array of species, with certain strains inhabiting soil and water sources (Araos *et al.* 2017: 2686). Strains may induce illness in individuals with compromised immune systems. *Pseudomonas aeruginosa* has the potential to infiltrate surface water through diverse origins, such as agricultural drainage, animal excrement, and human sanitation (Beceiro *et al.* 2013: 185). *E. coli* and *Pseudomonas aeruginosa* are capable of infiltrating surface water via nonpoint sources, including agricultural runoff and urban stormwater. Ingestion of these microorganisms by humans can result in waterborne illness (Wilson *et al.* 2020 and Aminian *et al.* 2023).

Microorganisms have the potential to significantly contribute to point source pollution, especially when untreated or insufficiently treated wastewater is released into bodies of water (Edokpayi *et al.* 2017: 402). Some of the most common microorganisms that contribute to point source contamination faecal coliforms and enterococci. Faecal coliforms and Enterococci are bacterial collections that is typically present in the gastrointestinal tract of metabolic animals, such as humans, domesticated animals, and wild fauna (Byappanahalli *et al.* 201: 685). The occurrence of these microorganisms in aquatic environments serves as an indicator of the existence of excremental pollution and the plausible existence of other noxious pathogens (Motlagh *et al.* 2019:1402). These microorganisms have the potential to induce gastrointestinal illness and other health complications in both humans and animals (Byappanahalli *et al.* 201:685). Faecal coliform and Enterococci pollution can be attributed to various sources such as sewage discharges, agricultural runoff, and stormwater runoff from urban areas. (Boehm and Sassoubre 2014).

Protozoa, although not commonly linked with point and nonpoint source pollution, may be detected in wastewater and have the potential to cause water quality problems if not adequately managed (Pandey *et al.* 2014: 3). The implementation of adequate measures for the treatment and disinfection of wastewater is imperative to mitigate the transmission of waterborne illnesses that are attributed to protozoan pathogens (Clasen *et al.* 2015: 2).

Table 3.1: Characteristics of selected microorganisms

Characteristics	***E. coli***	***Pseudomonas spp.***	***Cryptosporidium spp.***
Brief description	Gram-negative, non-spore forming bacteria.	Heterotrophic, motile, Gram-negative rod-shaped bacterium	*Cryptosporidium* are intracellular, extracytoplasmic epithelial cell parasites. Oocysts are round, thick-walled structures of about 4–5 µm in diameter and contain four sporozoites
Includes members that are not human pathogens	Yes	No	Yes
Includes human pathogenic members	Yes	Yes	Yes
Found within the intestinal tract of humans and warm-blooded animals	10^7 –10^9 cfu/g faeces in humans; 10^3 –10^9 cfu/g faeces in animals	Yes	Yes
Capable of growth in the aquatic environment	Yes, under specific conditions	Yes	No. *Cryptosporidium* species do not multiply outside the host
Applicable to fresh and marine waters	Yes	Yes	Yes
Association between GI and the indicator	Yes	yes	Yes
Rapid, easy, inexpensive, culture-based method available	Yes	Yes	Yes
Reference	(Odonkor *et al.* 2013), (Aminian et al. 2023) and (Edokpayi *et al.* 2017).	(Wilson *et al.* 2020), (Araos *et al.* 2017) and (Beceiro *et al.* 2013)	(Dixon, 2015), (Wanamaker, and Grimm, 2004).

3.2 METHODOLOGY

3.2.1 Site description

UMsunduzi River passes through highly industrialized areas, receiving run of from rural communities and the municipality along its course, as well as agricultural areas, which contribute to organic pollution in the river (Adeyinka 2014: 47). The river tributaries include, Dorpspruit, Slangspruit, and Baynespruit (Baker et.al 2015: 16). In addition, it is a major source of drinking water for the rural communities residing along with it and irrigation of crops and small gardens. It is also used for recreational sports (Munyengabe *et al.* 2017: 2).

3.2.2 Sample collection

Surface water was collected along the uMsunduzi River at defined sites (located along the route of the Dusi canoe marathon illustrated in Table 3.2), over 3 months that is, before (January 2022), during (February 2022) and after (May 2022) marathon. Six sampling locations along the uMsunduzi River were identified and monitored for the presence of specific microorganisms viz., *E. coli*, *Pseudomonas* and *Cryptosporidium* spp. The pattern of sampling sites followed a downstream path. The sampling sites were located using the route map for the Dusi canoe marathon of 2020 (Figure 3.1). In addition, the points were selected because they are the initial six checkpoints that all competitors pass through during the first day of the race. This ensured that a sufficient number of participants had contact with the selected sites. As the race progressed, the likelihood of participants failing to complete it rose . The names of the locations and their precise GPS coordinates are enumerated in (Table 3.2) below. Permission from all relevant stakeholders was sought before the commencement of the fieldwork (Appendices 2a and 3a).

Replicate sampling was employed to ensure variability and precision. Sediment samples were also utilized for this study. Samples were collected three times, one month apart, on a single day: before the marathon (17 January 2022), during the marathon (17 February 2022), and after the marathon (17 May 2022). The student governing body's striking activities caused an academic interruption, which delayed the samples obtained after the marathon by two months. There were three sets of samples collected: six before the marathon, six during the marathon, and six after the marathon. The samples were equivalent to a total of 18.

Samples were collected using sterilized 5L bottles at each site. The containers were rinsed with the water from the source, before collection and filled to the brim. The surface water was collected by holding the sterilised container by the handle and plunged at 0.5m below the water surface against the water current. All samples were protected from direct exposure to sunlight and transported to the laboratory, in cooler boxes where they were stored in a refrigerator at 2-4°C in the dark until analysis. Samples were analysed within 24hrs. Whether conditions remained the same during all sampling period.

Table 3.2: Sample Sites

Site name	***GPS Coordinates***	***Site description***	***Potential pollution source(s)***
Site A	29°37’19.22″ S 30°22’34.50″ E	Located inside the city, receiving surface runoff.	Anthropogenic activities
Site B	29°36’42.76″ S 30°23’15.54″ E	Located inside the city, within proximity to the taxi rank And receiving surface runoff.	Anthropogenic activities
Site C	29°36’6.28″ S 30°24’10.65″ E (Approximately 1.1 km from Mussons weir)	Surrounded by a township, community members. and also used for irrigation purposes by the community members additionally used as a source of water for livestock and domestic animals.	Anthropogenic Activities and Agricultural pesticides during rain wash off. Faecal, contamination from livestock.
Site D	29°59’62.730″ S 30°43’94.380″ E	The sampling site is located near the wastewater treatment facility (WWTP), with the discharge pipes being directed towards this vicinity.	Discharge from Darvill wastewater treatment facility
Site E	29°36’18.26″ S 30°28’59.85″ E (Approximately 3.8 km from Campsbell)	The sampling location is situated adjacent to the wastewater treatment plant (WWTP) downstream	Discharge from Darvill wastewater treatment facility
Site F	29°36’18.26″ S 30°28’59.85″ E	The sampling location is situated adjacent to the wastewater treatment plant (WWTP) downstream	Downstream, discharge from Darvill wastewater treatment facility

Figure 3.1 displays the Dusi canoe marathon map and the sample sites

Figure 3.1: Sample sites

3.3 ENUMERATION OF BACTERIAL INDICATORS

3.3.1 *E. coli*

Using the **IDEXX Colilert 18 method** (per the manufacturer's instructions), the presence and absence test as well as the enumeration of *E. coli* was accomplished. The Colilert-18 reagent (IDEXX Laboratories) was added to samples (final volume of 100ml) and thoroughly dissolved with active mixing. The **Colilert-18 reagent (IDEXX Laboratories)** was added to 100 mL of distilled water (negative control) and thoroughly dissolved. The mixture was poured into a **Quanti-tray/2000 (IDEXX Laboratories, Westbrook, United States),** sealed, and incubated for 18 hours at 38°C. The Colilert-18 method detects total *E coli* simultaneously by counting the number of yellow-coloured wells that fluoresce at 365 nm. This occurs when *E. coli* utilize β-glucuronidase to metabolise 4-methyl-umbelliferyl-β-D-glucuronide (MUG) and produce fluorescence. Since the majority of non-coliforms

lack these enzymes, they cannot develop and interfere. The matrix of the Colilert-18 test is specifically designed to suppress the few non-coliforms that possess these enzymes.
The MPN was determined by employing the MPN chart supplied by **IDEXX (IDEXX Laboratories, Westbrook, USA).** The presence of *E. coli* was quantified through the counting of wells exhibiting yellow coloration under fluorescence at 365 nm and interpreted against the MPN chart.

3.3.2 Pseudomonas aeruginosa

Using the **IDEXX Pseudalert** method (per the manufacturer's instructions), the presence and absence test as well as the enumeration of *Pseudomonas aeruginosa* was accomplished. The **Pseudalert reagent (IDEXX Laboratories)** was added to samples (final volume of 100ml) and thoroughly dissolved with active mixing. The **Pseudalert reagent (IDEXX Laboratories)** was added to 100 mL of distilled water (negative control) and thoroughly dissolved. The mixture was poured into a **Quanti-tray/2000 (IDEXX Laboratories, Westbrook, United States)**, sealed, and incubated for 24 hours at 38°C. Pseudalert detects *Pseudomonas aeruginosa* in water samples. The test is based on bacterial enzyme detection technology that detects *Pseudomonas aeruginosa* by hydrolyzing a substrate in the Pseudalert reagent. The cells of *Pseudomonas aeruginosa* exhibit a rapid rate of proliferation and multiplication, facilitated by the abundant availability of amino acids, vitamins, and other essential nutrients that are inherent in the Pseudalert reagent. *Pseudomonas aeruginosa* strains that are in the process of active growth exhibit the presence of an enzyme capable of cleaving the substrate within the reagent, thereby resulting in the emission of blue fluorescence when exposed to ultraviolet light.

The MPN was determined by employing the MPN chart supplied by **IDEXX (IDEXX Laboratories, Westbrook, USA)**. UV light was used to read the **Quanti-tray/2000 (IDEXX Laboratories, Westbrook, United States).** The presence of *Pseudomonas aeruginosa* was indicated by blue fluorescence. Subsequently, the wells that exhibited a positive (blue fluorescent) reaction were counted and interpreted against the MPN chart.

3.4 DETECTION AND QUANTIFICATION OF PROTOZOA

3.4.1 Cryptosporidium spp.

Membrane Filtration

In the context of this study, a vacuum filtration system was employed in conjunction with a nitrocellulose membrane filter (filter paper) having a diameter of 47mm and a pore size of 0.2µm. the small pore size in the membrane facilitates capturing the microorganism present in the sample of water during filtration. The aforementioned system was employed to extract *Cryptosporidium spp*. from the water samples.

The filtration column was sterilised through the process of autoclaving. The filtration column was then connected to a vacuum pump. The nitrocellulose membrane filter was placed between the chamber and catchment vessels of the column. When the vacuum was turned on, the water filtered through the nitrocellulose membrane filter, into the catchment vessel. Any microorganism present in the water was trapped on the nitrocellulose membrane filter.

A volume of 1 litre of the water sample underwent filtration using a nitrocellulose membrane filter with a pore size of 0.2µm and 47mm diameter. Subsequently, the filter paper trapped microorganisms and other particulate matter. Consequently, the filter paper that had entrapped the particles was utilized to examine the presence of *Cryptosporidium spp.* in the water samples. Using sterile forceps, the filter paper was transferred into sterile petri plates containers, which were subsequently labelled and used for the DNA extraction procedure.

DNA extraction and purification

For this study. **The ZymoBIOMICS ™ DNA Miniprep Kit** was used for the extraction and purification of *Cryptosporidium spp.* Using the ZymoBIOMICS ™ DNA Miniprep Kit method (per the manufacturer's instructions), the DNA extraction and purification of

Cryptosporidium spp. was accomplished. The Kit comprised a series of five fundamental procedures. The process involved five steps:

- The cellular structure was disrupted to produce a lysate. This was achieved by transferring the filter paper trapped with microorganisms (From the filtration step) into the **ZR BashingBead ™ Lysis Tubes (0.1 & 0.5mm)** and then adding the **750µL ZymoBIOMICS ™ Lysis solution** into the tube.
- The soluble DNA was separated from cell debris and other insoluble material. This was achieved through, bead beating the, **ZR BashingBead ™ Lysis Tubes (0.1 & 0.5mm),** containing the lysate with an **Omni Bead Ruptor**. The **ZR BashingBead ™ Lysis Tubes (0.1 & 0.5mm),** were then consequently centrifuged at 10,000 *g* for 1 minute.
- The DNA of interest was bound to a purification matrix. This was achieved by transferring the supernatant from the previous step into the **Zymo-Spin ™ III-F Filter** in a **Collection** Tube and was centrifuged at 8,000 *g* for 1 minute. The **ZymoBIOMICS ™ DNA Binding Buffer** was then added to the filtrate in the collection tube. The mixture was then transferred into **a Zymo-Spin ™ llCR Column** in a collection tube and centrifuged at 10,000*g* for 1 minute.
- Proteins and other contaminants were washed away from the matrix. This was achieved through the addition of the **ZymoBIOMICS ™ DNA Wash Buffer** to the **Zymo-Spin ™ llCR Column** in a **collection tube** from the previous step and centrifuged at 10,000g for 1 minute. The **ZymoBIOMICS ™ DNase/RNase Free Water** was then directly added to the **Zymo-Spin ™ llCR Column** matrix, incubated for 1 minute and centrifuged at 10,000g for 1 minute. The eluted DNA was then transferred into the **Zymo-Spin ™ lll-HRC Column Filter** containing the **ZymoBIOMICS ™ HRC Prep Solution** and centrifuged at 16,000*g* for 3 minutes.
- The DNA was then eluted. The **Implen NanoPhotometer™ NP80 UV/VIS spectrophotometer** was then used to measure the purity of the eluted DNA sample. 2µL of sample was pipetted onto the measurement surface. The measurement surface was clean and devoid of any impurities or debris that would have impacted the measurement's accuracy. Additionally, **ZymoBIOMICS ™ DNase/RNase Free Water** was used as a blank solution before the sample was analysed. To make

sure the graph of the blank spectra was a flat line, a sample of the blank solution was measured. To provide the NP80 with a negative control reference. This was to ensure that the measurement surface was devoid of any contaminants before measuring the eluted DNA sample.

Polymerase Chain Reaction (PCR)

Two microliters of eluted DNA from each sample column (total number of 18 samples) were added to a microcentrifuge tube containing the PCR mixture (Master mix (12.5uL), distilled water (8.5uL) and each *Cryptosporidium*-specific primers [primer forward (1uL) and primer reverse (1uL) to make a final volume of 25mL. PCR amplification was carried out on a **T100 thermal cycler (Bio-Rad),** using the conditions: 4 min at 94 °C, denaturation at 94 °C during 40 s and annealing at 55°C–56 °C for 40 s, 72 °C for 1 min, final extension at 72 °C for 7min, 45 cycles. Five microliters from each PCR amplification were electrophoresed on 1% agarose gel and stained with ethidium bromide.

Droplet Digital PCR (ddPCR) assay optimization for the detection of Cryptosporidium spp.
Using the **QX200 Droplet Digital PCR** equipment **(Bio-Rad),** the eluted DNA was amplified. Each ddPCR assay mix had a final volume of 22 µL and was made up of 10 µL of ddPCR MasterMix, 1µL of forward and reverse primers (Table 3.3), 2 µL of template DNA, and 8µL dH_2O. The reaction also included a no-template control (NTC). Using the **QX200 Droplet Generator (Bio-Rad),** droplets were produced. The **QX200 Droplet Reader** and **QuantaSoft software (Bio-Rad)** were utilised for the analysis of the plates. The threshold line was established through the segregation of negative and positive droplets.

Specificity of primers used

The study successfully detected and amplified the targets through the utilisation of the generic species-specific gp60 primer pair, *for Cryptosporidium parvum*. as outlined in Table 3.3. The amplified DNA was subjected to gel electrophoresis to ensure the specificity. The outcome of the analysis demonstrated the presence of singular bands (60 kDa), which is indicative of targeted amplification. A positive synthetic DNA control was incorporated to verify the amplification of the target product and the specificity of the primers. After conducting primer specificity analysis, the gp60 primer assay was selected for methodology optimization in this study. The above methodology was appreciated by (Mthethwa *et al.* 2022).

Table 3.3: Primers used for the PCR amplification of the eluted DNA of *Cryptosporidium spp.*

Primers	**Forward**	**Reverse**	**Size (bp)**	**Target**	**References**
Cryptosporidium (gp60)	GCCGTTCCACTCAGAGGAAC	CCACATTACAAATGAAGTGCCGC	150	*C. parvum*	Mthethwa *et al.* (2022)

3.5 Results

3.5.1 Microbial water quality

E. coli

Table 3.4 presents the average concentration of *E. coli* (MPN/100ml) observed at all sampling locations, irrespective of the time of measurement. The mean (m) concentration was 82.2(±112). MPN/100ml. There was no statistically significant difference observed in *E. coli* concentrations among all sites, therefore these were combined, and their means used to represent the *E. coli* concentrations in uMsunduzi River. **Site E** was observed to have the highest mean concentration (144(±219)MPN/100ml), however, it should be noted that, it is not significantly higher than other sites. In addition, **Site A** accounted for the least mean concentration of (42.0(±48)MPN/100ml). The concentrations varied, ranging from 397 MPN/100ml in **Site E** to 1.00MPN/100ml in **Site F**, with their means (143 (±279) and 58(±65)) respectively.

Table 3.4: Mean (±SD) concentration of *E. coli* (MPN/100ml) From uMsunuduzi river

Sample site	**No. of samples**	**Mean**	**(±SD)**	**Minimum**	**Maximum**
Site A	3	42.0	(±48)	3.00	95.9
Site B	3	98.4	(±87)	16.8	189
Site C	3	108.	(±164)	7.50	298
Site D	3	42.4	(±63)	2.00	114
Site E	3	143.5	(±219)	6.20	396
Site F	3	57.9	(±65)	1.00	129
Total	18	82.2	(±112)	1.00	396

The analysis encompassed a total of 18 samples. Table 3.5 presents the average concentrations of *E. coli* recorded across three-time intervals: before the marathon, during the marathon, and after the marathon. The period after the marathon demonstrated the highest average concentration of *E. coli* recorded at 189 (±134.51) MPN/100ml. In contrast, the lowest average concentration was observed before the marathon, recorded at 11.3 (±11.3) MPN/100ml. The average values varied from 397 MPN/100ml after the marathon to 1.00 MPN/100ml before the marathon.

Table 3.5: Summary statistics of *E. coli* concentrations along uMsunduzi River (Before the marathon, during the marathon and after the marathon)

Months	No. of samples	Mean(±SD)	(±SD)	Minimum	Maximum
Before the marathon	6	11.3(±11.3)	(±11.3)	1.00	27.5
During the marathon	6	44.1(±51.8)	(±51.8)	6.20	129.
After the marathon	6	189(±134.)	(±134)	43.5	397.
Total	**18**	**82. 2(±112)**	(±111)	**1.00**	**397**

Figure 3.2 below, illustrates the fluctuations in the average concentrations of three-time intervals. The levels of *E. coli* (MPN/100ml) exhibited a progressive increase during the studied period.

There is a notable disparity in the concentrations of *E. coli* observed in uMsunduzi River throughout specific measurement periods, as indicated by the statistical analysis (Welch's $t(2, 7.013) = 5.747$, $p = .033$). A post hoc analysis reveals that the measurements observed in the period after the marathon (May 2022) exhibited the highest concentration with a statistically significant increase compared to those recorded before the marathon (January 2022) ($p=.006$) and during the marathon (February 2022) ($p=.023$).

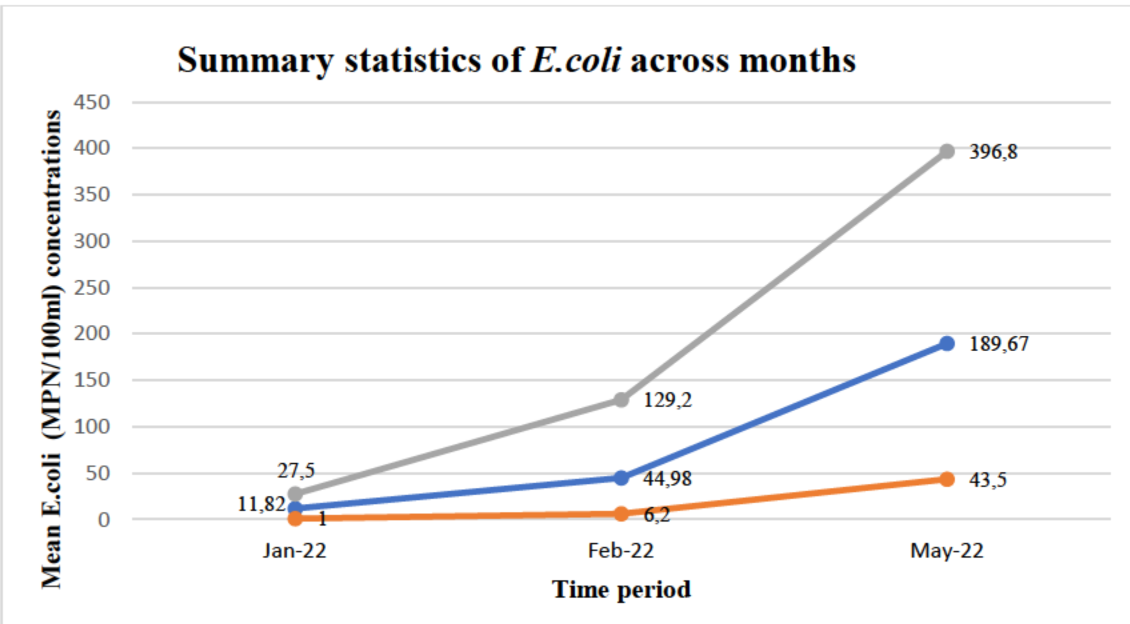

Figure 3.2: Summary of *E.coli* concentrations

Pseudomonas aeruginosa

The data in Table 3.6 displays the mean concentration of *Pseudomonas aeruginosa* (MPN/100ml) observed at all six sampling sites, irrespective of the time of observation. The average concentration was calculated to be (26.6 (±38.5) MPN/100ml). No statistically significant variation was found in the concentration of *Pseudomonas aeruginosa* across all sites. Consequently, these data were summarized, and their means were utilized to represent the concentrations of *Pseudomonas aeruginosa* in the uMsunduzi River. The site with the greatest mean concentration wa **Site D** (45.9(±76.0)MPN/100ml), but it should be emphasized that this concentration is not considerably greater than that of other locations. Furthermore, **Site F** findings revealed the lowest average of (11.5 (±9.10) MPN/100ml). The concentrations ranged from (134. MPN/100ml) in the Braai takeout to (1.00 MPN/100ml) in **Site C**, **Site F**, and **Site B**, with their respective averages being (45.9 (±76.0), 11.5 (±9.10), and 34.8 (±43.9)).

Table 3.6: Mean(±SD) concentration of *Pseudomonas aeruginosa* (MPN/100ml) from uMsunduzi River

Sample site	**No. of samples**	**Mean (±SD)**	**(±SD)**	**Minimum**	**Maximum**
Site A	3	13.4	(± 6.1)	9.5	20.4
Site B	3	34.8	(±43.9)	1.00	84.5
Site C	3	12.5	(±19.1)	1.00	34.5
Site D	3	45.9	(±76.0)	2.00	134
Site E	3	42.4	(±49.5)	3.00	98.0
Site F	3	11.5	(±9.10)	1.00	20.9
Total	18	26.8	(±38.5)	1.00	134

The research comprised 18 samples in total. Table 3.7 presents the average results from the study period, revealing that the peak recorded average was 65.3(±47.0) MPN/100ml observed after the marathon, with the average concentration attaining its maximum at 134 (±47.0) MPN/100ml. Conversely, the period before the marathon displayed the lowest mean concentration, recorded at 3.23 (±3.59) MPN/100ml reaching its maximum at 10.4 MPN/100ml. The total average for the specified period was 26.8 (38.5) MPN/100ml, with a range from 134 MPN/100ml to 1.00 MPN/100ml.

Table 3.7: Summary statistics of *P. aeruginosa* concentrations along uMsunduzi River (before, during, and after the marathon)

Months	No. of samples	Mean(±SD)	(±SD)	Minimum	Maximum
Before the marathon	6	3.23	(±3.5)	1.00	10.4
During the marathon	6	11.7	(±9.74)	1.00	26.1
After the marathon	6	65.3	(±47.0)	20.4	134
Total	**18**	**26.8**	(±38.5)	**1.00**	**134**

Figure 3.3 depicts the variations in time, in the mean concentrations observed during the study period. The data strongly indicates a gradual rise in the concentrations of *Pseudomonas aeruginosa* (MPN/100ml) throughout the study. According to the statistical analysis (Welch's $t(2, 7.484) = 6.451$, p =.0.24), there is a significant variation in the quantities of *Pseudomonas aeruginosa* found in the uMsunduzi River during various measurement periods. A post hoc analysis indicates that the measurements after the marathon (May 2022) exhibited a statistically significant increase when compared to the data recorded before the marathon (January 2022) (p=.004) and during the marathon (February 2022) (p=.012).

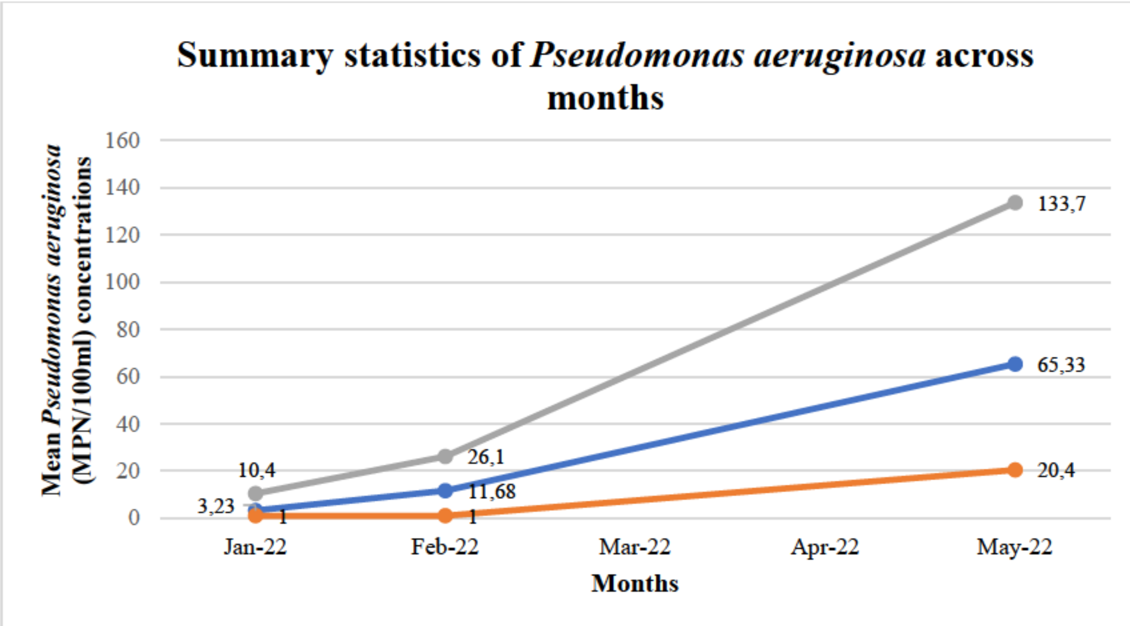

Figure 3.3: Summary of *P. aeruginosa* concentrations

Protozoan water quality

A notable fluctuation in the concentration of *Cryptosporidium spp*. was observed throughout the study. The mean concentrations observed before the marathon were 1200 (±706) copies/20µL. The levels ranged between 2040 copies/20µL to 143 copies/20µL. The average concentrations measured during the marathon were 629 (±422.2) copies/20µL, with a range of 1290 to 119 copies/20µL. The mean concentration of those observed after the marathon was 2340 (±1153.4) copies/20µL, whilst the range was from 4310 copies/20µL to 1180 copies/20µL. These depictions were the average concentrations irrespective of site. The concentrations of *Cryptosporidium spp.* in the water exhibited significant temporal variation. A one-way ANOVA was performed to compare the means of time intervals, $F(6.775) = 2$, $p = .008$. This result indicated that the variation in *Cryptosporidium spp.* concentrations were statistically significant with those recorded after the marathon being the highest. Following the initial ANOVA, additional tests were performed to compare time intervals, yielding a post hoc analysis result of $p = .007$, signifying a statistically significant difference as indicated in Figure 3.4.

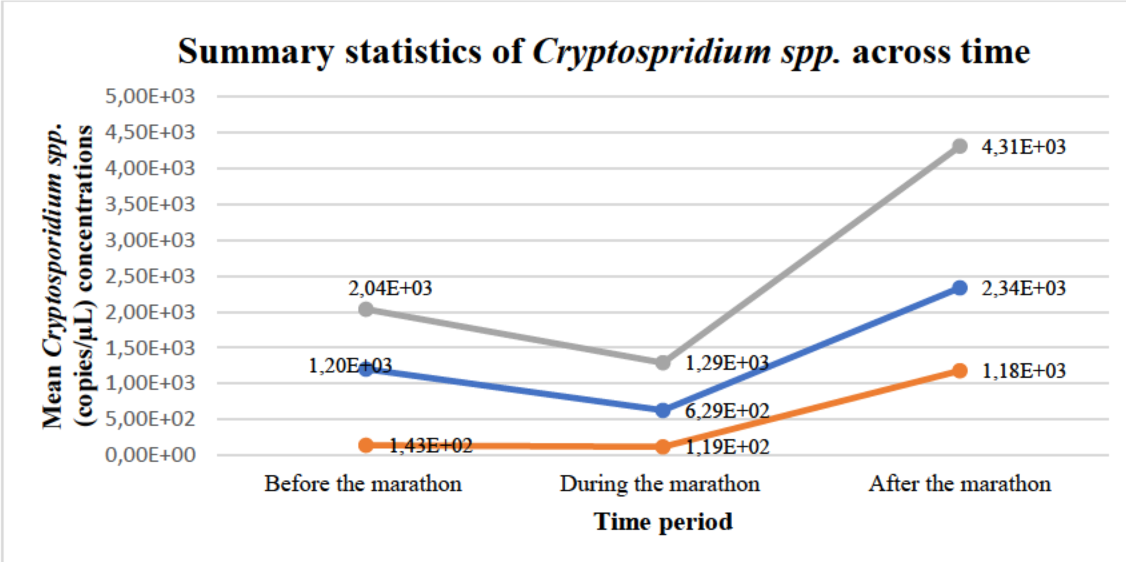

Figure 3.4: Mean *Cryptosporidium spp.* concentrations over time

The data in Table 3.8 displays the mean concentration of *Cryptosporidium spp.* (copies/20µL) detected across all sampling locations, independent of the time of measurement. The cumulative average concentration across all sites was 1390 (±1059) copies/20 µL. We observed no statistically significant difference in the concentrations of *Cryptosporidium spp.* across all sites. We aggregated the data from these sites and used the mean values to represent the concentrations of *Cryptosporidium spp.* within the uMsunduzi River.

Site B exhibited the highest mean concentration (2230 (±1885) (copies/20µL)). indicating that it had the greatest average quantity of *Cryptosporidium spp.* detected among all tested sites. It is essential to recognize that the observed difference lacks statistical significance. This indicates that although Site B exhibited the highest mean concentration, the difference between Site B and the other sites is not statistically significant, suggesting that it may be attributable to biological or environmental factors. Factors such as population heterogeneity, environmental influences, and biological variability can obscure significant differences.

Furthermore, the trend of Site A was observed to have the lowest average value of (840 (±952.) copies/20µL)). The concentrations demonstrated a range, spanning from (4310 (copies/20µL)) in Site A to (119 (copies/20µL)) in Site C.

Table 3.8: Mean (±SD) concentration of *Cryptosporidium spp.* (copies/20µl) across samples (*n*=18)

Sample site	**No. of samples**	**Mean (±SD)**	**(±SD)**	**Minimum**	**Maximum**
Site A	3	840 (±952.)	(±952)	143	1920
Site B	3	2230	(±1885)	633	4310
Site C	3	953 (±747.)	(±747)	119	1560
Site D	3	987 (±641)	(±641)	353	1640
Site E	3	1740 (±394)	(±1740)	1290	2040
Site F	3	1390 (±1059.)	(±1390)	755	3080

3.6 DISCUSSION

3.6.1 Water quality analysis before the marathon

The WHO and the DWAF in South Africa have established standards for the permissible concentrations of *E. coli* in surface water for recreational use (WHO 2003 and DWAF 1996). As per the guidelines established by the WHO, the permissible threshold for *E. coli* in surface water intended for primary contact recreational activities, such as swimming, is equal to or less than 200 colony forming units (CFUs) per 100 millilitres of water. The DWAF in South Africa has established a comparable criterion of 130 CFU per 100 millilitres for *E. coli* in surface water intended for recreational use. The aforementioned levels function as a valuable measure of the possible health hazards linked with engaging in recreational activities in bodies of water (WHO 2003; DWAF 1996).

The United States GDP (EPA) reports that one MPN per 100 millilitres (mL) of water is equivalent to one CFU per 100 mL of water. If a given water sample harbours a single colony-forming unit (CFU) of bacteria in every 100 millilitres of water, it is approximated to contain one most probable number (MPN) of bacteria per 100 millilitres of water (Gibson *et al.* 2021). The lowest average concentration of *E. coli* observed before the marathon, was recorded at 11.3 (±11.3) MPN/100ml. The average values varied between 27.5MPN/100ml to 1.00 MPN/100ml before the marathon. These findings suggest that effective wastewater treatment, natural attenuation processes, and low human impact may be contributing to the low *E. coli* levels in these water bodies (Gemmell *et al.,* 2013).

It is noteworthy that the mean *E. coli* concentration before the marathon was below the acceptable range for recreational water usage as defined by DWAF (1996) and WHO (2003). Additional credible explanations for the observed low *E. coli* levels in the river include natural attenuation, which encompasses the river's inherent processes like sedimentation, filtration, and UV radiation that may have successfully reduced *E. coli* concentrations. During seasonal variations, *E. coli* levels may change based on rainfall, temperature, and water flow. In South Africa, January marks the summer season, which may have contributed to reduced *E. coli* levels as a result of heightened water flow and increased UV radiation. Effective water quality management, local authorities or conservation initiatives may have been employing successful strategies for managing water quality, including river cleanups or restoration projects. Such as the initiatives implemented by the DUCT and the Trans-Caledon Tunnel Authority (TCTA), a South African state-owned entity responsible for water resource management and development.

The DUCT has initiated multiple projects aimed at preserving the uMsundusi River and its surrounding ecosystem. In 2020, DUCT undertook a river clean-up initiative, successfully removing litter and debris from the riverbanks (DUCT, 2020). A water quality monitoring program was also implemented to track changes in the river's health (DUCT, 2020). Furthermore, DUCT has focused on reducing faecal waste in rivers, minimizing solid waste, addressing industrial pollution, and managing invasive alien plant species, all while enhancing land care and mitigating soil erosion (DUCT, 2020). They have implemented education and awareness programs (DUCT, 2020).

In 2023 (TCTA), working alongside stakeholders from KZN for an important clean-up of the Msunduzi River in Pietermaritzburg (DUCT, 2023). In addition, to commemorate Heritage Day 2023, TCTA organized a clean-up of the Msunduzi River and conducted water-quality testing in partnership with several stakeholders in Pietermaritzburg (DUCT, 2023).

Geological factors, that pertain to the river's geology, including the presence of aquifers or natural filters, may have played a role in the reduction of *E. coli* levels (Gomaa *et al*., 2022: 3471) River aquifers and natural filters are essential for preserving water quality and sustaining aquatic ecosystems. The underground layers composed of permeable rock, sand, or soil are capable of storing and transmitting significant volumes of water, effectively filtering out sediment, bacteria, and various contaminants (Giri *et al.,* 2021:116332). The natural filtration process includes sand and gravel eliminating particulate matter (Ihekweme *et al.,* 2023: 258), rock formations purifying contaminants via chemical reactions, and soil and sediment taking in excess nutrients and sediments (Giri *et al.,* 2021:116332). Vegetation plays a crucial role in absorbing excess nutrients and sediments, thereby aiding in water purification (Ihekweme *et al.,* 2023: 258).

A comparable pattern was noted with *Pseudomonas aeruginosa*; the average concentrations measured before the marathon were the lowest in comparison to the other time intervals. It is crucial to highlight that, despite the low average concentrations of these measures, they surpassed the thresholds set by the WHO (2003) and DWAF (1996). In surface water, even at low concentrations, *Pseudomonas aeruginosa* is a potent opportunistic pathogen that may be hazardous to human health (Kumar *et al.* 2022: 123). Its potential for infection in individuals with weakened immune systems, such as those suffering from HIV/AIDS or cancer (Mishra *et al.* 2022: 156), increases concerns about waterborne transmission (WHO, 2022:23). *Pseudomonas aeruginosa* synthesizes toxins such as exotoxin A and pyocyanin (Singh *et al.* 2022: 78) and possesses a low infectious dose (Kumar *et al.* 2022: 125). The organism's resistance to various antibiotics (Mishra *et al.* 2022: 160) and its capacity to form biofilms (Singh *et al.* 2022: 92) render it a difficult pathogen to manage. *Pseudomonas aeruginosa* is capable of causing respiratory and wound infections, especially in immunocompromised individuals. The presence of *Pseudomonas aeruginosa* in surface water, even at low concentrations, presents a risk to human health and underscores the necessity for effective water management strategies (Kumar *et al.* 2024: 10).

The average recorded concentration of *Pseudomonas aeruginosa* before the marathon equated to 3.23 (±3.59) MPN/100ml reaching its maximum at 10.4 MPN/100ml. As per the guidelines established by the WHO, the presence of *Pseudomonas aeruginosa* in recreational water at a concentration of 0-1000 MPN/100ml is regarded as a potential health hazard. In line with the guidelines set forth by the DWAF, the detection of *Pseudomonas aeruginosa* at a concentration of 10-1000 cfu/100ml is deemed to be indicative of a potential health hazard.

The low quantities of the microbe can be verified based on the identical rationales as those presented for *E. coli.* The reason for this rationale is that *E. coli* and *Pseudomonas aeruginosa* exhibit various similar traits that could restrict their growth and persistence in the uMsunduzi River. Both are gram-negative bacteria, facultative anaerobes and mesophilic which could limit their growth in specific water conditions (Cabral *et al.,* 2010:3657). Furthermore, both types of bacteria exhibit sensitivity to UV radiation variations in water pH and temperature changes. Moreover, they encounter competition from indigenous microorganisms and predation by protozoa and s other microorganisms. The low concentrations may also be influenced by factors such as adhesion to sediment or biofilm (Hassard *et al.,* 2016:1692 and Wanjugi *et al.,* 2013:517-526) and water treatment processes (WHO 2017: 23). The observed similarities indicate that environmental factors and local conditions could be constraining the growth and survival of *E. coli* and *Pseudomonas aeruginosa* in the uMsunduzi River before the marathon.

A general trend of low levels of all microorganisms employed as indicator organisms was observed before the marathon. *Cryptosporidium parvum* exhibited a trend analogous to that observed with *E. coli* and *Pseudomonas aeruginosa*. It is important to note that, although the average concentrations of *Cryptosporidium parvum.* observed before the marathon were low, they exceeded the thresholds established by WHO (2003). The mean concentrations observed before the marathon were 1200 (±706) copies/20µL. The levels ranged between 2040 copies/20µL to 143 copies/20µL.
As per the guidelines provided by the WHO, the detection of 10-10000 oocyst microbes/L of *Cryptosporidium spp.* is considered a significant health hazard to human beings. Applying the oocyst/20µL concentration equation to convert copies/20µL of *Cryptosporidium spp*. to oocyst count. The mean concentration recorded before the marathon was 6.0×10^1 oocysts. which surpass the predetermined threshold. The detected low levels of *Cryptosporidium spp.* in surface water align with findings from other studies that report comparable trends (Chique *et al.,* 2020). Low levels may be attributed to effective wastewater treatment natural attenuation processes and minimal human impact (Kinge *et al.,* 2017).

3.6.2 Water quality analysis during the marathon

The water quality results during the marathon revealed a notable upward trend in microorganism concentrations compared to those observed before the marathon. This observable trend was consistently seen across all microorganism concentrations during the marathon, suggesting a potential correlation between the event and the elevated microbial levels.

The observed average concentration of *E. coli* during the marathon was 44.1 (±51.84) MPN/100ml. The observed average values differed from 129 MPN/100ml to 6.20 MPN/100ml during the marathon. During the marathon, the average concentration of *Pseudomonas aeruginosa* was 11.6 (±9.74) MPN/100 ml. The measured average concentrations varied between 26.1 MPN/100ml to 1.00 MPN/100ml. The measured average concentrations of *Cryptosporidium spp.* during the marathon were 629 (±422.) copies/20µL, with a range spanning between 1290 to 119 copies/20µL.

Despite the fact that *E. coli* levels are maintained within the limits established by WHO (2003) and DWAF (1996), *Pseudomonas aeruginosa* and *Cryptosporidium spp.* surpass the threshold established by these organizations. Both species continue to represent a significant hazard to human health.

The increase in microorganism concentrations could be attributed to several factors. One possible explanation is the mixing of sediment and water particles due to increased activity during and after the marathon in the water. Furthermore, the increased human activity during the marathon may have played a role in the increase of microorganism concentrations. For instance, a higher number of individuals in and around the water may have resulted in a rise in wastewater production, including urine, faeces, and sweat, which can introduce microorganisms into the water. This notion is further supported by the studies below.

Studies indicate that the combined effects of heightened sediment disturbance, human activity, and the possibility of wastewater generation during the marathon may have a role in the rise in microorganism concentrations in the water (Smith *et al.,* 2018:123; Johnson *et al.,* 2019:156). The disturbance of sediment, resulting from canoeing and various recreational activities, has the potential to release microorganisms into the water column

(Davis *et al.,* 2020:101). Furthermore, activities such as swimming and boating can elevate the concentrations of microorganisms present in the water (Taylor *et al.,* 2017: 78).

3.6.3 Water quality analysis after the marathon

The period after the marathon demonstrated the highest average concentration of *E. coli* recorded at 189 (±134.51) MPN/100ml, *Pseudomonas aeruginosa* recorded at 65.3(±47.0) MPN/100ml, and *Cryptosporidium spp,* recorded at 2340 (±1153.4) Copies/20µL. The progressive increase was statistically significant as evidenced by the post hoc analysis for the respective microorganisms and respective time- intervals. It is important to highlight that all concentrations measured for the respective microorganisms after the marathon exceeded the thresholds established by the WHO (2003) and DWAF (1996) for the recreational use of surface water. The elevated concentrations of *E. coli, Pseudomonas aeruginosa*, and *Cryptosporidium spp.* detected in the uMsunduzi River after the Dusi Canoe Marathon in 2022 may be linked to various contributing factors.

The interplay of various elements, including recreational activities stormwater runoff, sediment load, nutrient loading, flooding, agricultural runoff, urban runoff, and combined sewer overflows (Smith *et al.* 2019: 123), may have contributed to the elevated levels of microorganisms observed in the river.
Furthermore, the significant rainfall in KZN from March to May 2022 presumably contributed to amplifying stormwater runoff, sediment load, nutrient loading, flooding, agricultural runoff, urban runoff, and combined sewer overflows, all of which have the potential to introduce microorganisms into the river. The interplay of these factors might have contributed to the elevated levels of microorganisms observed in the river. Given that, no statistically significant difference was found in *E. coli, Pseudomonas aeruginosa,* and *Cryptosporidium spp.* concentrations across all sites, the values from Site E and Site D respectively have been utilized to illustrate ineffective wastewater management, as they are similar to those from other locations.

Site E exhibited the highest *E. coli* concentration of 397MPN/100ml MPN/100ml, after the marathon. The findings were not within the acceptable range according to WHO (2003) and DWAF (1996). Site E is part of the route network for the Dusi Canoe Marathon. Figure 2 illustrates the location of Site A, situated in the downstream area of Darvill Wastewater Treatment Works. The Darvill wastewater treatment facility is engineered for the treatment of wastewater, which is then discharged into the uMsunduzi River. The discharge takes place near Site E.

In the same vein, it was observed that Site D exhibited the most substantial presence of *Pseudomonas aeruginosa* after the marathon, with a recorded measurement of 134 MPN/100ml. Additionally, it had the highest average concentrations, which came to 45.9 (±76.0) MPN/100ml. Similar to the observations made at Site E, Site D is located near the discharge point of Darvill WWTP, as illustrated in Figure 3.1. The aforementioned trend was found with *Cryptosporidium spp.* Site D exhibited high concentrations after the marathon, reaching a maximum concentration of 1640 copies/20µL.

These results provide further evidence in favour of inefficient wastewater management from Darvill WWTP, due to flooding in KZN after the marathon resulting in sewer overflows and the treatment facility being overwhelmed. This suggests that a significant proportion of microorganisms and other contaminants may have not been removed effectively from the water by the wastewater treatment facility, indicating that it was operating ineffectively.

Engagement in recreational activities in surface water has been demonstrated to elevate the presence of microorganisms, such as *E. coli* and various other bacteria (Smith *et al.* 2019: 124). Research has consistently demonstrated that engaging in recreational activities like swimming, canoeing, and paddleboarding can result in elevated levels of microorganisms in surface water (Russo *et al.,* 2020: 115729). A study on a surface water river indicated that *E. coli* levels rose following recreational activities (O'Flaherty *et al.* 2019). In the same manner, an investigation into a river revealed that the concentrations of microorganisms and nutrients rose following recreational activities (Chávez-Díaz *et al*. 2020).

The study findings indicate that the uMsunduzi River exhibited significant pollution levels of indicator and potentially pathogenic organisms after the marathon. Consequently, utilizing untreated water from the river carries a substantial risk of infection. The microbiological condition of the uMsunduzi River is poor, as it fails to meet the standards set by the WHO (2003) and DWAF (1996) for recreational purposes. Our microbiological analyses indicated that the uMsunduzi River may be contaminated with faecal matter due to the frequent detection of pathogens such as *E. coli* and *Cryptosporidium spp.* associated with human and animal faecal matter.

3.7 SUMMARY OF THE CHAPTER

The objective of this study was to assess the microbial water quality of the uMsunduzi River over a three-month period, which included one month before the Dusi canoe marathon, during the marathon, and one month after the marathon. The water quality analysis in the uMsunduzi river was conducted successfully before, during, and after the Dusi canoe marathon. The detection and quantification of *E. coli*, *Pseudomonas aeruginosa,* and *Cryptosporidium spp.* were achieved by the study's objectives. Moreover, the study demonstrated that the microbial concentrations exceeded the thresholds established by the WHO, with the results being particularly pronounced after the marathon. The study has successfully established the rationales associated with the elevated concentrations observed.

The risks associated with exposure to *E. coli, Pseudomonas aeruginosa*, and *Cryptosporidium spp.* in surface water used for recreational activities are a significant area of concern. The identification of these microorganisms as causative agents of waterborne disease outbreaks is significant. Consequently, enhancing the quality of the river is crucial, and it may be necessary to dedicate further resources to the development of water treatment infrastructure and the establishment of reliable sanitation systems.

This chapter delineates the methodologies used to conduct the water quality analysis, along with the results and interpretations of the findings. Chapter 4 outlines the probabilistic microbiological risk assessment, the techniques utilized, the confounding outcomes, and the rationale for these results.

4. CHAPTER FOUR

A PROBABILISTIC MICROBIAL RISK ASSESSMENT FOR RECREATIONAL USE OF THE UMSUNDUZI RIVER

4.1. INTRODUCTION

In this study, a QMRA approach was employed to estimate the daily risk of gastrointestinal (GI) illness among individuals who utilize the uMsunduzi River. The daily risk was calculated for three, different periods, that is before the marathon, during the marathon, and after the marathon. QMRA is a methodological approach that provides a systematic and scientifically sound framework for evaluating water quality. Its primary objective is to ascertain whether the water sources under investigation present any potential health risks to individuals utilizing them (Sunger *et al.* 2015: 212). (QMRA) approach consists of four interconnected steps: a) identification of hazards, b) assessment of exposure, c) evaluation of dose-response relationship, and d) characterization of risk (Amoah 2018: 1022) as illustrated in Figure 4.2. The main aim of this analysis was to quantitatively assess the daily risk of gastrointestinal illness among individuals using the uMsunduzi River, specifically focusing on participants of the Dusi Canoe Marathon.

4.2. METHODOLOGY

4.2.1 Study Approach

The primary mode of exposure that was taken into consideration was ingestion, which included the estimations for incidental ingestion as well. The term incidental ingestion describes swallowing inhaled and trapped nasal and oral mists. The proportion of canoeing observed at a specific site was calculated based on a sample size of 100 users. The application of a dose-response model was applied to estimate the expected number of illnesses resulting from water-based recreational activity. A risk estimation distribution was created to depict the probable occurrences of illnesses that may arise on a randomly selected day at a recreational site. This distribution was generated by considering the relative frequencies of pathogen concentration, exposure time, the fraction of users engaged in canoeing, and the volumes of ingestion. QMRA estimates were then used to calculate Attributable risk for the participants for the period, during the marathon.

4.2.2. Site description

Six locations along the uMsunduzi River's route that had a high likelihood of being used by participants in the Dusi Canoe Marathon, the local population for domestic use, farmers for irrigation, and agricultural animals were monitored by using methods for Index pathogen enumeration, of three months that is, before, during, and after the marathon. Two of the locations are situated near an operational (WTTP), namely Site A and Site C. Chapter 3 provided a comprehensive analysis of site characteristics, encompassing both geographical and physical factors.

4.2.3 Data source

4.2.3.1 Hazard identification

Three microorganisms were selected as an index for waterborne illness linked to recreational exposure to surface water for this assessment. These microorganisms include *E. coli, Pseudomonas aeruginosa,* and *Cryptosporidium spp.* Several publications have revealed a connection between *E. coli, Pseudomonas aeruginosa Cryptosporidium spp*., and waterborne sickness resulting from recreational exposure to surface water (Gerdes *et al*. 2023; Islam *et al*. 2020; Gholipour *et al.* 2023; McKee *et al.* 2021). The risk associated with this association has been effectively evaluated using the QMRA approach (Sunger *et al*. 2018; Dorevitch *et al*., 2011; Dufour, 1984). In Addition, *E. coli*, *Pseudomonas aeruginosa Cryptosporidium spp.* have established dose response models.

4.2.3.2 Exposure Assessment

To assess the potential risks associated with engaging in recreational water activities, it was necessary to collect data about five specific input parameters. These parameters include 1) indicator bacterial concentration (MPN/100 ml) and protozoan concentration (oocysts/20ul), 2) ingestion rates (ml/hr), 3) exposure duration (hr/day), 4) total users (users/day). A three-month timeframe was designated for the collection and analysis of water samples to assess the quality of the water. A comprehensive account of the sampling and analysis processes has been provided in Chapter 3. The data regarding the number of users, the proportional distribution of user frequency, and the statistics on exposure duration at each site were collected using both in-person surveys and online surveys. The ingestion rate estimations from the Dorevitch study (Dorevitch *et al*. 2011) were utilized to create the exposure distributions for water-based activities with low contact. Table .1 &4.2 below present a summary of the final ingestion rate distributions utilized in the (QMRA) model. The assumption was made that accidental ingestion of the water would lead to gastrointestinal illness. Furthermore, it was believed that the worst-case scenario occurred, wherein there was no reduction in the concentration of pathogens before the individuals were exposed to or ingested.

Table 4.1: Ingestion rate estimates for recreational water sports

Observed activity	**Fitted distribution (Mean, SD)** **Ingestion rate (ml/hr)**	**Distribution source**
Canoeing	Natural log (5.5)+ Fixed intake (4ml/hr)	Sunger *et al.* (2015)
Fixed ingestion rate accounts for inhalation via mist and droplets in the nose and mouth		

Table 4.2: Exposure assessment parameters

Exposure scenario/ assumptions for dosage	**Ingestion rate (ml/hr)**	**Exposure duration (hrs/day)**	**Total volume ingested (ml/hr)**
Recreational/ accidental exposure to contaminated surface water: It was hypothesized that the participants of the Duzi Canoe Marathon intentionally or unintentionally ingested the water while canoeing.	4ml/hr	• 2hrs.45min/day • 8hrs.25min/3 days (multiple exposure)	• 11ml/2hrs.45min • 33ml/8hrs.25min

Dose-response assessment

The exponential (eq 1) and beta-Poisson (eq 2) models were chosen as probability models for developing the dose-response relationship due to their extensive utilization in QMRA (Sunger *et al.* 2015: 214). The beta-Poisson dose-response model, proposed by DuPont *et al*. (1971), was employed. Other researchers have utilized this model to estimate the risks of *E. coli* infection (Weir *et al*. 2017: 74). Hence, the risk of *E. coli* infection was evaluated utilizing the suggested formula. The exponential dose-response model as proposed by Ojileo *et al.* (2003) was in the estimation of *Pseudomonas aeruginosa* infection risk. For the risk estimation of *Cryptosporidium spp*. infection, the exponential dose-response model as suggested by Messner *et al.* (2001) was used. For *Cryptosporidium spp.* it was presumed that the copy number per gene was equivalent to the cell number.

$$P(d) = 1 - \left[1 + \frac{d}{N50}\left(2^{\frac{1}{a}} - 1\right)\right]^{-a}$$

Equation 1 Beta-poison dose-response model

where "*P(inf)*" is a risk of infection per exposure event; *"d" is* the concentration of *E. coli* ingested in a known volume; "*N50*" - median infection dose representing the number of *E.*

coli that will infect 50 % of the exposed population. A "*N50*" value of 2×10^{6} as reported by DuPont *et al*. (1971) was used for this study. "α" is a dimensionless infectivity constant, where an "α" value of 1.55×10^{-1} as suggested by DuPont *et al*. (1971) was used for the study.

$$Pinf = 1 - \exp(-k \times dose)$$

Equation 2 Exponential dose-response model

where "*Pinf*" is the infection risk per exposure event, "dose" the concentration of microorganisms (*Pseudomonas aeruginosa*)/ (*Cryptosporidium spp*.) ingested per that event and "k" the dimensionless infectivity constant. A "k" value of $3{,}22 \times 10^{-7}$ as reported by Ojileo *et al.* (2003) for the purpose of *Pseudomonas aeruginosa* and a "K" value of 5,72E-02 as reported by Messner *et al*. (2001) for *Cryptosporidium spp.* was used in this study.

4.3 PROBABILISTIC ANALYSIS

In the probabilistic approach, the uncertainty and variability for each input parameter were taken into account by generating input distributions rather than point estimations. Probability distributions were generated for the observed parameters, the users' proportional distribution and exposure duration distribution, using the distribution fitting feature available in the @Risk 7.5 (Palisade Corporation) software. Two risk estimates, total illnesses (illnesses/day) and illnesses per 100 users (illnesses/100 users/day), were generated for this study.

The cumulative number of illnesses was ascertained using the individual risk estimates. The estimation of individual risk for canoeing, specifically the risk of illness per day per person, was initially established through a Monte Carlo analysis. This analysis involved the random selection of values from distributions of concentration, ingestion rate, and exposure duration to calculate the dose. The provided data were utilized in the relevant dose-response models to estimate the likelihood of an individual being sick as a result of engaging in canoeing activities in the uMsunduzi River. The estimation of the likelihood of an illness occurrence at a particular site was determined by extracting data points from the frequency distribution. Similarly, the second set of analysis was carried out to predict the anticipated number of illnesses for 100 000 population . The individual risk estimates per activity, which were previously obtained, were multiplied by a factor of 100 to assess the risk of illness per activity per 100 000 population (Figure 4.1). The survey data, as referenced in Chapter 4, was utilized to estimate the proportion of user participants engaged in canoeing at each respective site.

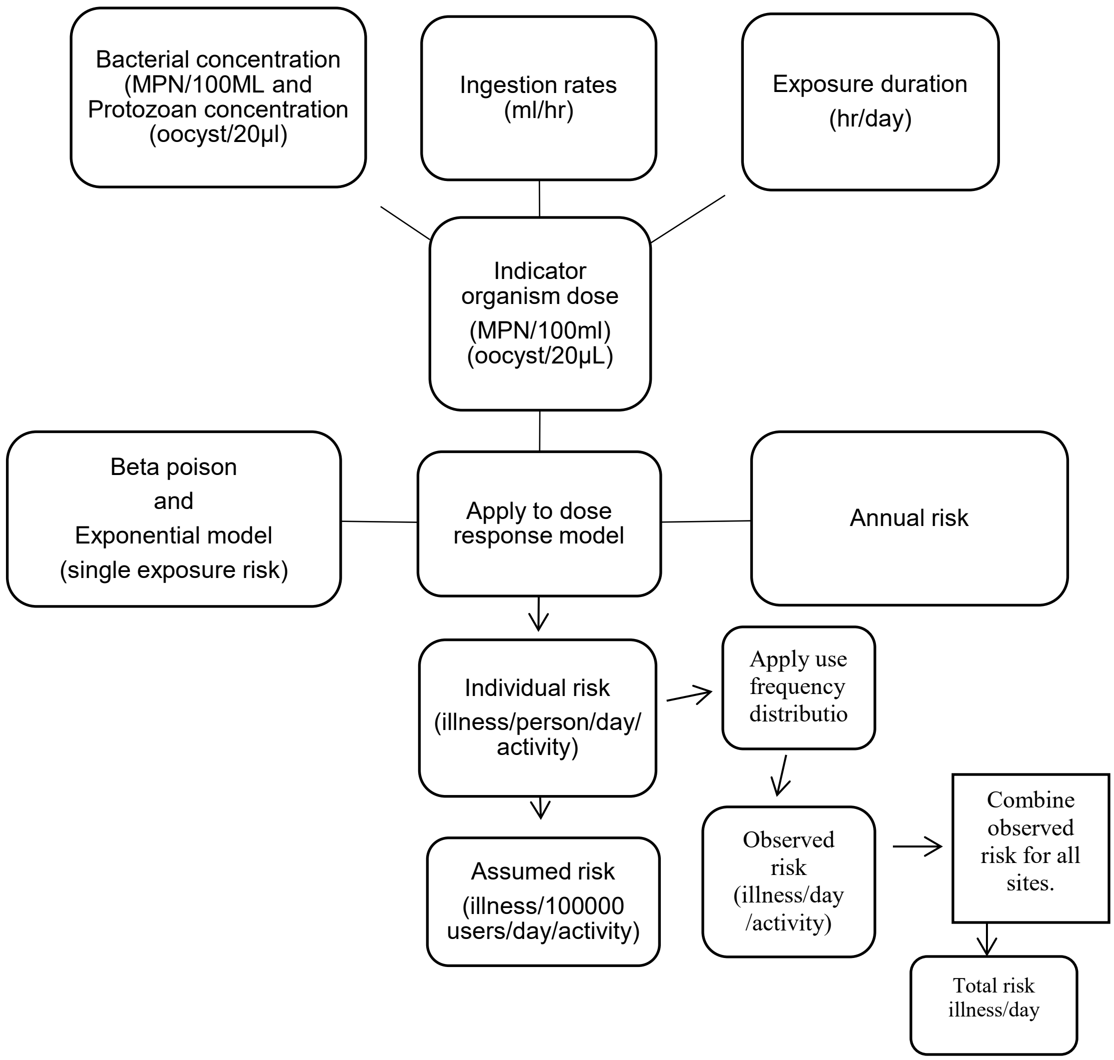

Figure 4.1: Flow diagram of probability

4.3.1. Risk characterization

The collective results of the hazard identification, exposure assessment, and dosage response assessment were utilized to ascertain the severity of infection caused by (*E.coli*, *Pseudomonas aeruginosa*, and *Cryptosporidium spp.*). in the given exposure scenario. The annual infection risk (P_A) associated with multiple exposures was determined using the following formula:

$$P(A) = 1 - (1 - Pinf)^n$$

Equation 3 Annual infection risk where P_{inf} is the risk of infection from a single exposure to a dose *(d)* of (*E.coli, Pseudomonas aeruginosa, and Cryptosporidium spp.*) and *(n)* being the number of days of exposure to the single dose *(d)* (Amoah, 2018:84).

4.3.2. Risk management

Deepnarain (2020) affirms that the objective of risk management is to mitigate or eliminate risks and their associated adverse outcomes.

4.3.3. Probability of infection following exposure to polluted river water

In this study, the risk of infection was calculated by assuming that 8% of the mean *E. coli* concentration was pathogenic, as supported by existing literature (Ramlal *et al.* 2022:166). For *Cryptosporidium spp.* it was presumed that the copy number per gene was equivalent to the cell number (Yang *et al.* 2014: 1105).

<u>Integration of the site-specific data for the QMRA</u>

The research included site-specific measurements of water ingestion volumes and laboratory-measured concentrations of pathogens. This allowed the estimattion to be more precise pathogen doses for the QMRA. The results of the study demonstrated that when site-specific exposure data was included, tailored estimated risks with greater variation were produced, in contrast to when literature-estimated water ingestion data is utilized. Using local data for risk assessments is crucial, highlighting its significance. In addition, the research directly assessed the presence of reference microorganisms in the water samples to quantify the potential risks they pose.

This study utilized laboratory measurements of the reference pathogens in water samples. This approach potentially minimized uncertainties associated with exposure in the QMRA.

Identifying the precise locations of each site was an essential element. As an illustration, certain locations are situated near urban structures, informal settlements, agricultural areas, and wastewater treatment plant facilities. This close contact has the potential to lead to a rise in contamination levels. The focused attention on this matter is significant as it can greatly impact the allocation of resources to areas that require close monitoring along the river. Figure 4.2 below outlines the steps of the QMRA utilized in this study.

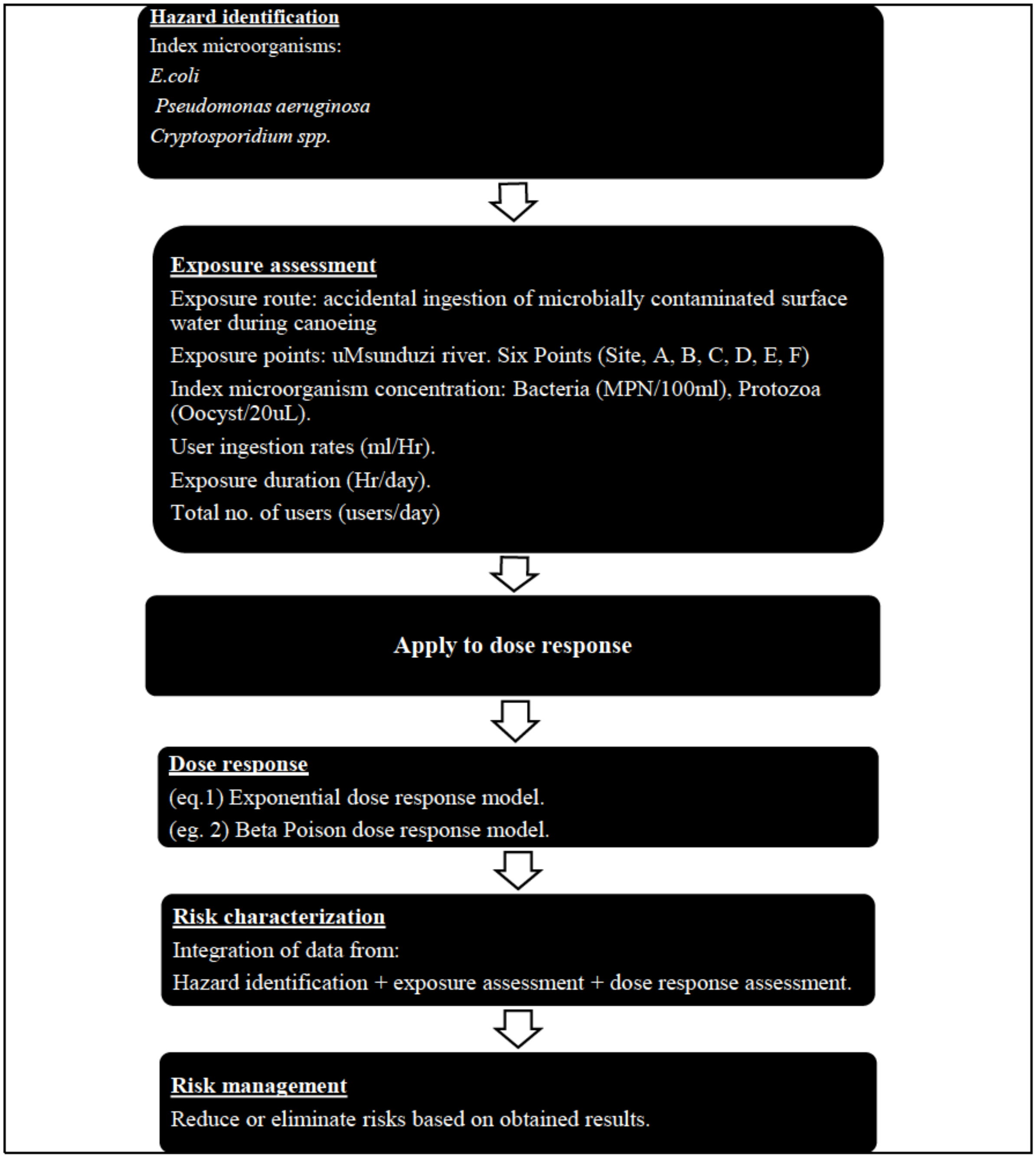

Figure 4.2: Flowchart of QMRA

4.4. RESULTS

Exposure assessment

Exposed population characteristics

A cross-sectional survey was conducted with 325 participants. Among them, 173 were unexposed and 152 were exposed to the uMsunduzi River. Only the participants who were exposed were included in the analysis for the QMRA. Four primary age classes were observed in the study, namely 25-39, and 40-64 years. Each of these age categories was represented by , (91.0%), and (9.0%) participants, respectively. The sample consisted entirely of male participants of White ethnicity (100%). The findings indicated that 100% of the population was exposed to health risks as a result of being exposed to river water contaminated with pathogenic microbes. Out of the 152 participants in the study, 108 (71%) reported experiencing diarrhea after being exposed to the river and accidentally ingesting its water. Additionally, 44 participants (29%) reported being exposed to the river and accidentally ingesting its water but did not experience diarrhea.

The risk estimate was based on a sample size of 152 participants, which included all individuals who participated in the study. Given that each person correctly completed the questionnaire's exposure and symptom categories that is, they all indicated whether or not they had been exposed to the river and whether or not they had developed diarrhea as a result of that exposure.

Quantitative risk assessment according to microorganisms and exposure scenario
Probability of *E.coli* infection for the Dusi Marathon participants using untreated surface water for recreational activities

Estimated risk of illness before the marathon

The uMsunduzi River poses a potential health risk for recreational users due to the possibility of *E. coli* infections. The risk of *E. coli* infection from a single exposure exhibited significant variation across different time intervals, with the lowest risk recorded before the marathon.

The estimated risk of *E. coli* infection from a single exposure before the marathon was 6.63×10^{-7} ($\pm6.35\times10^{-7}$), with a range between 1.55×10^{-6} and 5.59×10^{-8}. The data suggests that, among 100 individuals, roughly 0.0663% face a risk of *E. coli* infection from a single exposure before the marathon. This indicates that in a group of 100 000 population , fewer than 1 case or around 0.00663% would be predicted to get an *E. Coli* infection from a single encounter. This finding indicates that the risk of *E. Coli* infection before the marathon from a single exposure was relatively low. The estimated risk varied from 0.155% to 0.00559%. Furthermore, this implies that the actual risk of *E. coli* infection may be either higher or lower than the estimated average.

The average annual risk of *E.coli* infection before the marathon was 6.63×10^{-7} ($\pm6.35\times10^{-7}$) . The average annual risk of *E. coli* infection for a population of 100 000 population was estimated to be $1,27\times10^{-4}$ ($\pm2.32\times10^{-4}$) before the marathon. This is equivalent to an average annual risk of approximately 0.13%. That is, before the marathon approximately 0.13% of the 100 000 individuals, had a possibility of becoming infected with *E. coli* annually.

Estimated Risk of Illness During the Marathon

For 100 000 population , the estimated risk of contracting *E. coli* from a single encounter during the marathon was 2.52×10^{-6} ($\pm2.90\times10^{-6}$), with a range of $7,23\times10^{-6}$ to $3,47\times10^{-7}$ per person. This indicates that the estimated risk of contracting *E. Coli* from a single encounter is 0.00000252% per 100 000 population. This range implies that the actual risk of *E.coli* infection may be slightly higher or lower than the estimated average, with a range of approximately 0.000723% to 0.0000347%.

The estimated average annual risk of *E. coli* infection for a sample of 100 000individuals was 4.73×10^{-4} ($\pm1.06\times10^{-3}$) during the marathon. On average, approximately 0.0473% of a population of 100 individuals were estimated to be at risk of *E. coli* infection annually.

Estimated Risk of Illness after the marathon

For 100 000 participants, the estimated risk of contracting *E. coli* from a single encounter after the marathon was calculated to be 1.06×10^{-5} ($\pm7.53\times10^{-6}$) per person. This means that, out of 100 people, approximately 0.00106% were estimated to be at risk of *E. coli* infection from a single encounter.

The estimated average annual risk of *E. coli* infection for a sample of 100 000 individuals was calculated to be 3.02×10^{-3} ($\pm2.73\times10^{-3}$) after the marathon. This means that, on average, approximately 0.302% of the 100 individuals were estimated to be at risk of *E. coli* infection per year.

<u>Comparison of the estimated risk of illness</u>

There was a notable variation in the risk of one-time exposure among the three time periods (before, during, and after) with a statistically significant p-value of 0.044. The level of risk observed, after the marathon, was marginally greater compared to, before and during the marathon ($p=0.051$) (Table 16). This phenomenon may be attributed to the higher concentrations of *E.coli* detected after the marathon. According to the data presented in Table 17, the average annual risk of *E.coli* infection before the marathon was found to be significantly increased than the average one-time risk of infection after the marathon ($p=0.006$). It was observed that there is a significant positive correlation between *E.coli* concentration and the risk of infection ($p<0.001$). This suggests that higher concentrations of *E.coli* may lead to an increased risk of infection. Furthermore, a noteworthy disparity in annual risk was observed among the three time periods, with a statistically significant result ($p=0.044$). The level of risk observed after the marathon was found to be significantly increased compared to before the marathon. ($p=0.049$).

Probability of *Pseudomonas aeruginosa* infection for the Dusi Marathon participants using untreated surface water for recreational activities

<u>Estimated risk of illness before the marathon</u>

The estimated risk of *Pseudomonas aeruginosa* infection from a single exposure before the marathon was 1.15×10^{-7} ($\pm1.27\times10^{-7}$). The range of this risk was found to be between 3.68×10^{-7} and 3.54×10^{-8}. The analysis suggests that, among 100 000 populations , roughly 0.00000115% are at risk of *Pseudomonas aeruginosa* infection after a single exposure before the marathon. From a practical perspective, this indicates that in a group of 100 000 populations , fewer than 1 case or 0.0000115%, cases would be predicted to get a *Pseudomonas aeruginosa* infection from a single encounter. This finding indicates a relatively low risk of *Pseudomonas aeruginosa* infection before the marathon from a single exposure.

The estimated risk varied from 0.00000368% to 0.000000354%. This indicates that the actual risk of *Pseudomonas aeruginosa* infection may deviate from the estimated average, either being higher or lower. The estimated risk indicated a relatively decreased likelihood of infection from a single exposure. However, it's crucial to consider that continuous exposure to *Pseudomonas aeruginosa* can significantly elevate the probability of infection. This is particularly concerning, as even minimal concentrations of the bacteria in surface water can substantially threaten human health, as discussed in Chapter 3.

The estimated average annual risk of *Pseudomonas aeruginosa* infection before the marathon was calculated to be 4.18×10^{-5} ($\pm4.64\times10^{-5}$) within a sample of 100 individuals. This indicates that, on average, around 0.04% of the population faced the risk of developing a *Pseudomonas aeruginosa* infection annually before the marathon.

<u>Estimated risk of illness during the marathon</u>

The measured risk of *Pseudomonas aeruginosa* infection from a single exposure during the marathon was 4.14×10^{-7} ($\pm3.45\times10^{-7}$) for a population of 100 000 individuals. Approximately 0.0000414% of a population of 100 000 individuals is estimated to be at risk of *Pseudomonas aeruginosa* infection after a single exposure. The range of this risk was found to be between 9.24×10^{-7} and $3.54\times10^{-8.}$

The estimated average annual risk of *Pseudomonas aeruginosa* infection during the marathon was calculated to be 1.51×10^{-4} ($\pm1.26\times10^{-4}$) within a sample of 100 000 individuals. This means that, on average, approximately 0.151% of the 100 000individuals were estimated to be at risk of *Pseudomonas aeruginosa* infection per year.

<u>Estimated risk of illness after the marathon</u>

For a population of 100 000, the estimated risk of *Pseudomonas aeruginosa* infection from a single exposure after the marathon was determined to be 2.31×10^{-6} ($\pm1.67\times10^{-6}$). This implies that the estimated probability of contracting *Pseudomonas aeruginosa* from a single encounter was 0.000231%, out of 100 000 individuals. The estimated risk ranged between 4.74×10^{-6} (0.000474%) and 7.23×10^{-7} (0.000072%).

The estimated average annual risk of *Pseudomonas aeruginosa* infection after the marathon was calculated to be 8.44×10^{-4} ($\pm6.08\times10^{-4}$) within a sample of 100 individuals. This means that, on average, approximately 0.0844% of the 100 000 individuals were estimated to be at risk of *Pseudomonas aeruginosa* infection per year.

<u>Comparison of the estimated risk of illness</u>

The statistical analysis revealed significant variance in the risk of infection resulting from one-time exposure during canoeing (Table 4.3). A statistically significant disparity in the risk of one-time exposure was observed across the three instances ($p=0.011$). The risk after the marathon, exhibited a slight increase compared to before the marathon ($p=0.077$). Based on the data provided in Table 4.5, it was seen that the mean annual risk of infection exhibited a statistically significant increase compared to the mean one-time risk of infection ($p=0.009$). A statistically significant positive connection was observed between the concentration of *Pseudomonas aeruginosa* and the risk of infection ($p<0.001$). These findings indicate that elevated levels of *Pseudomonas aeruginosa* might potentially result in a heightened susceptibility to infection. Moreover, there was significant variation in annual risk among the three periods (p=028.) There was a slight increase in risk after the marathon compared to before the marathon (p= 0.077).

Probability of *Cryptosporidium spp.* infection for the Dusi Marathon participants using untreated surface water for recreational activities

Estimated risk of illness before the marathon

The average infection risk for *Cryptosporidium spp.* from a single exposure before the marathon was 7.48×10^{-1} ($\pm2.87\times10^{-1}$). The infection rate was between 9.59×10^{-1} and 2.01×10^{-1}. The data indicates that, among 100 000 individuals, roughly 75 (75%) people are at risk of *Cryptosporidium spp.* infection from a single exposure before the marathon. The estimated number of individuals who may become infected could vary from a minimum of 20 (20.1%) to a maximum of 96 (95.6%). This range suggests that certain individuals could have a greater susceptibility to infection, or that there may be differing levels of exposure to the pathogen.

The estimated average annual risk of *Cryptosporidium spp.* infection before the marathon was calculated to be 1.00 (±0) within a sample of 100 000individuals. Based on the average annual, risk calculation, the data shows that, out of 100 000 individuals, around 1 person (or 1%), is projected to be at risk of contracting an infection with *Cryptosporidium spp.* annually. This estimate is characterized by the absence of uncertainty (±0), indicating a strong confidence in the calculation.

Estimated risk of illness during the marathon

The average infection risk for *Cryptosporidium spp.* from a single exposure during the marathon was 5.62×10-1($\pm2.51\times10^{-1}$). The infection rate was between 8,69E-(86.9%) and 1.71E-0 (17.1%). This depicts that, out of 100 000people, approximately 56.2% were estimated to be at risk of *Cryptosporidium spp.* infection from a single exposure. The range suggests that certain individuals could have a greater susceptibility to infection, or that there may be differing levels of exposure to the pathogen.

The estimated average annual risk of *Cryptosporidium spp.* infection during the marathon was calculated to be 1.00 (±0)within a sample of 100 000 individuals. Based on the average annual, risk calculation, the data shows that, out of 100 000 individuals, around 1 person (or 1%), is projected to be at risk of contracting an infection with *Cryptosporidium spp.* annually. This estimate is characterized by the absence of uncertainty (±0), indicating a strong confidence in the calculation.

<u>Estimated risk of illness after the marathon</u>

The overall estimated infection risk for *Cryptosporidium spp.* from a single exposure after the marathon was 9.43×10^{-1} ($\pm5.62\times10^{-2}$) for a sample size of 100 000 people. Approximately 94.3% of individuals, were estimated to be at risk of *Cryptosporidium spp.* infection after a single exposure. The risk of infection ranged between 9.99×10^{-1} and 8.44×10^{-1}. The estimated average annual risk of *Cryptosporidium spp.* infection during the marathon was calculated to be 1.00 (±0) within a sample of 100 000 individuals. Based on the average annual, risk calculation, the data shows that, out of 100 000individuals, around 1 person (or 1%), is projected to be at risk of contracting an infection with *Cryptosporidium spp.* annually. This estimate is characterized by the absence of uncertainty (±0), indicating a strong confidence in the calculation.

<u>Comparison of the estimated risk of illness</u>

It was established that the variation in infection risk attributed to by a one-time exposure while canoeing was statistically significant. A notable disparity in the one-time exposure risk was observed among the three periods ($p=0.033$). The risk was marginally higher after the marathon compared to before the marathon) ($p=0.020$) (Table 4.3). The annual risk of infection for *Cryptosporidium spp*. was found to be significantly higher than the average single point risk ($p=0.001$). Higher concentrations of *Cryptosporidium spp.* significantly increased the probability of infection ($p<0.001$). These findings suggest that a greater concentration of *Cryptosporidium spp*. could potentially increase the risk of infection. The annual risk remained constant throughout the three periods.

4.5. Discussion

To estimate the risk of gastrointestinal illness associated with recreational water exposure during secondary contact. The risk assessment examined different concentrations of microorganisms, specifically *E. coli, Pseudomonas aeruginosa*, and *Cryptosporidium spp.,* along with the exposure scenarios discussed in this study. The risk was quantified and then analysed in relation to the WHO guidelines (2003). The observed risk values are accompanied by rationales.

Analysis of the estimated Risks of Gastrointestinal Illness Associated with *E.coli* concentrations in Surface water

The estimated risk of *E. coli* infection before the marathon for a single exposure scenario was 6.63×10^{-7}. This value represents the probable risk of illness from *E. coli* infection per person per day. The WHO (2003) threshold for secondary contact recreation is 1×10^{-5}. The estimated risk was below the WHO threshold, indicating that the risk of *E. coli* infection from recreational water use was deemed safe for secondary contact recreation. The QMRA estimated risk was approximately 15 times lower than the WHO threshold, suggesting a relatively low risk of illness.

This low risk may be attributed to the concentration amount of *E.coli* detected before the marathon. The *E.coli* levels as depicted in chapter 3, were within acceptable limits as set by WHO and DWAF. As depicted, that there was a significant positive correlation between *E.coli* concentration and the risk of infection ($p<0.001$). This suggests that low concentrations of *E.coli* may lead to decreased risk of infection. Rodrigues *et al.* (2020) identified a substantial positive association between *E. coli* concentrations and QMRA risk levels for recreational water users. The study indicated that *E. coli* concentrations over 100 CFU/100mL were associated with QMRA risk levels surpassing 1×10^{-2} (Rodrigues *et al*., 2020). These results support that concentration levels of *E. coli* are directly proportional to estimated risks, suggesting a linear relationship between microorganism concentration and observed risks. The reasons for Low concentrations of *E.coli* detected in uMsundunzi river have been extensively explored in Chapter 3.

During the marathon, concentrations of *E. coli* exhibited a progressive increase, leading to a higher estimated risk compared to levels observed before the marathon. However, the estimated risk of *E. coli* infection during the marathon remained within the thresholds established by the WHO, approximating 25% of the limit value.
The probability of illness due to *E. coli* in recreational waters for secondary contact was 1.06×10^{-5} after the marathon. This value exceeded the WHO threshold of 1×10^{-5}, suggesting a marginally increased risk. The risk exceeds the threshold value by approximately 6%. Individuals participating in secondary contact activities in recreational waters with *E. coli* concentrations at this risk value might be exposed to a marginally elevated risk of illness.

The level of risk observed after the marathon was found to be significantly increased compared to before the marathon ($p=0.049$). Given that the period after the marathon recorded the greatest *E. Coli* concentrations, the result is consistent with those reported in Chapter 3.

In accordance with literature, *E. coli* contamination is fuelled by both human and nonhuman waste (Gitter *et al.* 2020:13). This contamination could potentially occur due to sewage discharge into the uMsunduzi river, as well as the presence of people who reside along the river. It is worth noting that the uMsunduzi river is surrounded by the farming community and informal settlements. Consistent with the findings of a risk assessment study (Mena *et al* 2019).

Analysis of the estimated Risks of Gastrointestinal Illness Associated with *Pseudomonas aeruginosa* concentrations in Surface water

The WHO (2003) established a risk limit of 1.3×10^{-4} (1 in 7.700) for *Pseudomonas aeruginosa* in recreational secondary contact with surface water. The estimated infection risk for *Pseudomonas aeruginosa* before the marathon was 1.15×10^{-7}, approximately 50% of the target risk value, indicating a significantly reduced risk of illness. Similarly, the estimated infection risk for *Pseudomonas aeruginosa* during the marathon was $4.14 \times 10^{-}$

^{7}, approximately 50% of the target risk value, indicating a reduced risk. Due to its potentially low numbers in surface water, ingesting *Pseudomonas aeruginosa* from surface water does not constitute a significant colonization risk. While *Pseudomonas aeruginosa* is not often recognized as a faecal pathogen, it can provide significant health hazards. Therefore, it is valuable to analyse this bacterium in relation to the risk standards established by the USEPA and WHO (Januário *et al.* 2019:13).

The estimated infection risk for *Pseudomonas aeruginosa* after the marathon was 2.31 x 10^{-6}, approximately 2 folds (or 78%) of target risk value, indicating an increased risk. There was a progressive increase in the observed risk for *Pseudomonas aeruginosa* throughout the study period. This can be attributed to the continual increase in *Pseudomonas aeruginosa* concentrations observed in the water quality analysis reported in Chapter 3.

Analysis of the Estimated Risks of Gastrointestinal Illness Associated with *Cryptosporidium spp.* concentrations in Surface water

The estimated risk of *Cryptosporidium spp.* infection before, during, and after the marathon was compared to the USEPA's recommended target risk of 1×10^{-4} for recreational water activities. The estimated risk before the marathon 7.48×10^{-1}, during the marathon 5.62×10^{-1}, and after the marathon 9.43×10^{-1} significantly exceeded the USEPA (2012) recommended target risk, by factors of 85%, 72%, and 104%, respectively. The findings suggested an increased risk of illness from *Cryptosporidium spp.* associated with canoeing in surface water.

The elevated risk estimates observed may be correlated to the high concentrations of *Cryptosporidium spp.* detected throughout the entire study period, as detailed in Chapter 3. The study's results revealed that the nonhuman source, specifically cattle, posed the highest risk of illness, as quantified by the reference organism *Cryptosporidium spp.* This notion is also supported by Devan *et al.* (2020), who states that land use activities that contribute faeces, such as cattle and sewage discharges, result in significantly higher concentrations of *Cryptosporidium* oocysts in water bodies. These findings were consistent with Gitter *et al.* (2020), who found that *Cryptosporidium spp.* exceeded the risk for GI illness linked to contaminated recreational water bodies located in Walnut Creek River (Texas), the results of this study was one magnitude higher (10-1) when compared to the USEPA (2012) guidelines. The contamination of surface water by cattle and domestic animals increased the likelihood of contracting illness as their faeces contain a greater quantity of *Cryptosporidium spp.* oocysts.

The results of the QMRA presented here indicate that the probability of exposure to *Cryptosporidium spp.* in the uMsunduzRiverexceeds the acceptable threshold. Because this poses severe health risks not only to canoeists but also to the neighbouring community that employs uMsunduzi river for domestic and agricultural purposes, this demonstrates that the stakeholders should implement action towards mitigating this critical hazard. Safeguarding safeguarding oSouth African rivers is a fundamental aspect of both the National Water Resource Strategy 3 (2023) and the National Water Act, 1998 (Act No 36 of 1998). These regulations aim to guarantee the preservation, use, and development of the country's water resources (DWS 2023).

Table 4.3: Mean probability (±95% Cl) of infection for Dusi marathon participants canoeing in uMsunduzi River (n=3)

TIME PERIOD	***E. COLI***			***PSEUDOMONAS AERUGINOSA***			***CRYPTOSPRIDIUM SPP.***		
	One-time exposure	**Annual risk**	**Established Thresholds**	**One-time exposure**	**Annual risk**	**Established Thresholds**	**One-time exposure**	**Annual risk**	**Established Thresholds**
Before the race (January 2022)	6.63×10^{-7} (±6.35×10^{-7})	$1{,}27\times10^{-4}$ (±2.32×10^{-4})	1×10^{-5} (WHO 2003)	1.15×10^{-7} (±1.27×10^{-7})	4.18×10^{-5} (±4.64×10^{-5})	1.3×10^{-4} (WHO 2003)	$7{,}48\times10^{-1}$ (±2.87×10^{-1})	1.000 (±0)	1×10^{-4} USEPA (2012)
During the race (February 2022)	2.52×10^{-6} (±2.90×10^{-6})	$4{,}73\times10^{-4}$ 1.06×10^{-3}	1×10^{-5} (WHO 2003)	4.14×10^{-7} (±3.45×10^{-7})	.000 (±1.26×10^{-4})	1.3×10^{-4} (WHO 2003)	$5{,}62\times10^{-1}$ (±2.51×10^{-1})	1.000 (±0)	1×10^{-4} USEPA (2012)
After the race (March 2022)	1.06×10^{-5} (±7.53×10^{-6})	3.02×10^{-3} 2.73×10^{-3}	1×10^{-5} (WHO 2003)	2.31×10^{-6} (±1.67×10^{-6})	.000 (±6.08×10^{-4})	1.3×10^{-4} (WHO 2003)	9.43×10^{-1} (±5.62×10^{-2})	1.000 (±0)	1×10^{-4} USEPA (2012)

Total	**4.6×10^{6} ($\pm6.25\times10^{6}$).**	**$1{,}67\times10^{3}$ ($2{,}27\times10^{-3}$)**	1×10^{-5} (WHO 2003)	**9.00×10-7 (±1.36×106)**	**$3{,}46\times10^{-4}$ ($\pm4{,}98\times10^{-4}$)**	1.3×10^{-4} (WHO 2003)	**7.51×10^{-1} (±2,63×101)**	**1.00 (±0)**	1×10^{-4} USEPA (2012)

4.6 SUMMARY OF THE CHAPTER

pathogens in contaminated waters can have significant implications for public health. This study assessed the potential health risks associated with the utilization of contaminated river water by communities, particularly the participants of the Dusi Canoe Marathon, who engage in recreational activities in the uMsunduzi river using *E.coli, Pseudomonas aeruginosa,* and *Cryptosporidium spp*. as model organisms. The average microbial count at all the sampling sites exceeded the guidelines set by South Africa and the WHO for recreational water use.

The results of the QMRA indicated a significant risk of infection for the populations who utilize uMsunduzi river. This heightened risk was mostly associated with *Cryptosporidium spp.* which was one order magnitude higher (10^{-1}) than the threshold set by the WHO and the USEPA. This underscores the criticality for continuous surveillance and control of microbial contamination in river systems This study makes a valuable contribution to the existing literature concerning the microbial hazards linked to the uMsunduzi River. Moreover, it underscores the criticality of precise risk assessments to regulate water quality effectively. This study has the potential to make a significant contribution to the South African National Water Resource Strategy 3. The strategy aims to effectively plan, develop, manage, protect, and control the use of water resources in South Africa for the future. Chapter 4 presented the probabilistic microbial risk assessment, detailing the methodology, results, and discussion. Chapter five below, presents the epidemiological assessment, the methodology employed, and the findings, accompanied by the discussion.

5. CHAPTER FIVE

INCIDENCE OF DIARRHEA AND ASSOCIATED RISK FACTORS AMONG THE DUSI CANOE MARATHON PARTICIPANTS: A PRIMARY DATA ANALYSIS OF PARTICIPANT DEMOGRAPHICS AND EXPOSURE ASSESSMENT SURVEY 2022

5.1 INTRODUCTION

Diarrhea, as defined by (WHO), is the occurrence of three or more loose or liquid stools within a 14-day time frame (Hussein, 2017:9). Diarrhea often indicates an infection in the intestinal tract, resulting from various bacteria, viruses, and parasites (WHO, 2013). *E. coli* is the leading cause of diarrhea. Microorganisms can be transmitted through contaminated water and person-to-person contact, particularly in environments with inadequate water quality (Ekici *et al.*, 2019:89). Diarrhea has been associated with various indirect risk factors, which can be categorized as environmental and behavioural risk factors in recreational settings (Reses *et al,* 2018:1071). The factors to consider are the water source for recreational purposes and the specific activities conducted in that water source. These risk factors are interconnected and can vary for various reasons (Yoder *et al.,* 2008:5).

In recent years, diarrhea among participants in the Dusi Canoe Marathon has drawn attention, prompting an in-depth review of the associated risk factors. This primary data analysis delves into participant demographics and exposure assessments to evaluate their level of exposure to better understand the complexities of this health issue during the 2022 marathon. Additionally, this study examined the potential correlation between the participants' exposure to uMsunduzi River and diarrhea among the Dusi canoe marathon attendees.

Figure 5.1 illustrates the conceptual framework of this study, which identifies the behavioural and environmental risk factors associated with diarrheal episodes among the participants. The diagram illustrates the linkage of these factors and demonstrates that a single factor alone may not be sufficient to cause a diarrheal episode, but rather a combination of factors is necessary.

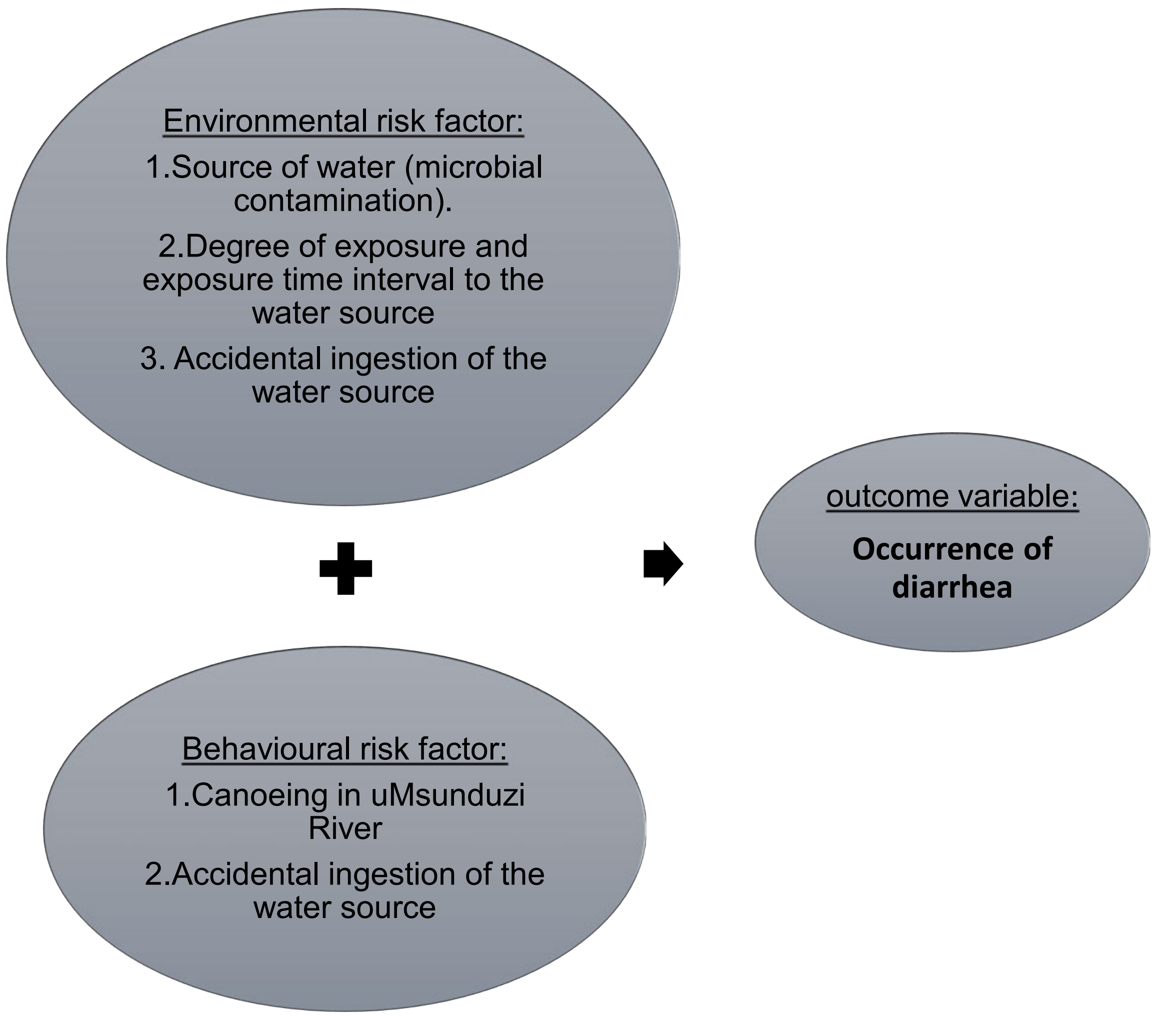

Figure 5.1: Conceptual framework: interacting interlinked risk factors leading to the occurrence of diarrhea among the Dusi Canoe Marathon participants

5.2 METHODOLOGY

5.2.1 Study design

The study employed a cross-sectional, descriptive survey to assess the population of participants in the Dusi Marathon. The survey examined the correlation between river exposure and infection occurrence using questionnaires (Appendices 6 & 7) and recorded instances of diarrhea, following established epidemiological methods.

5.2.2 Study setting

The survey was conducted using an online platform. The survey was conducted following the conclusion of the Dusi canoe marathon.

5.2.3 Study population

The investigation examined two cohorts of subjects, namely the exposed and non-exposed cohorts. The cohort of individuals subjected to the study were the participants of the Dusi marathon canoe race. The nonexposed cohort consisted of individuals who were not directly participating in the Dusi marathon, but rather were present as observers. This group encompassed a range of individuals, including family members, friends, event staff, and members of the general public who attended the study site (uMsunduzi River) during the marathon and had no contact with the water body. The exposed group, consisted of individuals who had complete immersion of their entire body, including their head, in the uMsunduzi River. Both groups were recruited to take part in the study during the marathon and interviews were carried after the marathon. The virtual interviews were carried out with a time delay of 7-9 days. This was done to ensure that any reported illnesses or symptoms were a result of their subsequent exposure to the aquatic environment.

5.2.3 Sampling

Assuming a population of 3200 individuals, consisting of 1600 participants and 1600 spectators, based on the estimated number of participants in the 2021 Dusi Canoe marathon and the assumption that each participant is accompanied by one spectator. The sample size was determined based on a margin of error of 0.05 and a significance level of 0.05. The sample size was determined using the Cochrane's formula and subsequently adjusted for the small population size. The stratified sampling method was employed, this resulted in a minimum sample size of 344 participants, with 172 participants in both the exposed and unexposed groups being required. Both the exposed and unexposed groups were required to satisfy the inclusion and exclusion criteria outlined in (Appendix 4)to be eligible for participation in the study.

5.3 DATA COLLECTION

The study participants were provided with a letter of information that comprehensively explicated the study particulars (refer to Appendix 4). Subsequently, they were asked to provide their consent by signing a consent form (Appendix 5). This procedure was carried out during the Dusi canoe marathon for both cohorts. The researcher and the research assistants personally approached the participants. Individuals who satisfy the predetermined criteria for participation were requested to peruse and complete the letter of information, followed by placing their signature on the informed consent document. Subsequent online interviews were carried out by the researcher and research assistant, 9 days after the recruitment phase. The follow-up virtual interviews were conducted using a comprehensive demographic questionnaire (Appendix 6), which was divided into three sections: population demographics (A), exposure determination (B), and infection history resulting from exposure (C). Additionally, a data collection sheet was utilized to gather detailed information on the characteristics, duration of self-reported diarrheal symptoms, and healthcare-seeking behaviour of the respondents were described and reported for only the most recent episode.

Each participant required approximately 30 minutes to complete the questionnaire. The preservation of confidentiality and anonymity was upheld through the assignment of numerical identifiers to the dataset and the avoidance of any association between identifiable information and said dataset.

5.4 PRE-TESTING OF THE DATA COLLECTION TOOLS

Undertaking a pilot study for the questionnaire constituted a crucial phase in the research process aimed at ascertaining the efficacy and validity of the survey tool. A total of 30 individuals were selected to partake in the preliminary investigation. The study's focus group did not include the participants. A diverse group of individuals with varying demographics, educational backgrounds, and geographical locations were recruited to participate in the pilot study. The investigator conducted a thorough evaluation of each stage of the survey, from beginning to end, using the pilot sample. The process involved distributing the survey, collecting responses, and analyzing the data. After the pilot sample has completed the questionnaire.

The researcher gathered data from participants regarding their experiences with the survey. Data was collected through structured interviews and written feedback. The researcher carefully observed the participants' understanding of the questions, the clarity of the instructions provided, and any difficulties they encountered.

As a means of identifying plausible issues or inconsistencies, the investigator compiled the preliminary group's feedback and assessed the data. Based on participants' feedback and data analysis, it can be concluded that they demonstrated a strong understanding and clarity regarding the survey instrument.

5.5 METHODS AND VARIABLES

Outcome variables: The primary outcome variable was the incidence of diarrhea as reported by the participants. Participants who reported experiencing diarrhea either during the marathon, within 1-7 days after the marathon, or within the last 24 hours prior to data collection.

Predictor variable: The study examined three independent variables that were identified to be associated with the occurrence of diarrheal episodes. These variables source of water used for recreational activities, degree of exposure to the water source, and accidental ingestion of the water source.

Source of water used for recreational activities: The water source utilized for canoeing was uMsunduzi River. In chapter 3, the water source was deemed to be of poor quality based on the guidelines set by (WHO 2021) and (DWAF 1996).

Degree of exposure to the water source: The level of exposure to the water source was assessed by measuring the extent of full body immersion, which includes submerging both the head and body in water, as well as the duration of exposure. This duration was measured in hours per day, reflecting the number of hours participants were exposed to the water, and in number of days, indicating the length of time participants were exposed to the water.

Accidental ingestion of the water source: The degree of accidental ingestion was assessed by quantifying the ingestion rates, which represent the volume of water (measured in millilitres) consumed per hour.

5.4 Statistical analysis

Data cleaning and variable management: The Recorded defined dataset of participants was imported into Excel. The dataset underwent a cleaning process where irrelevant variables were eliminated. Subsequently, 23 relevant variables were chosen and incorporated into the study. The relevant variables were recoded using Excel.

Statistical methods: The data analysis was conducted using IBM® SPSS® software, version 29.0.10 of the statistical package. Descriptive statistics were employed to summarize the study variables, while logistic regression was utilized to examine the impact of 21 predictor variables on the outcome variable.

Descriptive statistics were employed to summarize and present the characteristics of the participants. These findings were then presented in tabular form. Pearson's Chi-Squared test and two-way contingency tables were employed to evaluate the statistical significance and frequency distribution of the outcome variable about the predictor variables, respectively.

Logistic regression analysis was conducted to assess the relationship between the outcome variable and the predictor variables. The bivariate analysis was conducted to evaluate the relationship between each predictor variable and the outcome variable. The analysis yielded the Crude Odds Ratios (CORs) at a significance level of 95% confidence interval.

Missing values: The study initially estimated the number of 344 participants for inclusion in the study, with an equal distribution between the exposed and unexposed groups. Out of the initial 344 respondents, the study ultimately contained 325 participants as some individuals dropped out for personal reasons. The study included 325 participants, with 152 exposed and 173 unexposed individuals. Out of the 152 individuals who were exposed, 74 were deemed ineligible for the study because they failed to provide sufficient information in certain categories, such as age, gender, duration of exposure, water consumption, and a detailed description of diarrhea symptoms. leaving a total number of 78 eligible participants in the exposed cohort. Therefore, the total participant size was 251.

Bias: Accurate sample selection protocols were employed to mitigate the influence of selection bias. The research assistants effectively implemented the listings and selection process, following the selection protocol and utilizing the inclusion and exclusion criteria.

Ethical considerations: Approval was received from the Institutional Research Ethics Committee (IREC) (Reference No. IREC 021/21). Prior to the interviews, participants were presented with an informed consent statement containing information regarding the purpose, duration, and procedures of the interviews, as well as the potential benefits and risks involved. Participants were required to provide written consent to participate in the study. Those who did not provide written consent were not included in the study.

5.5 RESULTS

5.5.1 Participant flow

The participants' flow is summarized in the provided flow chart below (Figure 5.2). The study included 251 participants. This total includes both the exposed and unexposed groups. 74 respondents were excluded from the exposed group. The study included a final sample of 251 respondents, with 173 from the unexposed group and 78 from the exposed group.

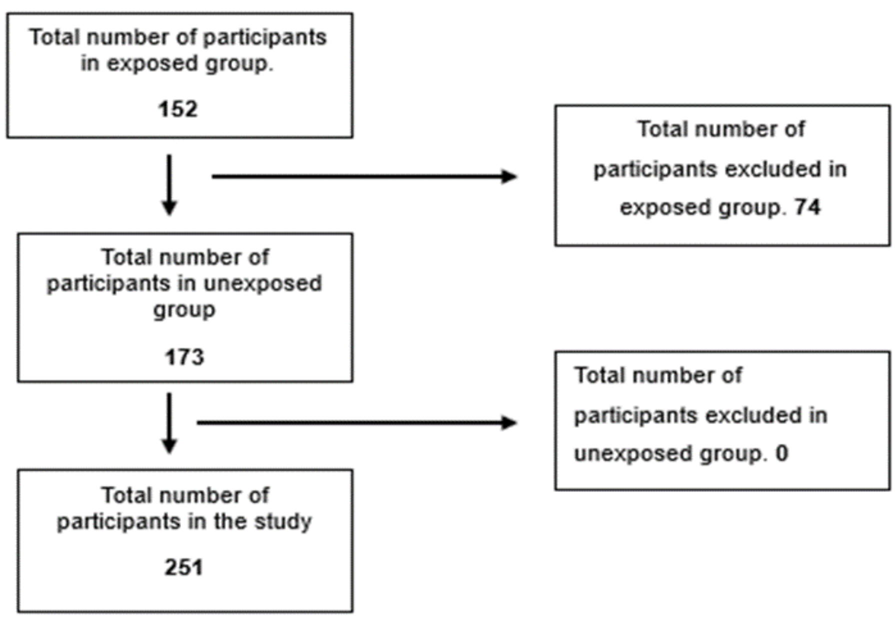

Figure 5.2: Flow of the participants included in the study

5.5.2 Characteristics of the exposed cohort

The subsequent analysis predominantly concentrated on the exposed cohort, based on that, the data gathered from the unexposed cohort was utilized primarily for risk estimate specifically, the odds ratio.

The study included male participants (100%) who were predominantly of White ethnicity (100%). Most participants were between the ages of 25 and 39 (91%), as shown in the frequency Table 5.1 below.

Table 5.1: Frequency and Percentage Distribution of participants demographics

Characteristics	**N=78 (%)**
Ethnicity	
Black	0(0%)
White	78(100%)
Indian	0(0%)
Coloured	0(0%)
Age	
18-24	0%
25-39	71(91.0%)
40-64	7(9.0%)
60 Above	0%
Gender	
Male	78(100%)
Female	0(0%)

5.5.3 Environmental and behavioural conditions

As stated in Chapter 3, the study results revealed that uMsunduzi River displayed noteworthy levels of pollution from indicator and pathogenic organisms. The microbiological quality of the uMsunduzi River was of poor quality, as it did not meet the recreational standards established by the WHO and DWAF. The microbiological analyses suggested that the uMsunduzi River may be contaminated with faecal matter, as evidenced by the consistent presence of pathogens including *E.coli* and *Cryptosporidium spp.* As depicted in the table below, one may appreciate the specific microorganisms detected and their average concentrations.

The results show that 100% of the participants were exposed to the uMsunduzi River. A significant portion of respondents (46.2%) reported being exposed to the river for three days. The minimum number of days accounted for only 1.3% of the total, equivalent to a single day. The binomial test revealed that a significant proportion of individuals, 100%, ingested water from the river. Additionally, a significant proportion of individuals, 99%, reported illness after being exposed. This may be seen in Table 5.2 below.

Table 5.2: Frequency Distribution of Environmental and Behavioural Conditions of the participants

<table>
<tr><td colspan="4">Characteristics</td></tr>
<tr><td colspan="4">Average microbial concentration detected in the river. Mean(±SD)</td></tr>
<tr><td rowspan="3">Environmental conditions</td><td rowspan="3">Source of water: Contaminated</td><td>E.coli</td><td>82.16(±111.64) MPN/100ML</td></tr>
<tr><td>P. aeruginosa</td><td>26.75(±38.50) MPN/100ML</td></tr>
<tr><td>Cryptosporidium spp.</td><td>1388.89(±1059.10) Copies/20µL</td></tr>
<tr><td colspan="4">N=78(%)</td></tr>
<tr><td rowspan="9">Behavioural conditions</td><td rowspan="5">Duration of exposure (In days)</td><td>1</td><td>1(1.30)</td></tr>
<tr><td>2</td><td>22(28.20)</td></tr>
<tr><td>3</td><td>36(46.20)</td></tr>
<tr><td>4</td><td>16(20.50)</td></tr>
<tr><td>5</td><td>3(3.80)</td></tr>
<tr><td rowspan="2">Ingestion of water</td><td>Yes</td><td>78(100)</td></tr>
<tr><td>NO</td><td>0(0)</td></tr>
<tr><td rowspan="2">Incident of illness after exposure</td><td>Yes</td><td>77(99)</td></tr>
<tr><td>No</td><td>1(10)</td></tr>
</table>

5.5.4 Incidence of diarrhea

In this study, diarrhea was defined as of three or more loose stools within 24 hours, or a frequency greater than what is considered normal for that particular adult. A total of 70 respondents (89.7%) from the exposed cohort indicated that they had encountered at least one episode of diarrhea. The median duration of diarrhea, as reported, was 3-4 bowel movements per day (n=50, 76.9%) and lasted for 1 day (n=26, 33%). Abdominal pain was the second most commonly reported symptom, occurring in (n=3, 3.8%) of cases. This was often accompanied by nausea, joint pain, and stomach pain, which accounted for (n=5,6.4%) of cases. In addition (47.5%) of the participants sought medical attention. A small proportion (n=3, 1.73%) of the unexposed cohort experienced symptoms of diarrhea.

5.5.5 Risk factors associated with diarrhea in the exposed cohort

In the bivariate analysis, a longer exposure time to the uMsunduzi River was associated with an increased likelihood of participants experiencing diarrhea. However, this association was not of statistical significance [COR: 1.3, 95% CI (0.86 - 1.71)].

5.5.6 Risk estimate

The risk estimate was based on a sample size of 325 participants, which included all individuals who participated in the study. Given that each person correctly completed the questionnaire's exposure and symptom categories that is, they all indicated whether or not they had been exposed to the river and whether or not they had developed diarrhea as a result of that exposure.

Logistic regression analysis was conducted to assess the relationship between the outcome variable and the predictor variables. The bivariate analysis was conducted to evaluate the relationship between each predictor variable and the outcome variable. The analysis yielded the Crude Odds Ratios (CORs) at a significance level of 95% confidence interval.

Out of the 152 participants in the study, 108 (71%) reported experiencing diarrhea after being exposed to the river and accidentally ingesting its water. Additionally, 44 participants (29%) reported being exposed to the river and accidentally ingesting its water but did not experience diarrhea. Out of the total 173 individuals who were not exposed to the river, only 3 (1.73%) reported experiencing diarrhea symptoms. Conversely, a larger proportion of the participants (n=170, 98.3%) who were not exposed to the river did not experience diarrhea as depicted in Table 5.3 below.

The bivariate analysis revealed a significant association between exposure to the river and the incidence of diarrhea. The risk of diarrhea was 97% higher in those who were exposed to the river and accidentally ingested the water compared to those who did not [COR:139, 95% CI (42.1 – 459.1)]. According to the COR, incidental ingestion of river water increases the risk of illness compared to not being exposed to the river. Therefore, the likelihood of a person being ill due to exposure and accidental ingestion of the river water is 139 times higher compared to an individual who is not exposed to the river. The correlation was statistically significant, with a p-value of (<0.001).

Table 5.3: Crude Odds Ratio of participant diarrhea about environmental and behavioural risk factors of the Dusi Canoe Marathon

Variable		**Diarrhea**		**Total**
		Yes	**No**	
Exposed to the river and ingested the water	**Yes**	108	44	152
	No	3	170	173
Total		111	214	325
Risk estimate		**[COR:139, 95% CI (42.1 – 459.1)] p<0.001**		

5.6 DISCUSSION

This study sought to analyse the relationship between exposure to the uMsunduzi River and the incidence of diarrhea among participants of the Dusi Canoe Marathon, after their exposure to the river. Utilizing data acquired from a cross-sectional survey.

The study revealed that the prevalence of diarrhea was 89.7% among the exposed group, whereas it was only 1.73% among the non-exposed group. Furthermore, the risk assessment showed that those exposed to the river and accidentally ingested water had a 97% greater chance of developing diarrhea than those who did not [COR:139, 95% CI

(42.1 – 459.1)]. The variation between the two cohorts may be attributed to the disparity in the environmental and behavioural settings to which the two groups were subjected. The exposed group had direct contact with water that was contaminated with microorganisms. They also accidentally ingested the contaminated water and had prolonged exposure to the water's conditions. In contrast, the unexposed cohort had no interaction with the river.

However, epidemiological studies have found a higher risk of diarrheal diseases linked to the use of shared sanitation facilities. A systematic review incorporating twelve studies on diarrhea conducted a meta-analysis, which revealed a 44% higher risk of contracting diarrhea among individuals who shared sanitation facilities compared to those who utilized individual household latrines (Just *et al.* 2018: 195). Consequently, individuals who were not previously exposed to the river may have developed diarrhea as a result of sharing toilets with those who were exposed to contaminated water and subsequently contracted diarrhea. The unexposed cohorts observed during the Dusi Canoe Marathon were presumed to be family members or friends of the participants, as mentioned earlier. The demographics of the individuals in both the exposed and unexposed groups did not show any association with the incidence of diarrhea. Moreover, the source of water used for recreational activities may potentially contribute to the probability of the exposed group experiencing diarrhea, in contrast to the unexposed group.

The Dusi Canoe Marathon is a multi-day competitive event involving paddling that spans a distance of roughly 120 kilometres on the uMsunduzi and Umngeni rivers located in KZN. The race commences in Pietermaritzburg and concludes in Durban Mars *et al.* (2000: 17). Foreman (1997), mentions that the Dusi Canoe Marathon is a prominent canoeing event in Africa, drawing a considerable number of paddlers annually, with participants ranging from 900 to 2,000. The race boasts a lengthy lineage, tracing its origins back to 1951, and has achieved global acclaim as a preeminent river marathon.

The research focused on the participants of the Dusi canoe marathon. The findings of the survey displayed that all participants were male (100%) and of White ethnicity (100%). The majority of participants were between the ages of 25 and 39 (91%). The event occurs

in South Africa, a nation characterized by its diverse population comprising multiple ethnic backgrounds. It is noteworthy that canoeing and other sporting events are inclusive of individuals from diverse ethnic and gender backgrounds, with no restrictions on participation based on ethnicity or gender. The study offered voluntary participation, and participants had the option to choose whether or not to take part. However, it was observed that a greater number of males participated in the study compared to females.
Farman (2002) conducted a study on the Dusi Canoe Marathon, which evaluated the pre-race concerns of Dusi Canoe Marathon paddlers. The study revealed that a greater number of male participants aged between 18-35 years participated in the research. According to Farman (2002: 9), the quantity of female participants was scarce. The results of this investigation are consistent with those of the present study.

This study hypothesized that the accidental ingestion of contaminated surface water from the uMsunduzi River by the participants of the Dusi Canoe Marathon was a potential occurrence that may result in a higher susceptibility to infection and consequent diarrhea. The findings of the study indicate that a significant proportion of the respondents (46.2%) reported being exposed to uMsunduzi river for three days. The minimum number of days was ascribed to a single day, constituting 1.3% of the total. Additionally, a significant proportion of 100% of individuals ingested water from the river, while a statistically significant proportion of 99% of individuals experienced illness following exposure.

The symptoms most commonly reported were diarrhea, accounting for 89.7% of cases. Moreover, a significant percentage of the respondents (76.9%) indicated that they had a frequency of 3-4 bowel movements per day. The majority of participants, specifically 93.6%, exhibited symptoms of loose watery stool. Furthermore, a substantial proportion of the participants indicated experiencing incontinence and urgency symptoms (84.6%).

As per the WHO definition in 2023, Diarrhea is characterized by the occurrence of three or more loose or liquid stools per day or a frequency of bowel movements that exceeds the individual's normal pattern. Typically, it presents as a manifestation of an infection within the gastrointestinal tract, which may stem from a diverse range of bacterial, viral, and parasitic microorganisms. The transmission of infection occurs via ingestion of contaminated food or water, or through interpersonal contact facilitated by inadequate hygiene practices.

Mars (2000: 18) conducted a study to evaluate the impact of pollution on the health risks associated with the Dusi Canoe Marathon, which was held during flood conditions. The findings revealed that a significant proportion (43.7%) of the participants experienced health issues, with diarrhea and infected wounds being the most prevalent ailments. Approximately 35.6% of cases of diarrhea required medical consultation. Throughout the 50-year history of the Dusi Canoe Marathon, cases of gastro-intestinal infection have been found to surpass other potential hazards of the race, including snakebite and, in the earlier years, the presence of crocodiles. Diarrhea has become part of the folklore of the race Mars (2000: 17).

As per the findings of the Dusi survey statistics 2019, a significant proportion of the participants, that is, 60%, developed "Dusi Guts", which to a condition of diarrhea resulting from the involuntary and excessive consumption of river water during the race. The condition is attributed to the elevated levels of *E.coli* bacteria present in the Msundusi River. It should be noted that in the year 2020, a significant proportion of paddlers experienced varying degrees of Dusi Guts, with 27.7% reporting severe symptoms and 32.49% reporting mild symptoms (Dusi Canoe Marathon: 2020).

The results of the study are consistent with the previously published findings of the aforementioned studies. The findings of this study indicate that the incidence of diarrhea among the participants of the Dusi Canoe Marathon correlates with the elevated levels of *E. coli*, *Cryptosporidium spp*., and *Pseudomonas aeruginosa* detected in the uMsunduzi river before, during, and after the Dusi Canoe Marathon. The results signify an elevated probability or higher likelihood for the onset of diarrhea upon exposure to the uMsunduzi River. As aforementioned, the consumption of river water with substandard quality leads to the emergence of diseases among individuals who come into contact with it.

The study's sample was representative of the larger Dusi Canoe Marathon participants, allowing for generalization of our findings to the larger population engaged in recreational activities with minimal contact in surface water, despite the small sample size. These findings have implications for public health due to the presence of viable *E. coli*, *Pseudomonas aeruginosa*, and *Cryptosporidium spp.* in the river water. This water is frequently used by our respondents and others in the region for recreational activities, domestic purposes, and irrigation.

The incidence of diarrhea among adults engaging in recreational activities in river water is significant. Field studies demonstrate that the prevalence of diarrhea among those who come into contact with recreational water ranges from 3% to 8%, highlighting the widespread occurrence of sickness resulting from these activities (Sanborn *et al.* 2013:491). Recreational Water Illnesses (RWIs) are a significant concern due to the potential risk posed by contaminated water. Both men and women participate in recreational water activities at nearly equal rates, indicating a mutual susceptibility to waterborne pathogens (McOliver *et al.* 2009:1116).

The WHO classifies Recreational Water Illnesses (RWIs). These illnesses mainly consist of diarrhea resulting from different pathogens such as *Cryptosporidium spp., Giardia, Shigella*, and *norovirus* (Yoder *et. al* 2008: 9). (RWIs) are transmitted through ingestion, inhalation, or contact with contaminated water in various aquatic environments such as swimming pools, spas, lakes, rivers, or oceans. Diarrhea is the most frequently reported (RWI). Diarrheal illnesses can result from the presence of pathogens such as *Cryptosporidium spp.* and *E. coli* (McOliver *et al.* 2009:1116).

From 1978 to 2008, there has been a consistent rise in the number of reported outbreaks of (RWIs). The number of outbreaks increased from a minimum of 5 in 1986 to a maximum of 84 in 2007. The number of RWI outbreaks experienced significant increases post-1997. The overall increases can be attributed to a rise in reported outbreaks of acute diarrheal illness (gastroenteritis) associated with recreational water venues. Additionally, the rise in reported outbreaks of acute diarrheal illness (gastroenteritis) may be attributed to the increasing reporting of outbreaks caused by *Cryptosporidium spp*. (Centres for Disease Control and Prevention, 2010).

This aligns with the findings of the study, which observed a significant presence of *Cryptosporidium spp.* together with of *E. coli*. Furthermore, a significant proportion of the exposed group had diarrhea after their exposure to the river. Assessing the risk of diarrhea among recreational users is crucial in reducing the elevated incidence of diarrhea within this population group. The study found a significant incidence of diarrhea (89.7%) among participants of the Dusi Canoe Marathon over a three-day period. These findings will contribute to the development of evidence-based strategies for reducing the incidence of diarrhea among individuals engaging in recreational activities in water sources.

Recreational users and a subset of the local community frequently utilize uMsunduzi River. Unfortunately, the presence of pathogens in this river poses a risk to both individuals with healthy immune systems and those with compromised immune systems. *Cryptosporidium spp*. is not regularly monitored in surface waters (McOliver *et al.* 2009: 1122). Consequently, there is a lack of public awareness regarding the potential hazards associated with exposure to contaminated rivers and infection from pathogens present in river water. Regulatory agencies, environmental associations, and other stakeholders in recreational water programs need to familiarize themselves with the impact of infectious pathogens in recreational waters. They should also establish regular surveillance, monitoring, and notification systems to safeguard the safety of metropolitan waters.

The conceptual framework hypothesized that there was a correlation between various variables and the incidence of diarrhea among the participants of the Dusi Canoe Marathon. The risk variables considered included both environmental and behavioural aspects. All of these elements have been thoroughly examined and explained in the preceding sections. All components within the conceptual framework were linked to the incidence of diarrhea in the Dusi Canoe Marathon participants. There may be more risk variables that have not been highlighted, which might possibly be associated with diarrhea in this group.

5.7 SUMMARY OF THE CHAPTER

This study revealed a significant occurrence of diarrhea among participants of the Dusi Canoe Marathon. Various important environmental and behavioural risk factors contributed to the incidence of diarrhea among the participants. The identified independent variables associated with increased risk were exposure to a water source (specifically uMsunduzi River) with elevated levels of indicator organisms and other pathogenic organisms, as well as accidental ingestion of this water source during recreational activities such as canoeing. Frequent surveillance is essential to guarantee the safety of water for recreational uses such as swimming, boating, and fishing. Monitoring programs can detect potential health hazards and implement appropriate measures to mitigate them by conducting tests for pathogens, biological contaminants, and chemical pollutants. It is of utmost importance to note that recreational bodies of water have the potential to serve as a reservoir for waterborne illnesses, thereby exposing individuals to considerable health hazards.

Systematic observation and analysis of water quality over time can yield significant insights that can guide the formulation of effective management plans. Through the examination of patterns and alterations in water quality across a remove redundancy period, governing bodies can pinpoint the origins of pollution, evaluate the efficacy of measures taken to prevent contamination, and arrive at well-informed determinations aimed at preserving water resources. The utilization of a data-driven methodology is of paramount significance in guaranteeing the viability of water consumption practices and executing suitable strategies to counteract the hazards of pollution.

In conclusion, this primary data analysis provided a comprehensive overview of the incidence of diarrhea among Dusi Canoe Marathon participants in 2022. By scrutinizing participant demographics and exposure assessments, key factors were identified in contributing to this health concern. As the Dusi Canoe Marathon continues to attract a diverse array of participants, understanding and addressing health challenges is paramount. This analysis serves as a valuable resource for future event planning and underscores the commitment to participant welfare. Chapter 6 will address the proposed risk reduction strategies aimed at decreasing the incidence of diarrhea among participants of the Duzi Canoe Marathon.

6. CHAPTER SIX

A RISK REDUCTION STRATEGY FOR RECREATIONAL USE OF THE UMSUNDUZI RIVER

6.1 INTRODUCTION

Exposure to poor water quality can lead to health consequences in the short term. Therefore, it is crucial to establish standards, monitoring systems, and implementation strategies that allow for timely preventive and remedial measures (WHO 2011: 14). The focus of risk reduction measures is on identifying circumstances and procedures that promote a consistently safe recreational environment. This approach prioritizes the monitoring of conditions and practices, utilizing threshold values as crucial indicators. These indicators are evaluated through monitoring and assessment processes.

This chapter aims to provide a clear definition of risk reduction measures and propose strategies to mitigate illness among participants in the Dusi Canoe Marathon. These measures have the potential to decrease illness among individuals who engage in recreational activities in surface waters. The subsequent flow diagram (Figure 10) delineates an approach for mitigating risks.

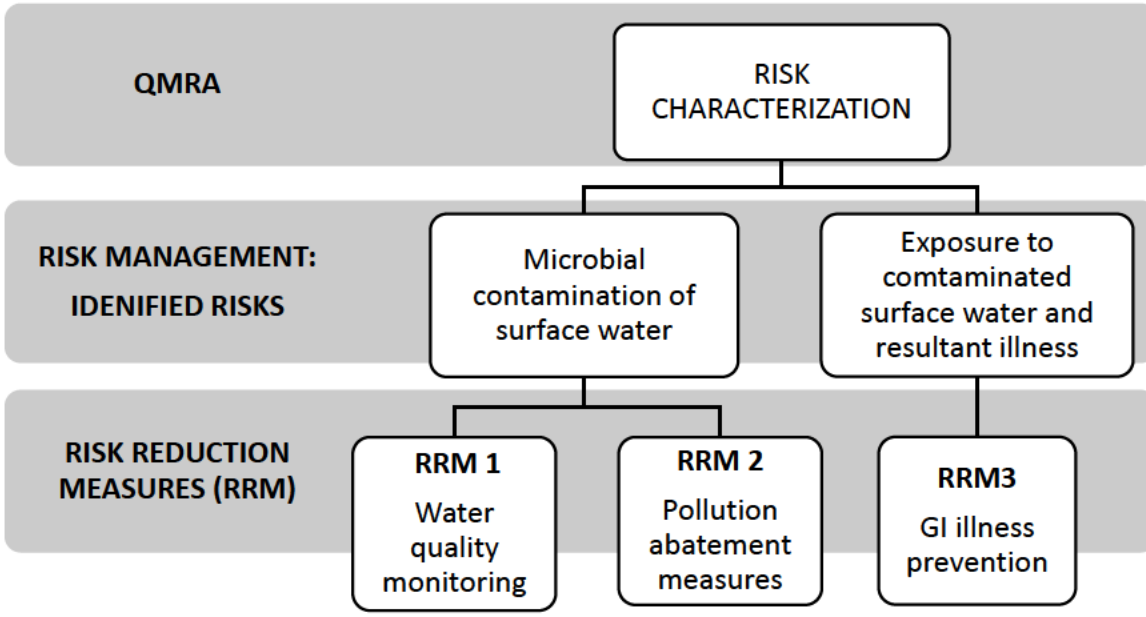

Figure 6.1: Risk reduction measure approach

6.2 APPROACHES TO REDUCE THE PRESENCE OF MICROORGANISMS IN SURFACE WATER

6.2.1 Risk reduction measure one: Medium measures

Water quality monitoring

Managing the risks related to microbial contamination is a crucial component. The criticality of this approach derived from the observation that the elevated risk was primarily attributable to the uMsunduzi River's high prevalence of microbial contamination as depicted in Chapter 3. Accordingly, this compels the initial approach to prioritize the mitigation of challenges associated with the primary water environment.

Before the Dusi Canoe Marathon, there is a heightened focus on monitoring the water quality in the Msunduzi River. Before the marathon, preparations are made to guarantee the safety of the water for the paddlers (Dusi Canoe Marathon: 2023).

The organizing committee of the Dusi Canoe Marathon consistently works to tackle these challenges and guarantee the water quality meets the necessary standards for the competition. Important sources for water quality monitoring are the National Department of Water and Environmental Affairs, the City of Pietermaritzburg, and the Dusi Canoe Marathon's water quality team (Dusi Canoe Marathon 2023).

The main focus of the water quality monitoring carried out by the Dusi Canoe Marathon committee and stakeholders is the detection of the primary pathogen, *E.coli*. While *E.coli* is commonly used as an indicator organism, it is important to note that this inquiry does not include other potential pathogens such as those modelled in this study. This omission is a significant limitation, as it means that the analysis does not provide an in-depth appreciation of the presence of other pathogens. As a result, immediate action must be taken to address this gap and allocate sufficient resources for mitigation efforts.

Water quality monitoring requires consistent testing and analysis of key parameters, including pH, temperature, dissolved oxygen, and contaminants (Silva *et al.* 2022:5059). Real-time data is crucial in assessing the suitability of a water body for recreational purposes (El-Shafeiy *et al.* 2023: 8613) Recreational water facilities and organizations have a legal obligation to maintain water quality standards and ensure the safety of the water for public use (WHO 2023). Regular monitoring is essential to determine if water is safe for human use and water sports, guaranteeing an illness-free recreational experience (Choi *et al.* 2021: 5).

6.2.2 Risk reduction measure two: Long-term measures

Pollution abatement strategies

The goal of pollution abatement measures is to restrict or reduce the release of sewage or faecal contaminants into the environment (Prata 2022: 2). Performing maintenance on sewers and storm water pipelines, resolving problems with sewage pumping stations, and adopting strategies to reduce sewer inputs to prevent the occurrence of overflows, such as by recycling wastewater (Sobieraj *et al.* 2022: 2109).

The main goals of a Wastewater risk abatement plan (W_2RAP) are to prevent contamination during the transportation of wastewater, storage, and disposal of sludge. Additionally, it aims to reduce or eliminate contamination through the treatment processes and minimize contamination of the resource where the treated effluent is returned (DWA 2011: 18).

The Darvill Wastewater Treatment Works is a wastewater treatment plant situated along the uMsunduzi River. It is specifically located at Pinetree, downstream of the Darvill Wastewater Treatment Works. The Darvill wastewater treatment facility is a specialized plant that effectively treats wastewater before it is released into the uMsunduzi River. The discharge takes place near the Pinetree Sample site. Pinetree's findings revealed a concerning lack of compliance and insufficient treatment of the plant's effluents. This was evident in the location's consistently high mean *E. coli* concentration (143.5MPN/100ml) throughout the entire study period. Consequently, it is enecessary to implement the Wastewater Risk Abatement Plan (2011), which was established by the South African Department of Water Affairs, to guarantee that WWTPs are compliant, thereby contributing to the mitigation of biological contamination of surface water.

The DWA (2011) aims to implement the W_2RAP by achieving the following objectives: 1. Understanding the wastewater treatment system and its ability to produce treated effluent that meets health and environmental standards. 2. Identifying and controlling potential sources of contamination. 3. Validating control measures used to manage hazards. 4. Establishing a monitoring system for the control measures within the wastewater treatment system. 5. Taking prompt corrective actions to ensure compliance with effluent standards and safe disposal of sludge. 6. Verifying the quality of effluent to ensure proper implementation of the W_2RAP and adherence to waste quality standards.

6.3. APPROACHES TO REDUCE THE RISK OF GASTROINTESTINAL ILLNESS IN PARTICIPANTS OF THE DUSI CANOE MARATHON

6.3.1 Risk reduction measure three: Medium measures

Preventative medicines

Bismuth subsalicylate is a medication that finds extensive application in sports medicine for the prevention and treatment of diarrhea resulting from water exposure during recreational activities (Himeno *et al.* 2022: 121). Bismuth has various medical applications, particularly in the treatment of gastrointestinal disorders. It is commonly used to address conditions such as gastric and duodenal ulcers, dyspepsia, and diarrhea (Gonçalves *et al*. 2024: 1600).

Bismuth is classified as the 83rd element in the modern periodic table, specifically belonging to Group 15 and Period 6. It is categorized as a heavy metal. Despite its classification as a toxic metal, bismuth and its compounds are generally regarded as relatively safe (Yu *et al.* 2021: 2038). Bismuth-based agents exhibit extensive antimicrobial properties against a wide range of bacterial pathogens, including both Gram-positive and Gram-negative strains (Wang *et al.* 2020: 183).

A study examining the impact of bismuth on several bacterial pathogens including *Clostridium difficile, Salmonella, Shigella,* and Shiga toxin-producing *E. coli* shown a decrease in bacterial growth in a laboratory setting after treatment with bismuth, as compared to a control group that did not receive therapy. Bismuth has been discovered to bind to the toxins produced by *E. coli* (Pitz *et al.* 2015:93 and National Library of Medicine National Centre for Biotechnology Information 2020).

In contrast bismuth subsalicylate is often used to treat travellers' diarrhea, some studies suggest that athletes, notably those engaged in water sports, may utilize bismuth subsalicylate as a preventative measure against diarrhea (Patel *et al.* 2018:152). There have been few studies conducted to establish the effectiveness of bismuth in treating diarrhea caused by exposure to contaminated water during recreational activities. However, its effectiveness in treating and preventing gastrointestinal illness, particularly travellers' diarrhea, has been extensively researched and documented (Leung *et al.,* 2019:38). Although there may be differences in the populations affected, the pathophysiology, microbiological origins, route of acquisition and transmission, and symptoms reported in travellers' diarrhea and diarrhea resulting from recreational water activities are equivalent (Osman *et al.* 2023: 2). This suggests the potential use of this element as a preventive measure against acquiring gastrointestinal illness from exposure to contaminated water during recreational activities.

Traveler's diarrhea is a common illness that often occurs during travel. It is caused by various types of pathogens, including bacteria, viruses, and protozoa (Giddings *et al.* 2016: 317). Bismuth has been found to have the potential for chemoprevention and can effectively reduce the occurrence of traveler's diarrhea by up to 65%. In a study that used a placebo control, the occurrence of diarrhea and the presence of enteropathogens in the stool were found to be decreased in individuals who were administered bismuth (Giddings *et al.,* 2016:318 and Lloyd *et al.* 2012: 40).

A study was conducted to evaluate the effectiveness of bismuth subsalicylate in preventing and treating infectious diarrhea. The findings indicated that individuals who received bismuth subsalicylate for a maximum of 21 days had significantly higher chances of preventing travelers' diarrhea compared to those who received a placebo (95% CI 2.1, 5.9; $p < 0.001$). Furthermore, individuals suffering from infectious diarrhea who were administered bismuth subsalicylate experienced a significantly higher likelihood of relief from diarrhea compared to those who received a placebo (95% CI 2.1, 6.3; $p < 0.001$) (Bram *et al.* 2021: 2323).

According to the WHO, it is recommended to use bismuth subsalicylate as a preventive treatment before or during exposure to reduce the risk of diarrhea in individuals who are at risk. A comprehensive analysis has determined that bismuth subsalicylate can provide benefits to individuals who are at risk or have been impacted by diarrheal disease caused by contaminated food and water (Bram *et al.* 2021: 2335).

Bismuth-based agents possess qualities that make them promising candidates for various medical applications. They are non-toxic to humans, cost-effective, stable, and have versatile medical properties. This suggests that they could play a significant role in future medical advancements (Rosário *et al.* 2023: 45).

6.3.2 Long-term measures

<u>Potential application of homeopathic preventive treatments</u>

Homeopathy emerged in the late 18th century and gained popularity in the 19th century for its effectiveness in treating epidemics. Today, it is widely used as a system of medicine worldwide (Gilla *et al.* 2020: 104). Homeopathic products can be derived from plants, animals, or minerals. They are distinct from allopathic medications due to their significantly lower concentrations, which makes them safer in terms of potential toxicity (Jaguezeski *et al.* 2020: 104269).

Homeoprophylaxis, also referred to as homeopathic prophylaxis, involves the utilization of homeopathy as a means of preventive medicine or immunization against severe infectious diseases (Nayak *et al.* 2020: 129). Research has demonstrated the efficacy of Homeopathic medicine in enhancing the immune system of individuals when administered as a prophylactic measure during a pandemic. In the past, Homoeopathy has been utilized for prevention during various epidemics such as cholera, Japanese encephalitis, Leptospirosis, Spanish Flu, yellow fever, scarlet fever, Diphtheria, typhoid, and more (Nayak *et al.* 2022: 1).

A clinical trial was conducted in León, Nicaragua to compare the effectiveness of homeopathic medicine and placebo in treating acute childhood diarrhea. The study found that the treatment group experienced a significant decrease in the duration of diarrhea, as defined by the number of days until there were less than three unformed stools daily for 2 consecutive days ($P < 0.05$). There was a notable disparity in the number of stools per day between the two groups after 72 hours of treatment, with statistical significance ($P < 0.05$) (Jacobs *et al.* 1994: 719).

Throughout the entire duration of the study, *Cryptosporidium spp.* consistently posed the highest risk with *E.coli* posing the second highest risk. This preventative medicine will primarily address the reduction or prevention of gastrointestinal illness among recreational users of uMsunduzi River, specifically related to the infection of *Cryptosporidium spp.* and *E.coli*. The preparation method of the Homeopathic agent plays a crucial role in ensuring effective prevention. In this investigation, the Homeopathic medication will be established as a Nosode for hypothetical purposes. Nosodes, derived from the Greek word nosos meaning sickness, are biological preparations used in homeopathic medicine for disease prevention (Munshi *et al.* 2022: 43). Homoeopathic Nosodes may be used for the treatment and prevention of both non-infectious and infectious disorders. Homoeopathic practitioners have used over 60 homoeopathic nosodes , which served a substantial part in treatment (Zulu 2023: 1).

Nosodes are diluted forms of pathogenic organisms, including fungi, parasites, viruses, or bacteria, as well as disease products (Varanasi and Nayak, 2020: 130). Nosode preparation entails a series of consecutive dilutions of biological material, often in increments of 100, which is then given to prevent or treat a health issue. Nosodes have gained significant popularity in the field of animal homeopathic medicine and are now being used as therapeutic agents in human medicine (Munshi *et al.*, 2022: 45).

Cuba is the global leader in the development and effective implementation of homoeopathic nosodes in public health. Cuba has extensive expertise in using nosodes either as a standalone therapy or in conjunction with non-nosode homeopathic treatments, such as prophylaxis. The nation has used nosodes to treat many maladies such as pneumonia, hepatitis, meningitis, dengue, cholera , leptospirosis, and other ailments (Bracho *et al*. 2010: 158 and Munshi *et al.* 2022: 44).

A comprehensive study was conducted to investigate the effectiveness of a nosodal preparation in preventing the spread of Leptospirosis during a potential epidemic in Cuba. The results indicated a significant reduction in infection rates among the population (Bracho *et al*. 2010: 157).

In Canada, Health Canada's Natural Health Products Regulations have approved the use of nosodes for humans. Currently, there are 179 approved products available for purchase, with 82 specifically indicated for the prevention of common and significant infections (Rieder *et al.* 2015: 219). For the objectives of this study, *Cryptosporidium spp*. and *E.coli* would be cultivated as nosodes. The preparation would be provided to the Dusi canoe marathon participants one month before the marathon. This would help prevent gastrointestinal illness caused by exposure to micro-biologically contaminated surface water. The efficacy of this modality would require validation through a retrospective survey. This would subsequently contribute to the advancement of public health knowledge in the prevention of waterborne gastrointestinal illness associated with recreational activities in surface water bodies.

Studies have been conducted to examine the potential of homeopathic nosodes, specifically *E. coli,* as a preventative measure for gastrointestinal illness caused by this bacterium. For instance, an in vitro study conducted by Xaba in 2018 sought to determine the antimicrobial efficacy of the homoeopathic remedy Colibacillinum, which is derived from a uropathogenic strain and an enteropathogenic strain of *E. coli*, against uropathogenic and enteropathogenic cultures of *E. coli*. The findings of this investigation indicated that the homoeopathic remedy Colibacillinum, exhibited statistically significant inhibitory effects against both uropathogenic and enteropathogenic strains of *E. coli* ($p<0.001$).

An additional study conducted in 2019 evaluated the effectiveness of homeopathy in preventing *E. coli* diarrhea through a randomized placebo-controlled trial. The results showed that piglets in the homeopathic treated group had significantly less *E. coli* diarrhea ($P = 0.0012$) compared to piglets in the placebo group. Furthermore, the severity of the diarrhea was reduced. Additionally, the ratio of diarrhea in the placebo group to that in the homeopathic treatment group was greater than sixfold (Ellinger 2019: 41, 45).

Although the effectiveness of using *E.coli* as a nosode has not been specifically studied in humans, the findings from these studies provide evidence of possible therapeutic benefits. Furthermore, these findings may be used as support to explore the use of this medication in humans for the prevention of gastrointestinal illness. Although there are no peer-reviewed studies on the efficacy of *Cryptosporidium spp.* as a nosode, the remedy has been successfully employed in private practice for the treatment and prevention of childhood diarrhea. This renders the remedy a prospective measure for the prevention of diarrhea in the study population.

6.4 SUMMARY OF THE CHAPTER

Waterborne diseases continue to be significant contributors to human disease and mortality on a global scale. Over 95% of waterborne infections are avoidable, and their eradication is a particular Millennium Goal. The objective of this study was to propose risk reduction measures and procedures to enhance safety in surface water recreational activities. These recommendations are based on the identified risks in chapters 3 and 4, as well as the findings of the QMRA in chapter 5. As indicated in earlier chapters, it was found that the microbial count at all the sampling sites exceeded the guidelines set by South Africa and the WHO for recreational water use. Factors contributing to a higher risk included being exposed to a water source (specifically uMsunduzi River) with elevated levels of indicator organisms and other pathogenic organisms, as well as unintentionally consuming this water source during recreational activities like canoeing. Moreover, the QMRA findings revealed a noteworthy infection risk for the populations that rely on the uMsunduzi river. The increased risk was primarily linked to *Cryptosporidium spp.*, which exceeded the threshold established by WHO and the USEPA by an order of magnitude (10^{-1}).

The risk reduction measures in question primarily aimed to decrease the presence of microorganisms, as discussed in the chapter. Additionally, they sought to prevent gastrointestinal illnesses among the participants of the Dusi Canoe Marathon by utilizing complementary medicine techniques. These measures have the potential to contribute to the corpus of knowledge in public health and mitigate the issues disclosed in this study assuming they are further explored. Chapter seven provides a critical overview of the study and concludes the thesis.

7. Chapter seven

Critical overview

7.1 ASSESSMENT OF THE MICROBIAL WATER QUALITY OF THE UMSUNDUZI RIVER OVER THREE MONTHS, INCLUDING ONE MONTH BEFORE THE COMMENCEMENT OF THE DUSI CANOE MARATHON, ONE MONTH DURING THE MARATHON, AND ONE MONTH FOLLOWING THE MARATHON

The present study aimed to assess the microbiological water quality of the uMsunduzi River throughout a six-month monitoring period, notably before, during, and after the Dusi Canoe Marathon. For the aims of this study, microorganisms were used as principal indicators to evaluate water quality. An analysis was conducted on the following microorganisms: *E. coli*, *Pseudomonas aeruginosa*, and *Cryptosporidium spp.*

A total of six sampling points along the uMsunduzi river were identified and monitored for the prevalence of specific microorganisms. The sample points were distributed in a downstream pattern. The sampling sites were identified using the route map provided in Appendix 3, which corresponds to the Dusi Canoe Marathon of 2020. The locations were labelled based on the Dusi Canoe Marathon Map. Furthermore, these points were chosen as they represent the six essential checkpoints that every participant must navigate on the race's opening day. By doing so, a satisfactory number of participants were able to interact with the chosen locations.

This study employed quantitative water quality detection and quantification methods to evaluate the biological integrity of the uMsunuduzi river. The liquid culture method was utilized to detect and quantify bacterial organisms, and the molecular technique (ddPCR) was employed for the detection and quantification of protozoa. Descriptive statistics and non- parametric approaches were used to analyse the data collected gathered from uMsunduzi river. Subsequently, the data was described and presented.

The water quality of the uMsunduzi river was appraised based on the criteria for water intended for recreational use established by the WHO (2003) and DWAF (1996). The analysis revealed that the concentrations of *Cryptosporidium spp*. peaked at 215.5 Oocyst Microbes/L in May 2022, following the Dusi Canoe Marathon. This level is considered hazardous according to the criteria established by the WHO (2003), which indicates that 10-10000 Oocyst Microbes/L of *Cryptosporidium spp.* poses a significant health risk to humans.

These results were comparable to those of the bacterial analysis, given that both the levels of *E.coli* and *Pseudomonas aeruginosa* were greater than the benchmark established by the WHO (2003) and DWAF (1996). The acceptable limit for *E. coli* in surface water allocated for primary contact recreational activities, is 200 colony forming units (CFU) or fewer per 100 millilitres of water. An equivalent threshold of 130 colony-forming units (CFU) per 100 millilitres for *E. coli* in surface water designated for recreational use as set by the DWAF in South Africa. The data revealed that Pinetree had the highest mean *E. coli* concentration of 396.8 MPN/100ml in May 2022.

Similar to *Pseudomonas aeruginosa*, a notable rise in levels was seen in May 2022, reaching 133.7 MPN/100 ml, above WHO (2003) and DWAF (1996) recommendations. According to the standards set by the World Health Organisation (WHO), the contamination of recreational water with *Pseudomonas aeruginosa* at a concentration ranging from 0 to 1000 MPN/100ml is considered a possible risk to human health. Conforming to the standards established by the DWAF, the identification of *Pseudomonas aeruginosa* at a concentration ranging from 10 to 1000 colony-forming units per 100 millilitres is considered to suggest a possible risk to human health.

The data indicates that the highest concentrations of the organisms occurred in May 2022, with Pinetree and Braaitakeout sampling sites being the most noteworthy areas. Both of these sample locations are situated near the downstream area of Darvill Wastewater Treatment Works. The Darvill wastewater treatment facility is a specialized process plant specifically designed to treat wastewater before it is released into the uMsunduzi River. In the second chapter, it was shown that untreated sewage, wastewater, and stormwater runoffs are the primary contributors to microbiological pollution in surface water worldwide. The region of KZN had substantial rainfall throughout the early and mid-months of January 2022. Consequently, the malfunctioning and blocked sewage systems within Pietermaritzburg's municipal infrastructure resulted in issues that adversely impacted the water quality in some sections of the river network. The above-characterized causes are attributed to the inadequate water quality reported at both sample sites and time frame.

Based on our microbiological analyses, it appeared that the uMsunduzi River could be contaminated with faecal matter. This is supported by the consistent presence of organisms including *E.coli* and *Cryptosporidium spp.* The water analysis results for the uMsunduzi river suggest that the primary sources of water pollution in this region are non-point pollution sources, specifically agricultural and urban land uses. Thus, to enhance water quality, it is crucial to prioritize the reduction of these sources.

This research demonstrates that information regarding potential environmental impacts on water quality may be effectively extracted from the data set using environmental metric approaches. These instruments are beneficial for appreciating the intricate nature of water quality concerns. These methods are thought to aid decision-makers in evaluating water quality and establishing priorities in pollution mitigation efforts.

7.2 Analysis of the correlation between the exposure to uMsunduzi River and the occurrence of pathogenic illnesses among the participants of the Dusi Canoe Marathon.

Utilizing canoeists as a sample group of river users, this thesis intended to determine the scope of concerns about river health and water-related illness in the uMsunduzi River Catchment utilizing data acquired from a cross-sectional survey. Canoeists frequently utilize the uMsunduzi River for training and competitive purposes, dedicating a significant portion of their week to these activities. Moreover, this community tends to have a high level of education and financial stability, granting them access to extensive medical and scientific resources. They also demonstrate a strong willingness and interest in actively participating in research endeavours (Kirsten 2006).

Due to this factor, they represented a readily available group of individuals from whom one could obtain precise and comprehensive data. It was supposed that the potential for canoeists to contract illnesses would have implications for the communities that rely on the river for daily domestic activities. Therefore, this research provides a basis for determining the necessity of conducting additional studies and implementing resource management strategies in the catchment area.

The study's findings revealed that a considerable number of participants (46.2%) reported being exposed to the uMsunduzi River for a period of three days. Additionally, a statistically significant majority of individuals (99%) experienced illness after being exposed. Additionally, the study found that the incidence of diarrhea was 89.7% in the group that was exposed to the river. The results are comparable with those indicated below however; the findings of this research demonstrate a substantial rise in the number of individuals who reported GI illnesses when compared (Chapter 4).

In a study conducted by Hay (2017), it was found that a survey conducted in 2016, immediately following the race, indicated that 40% of the paddlers experienced varying degrees of gastrointestinal infections. Wilson et al. (2017) also discovered that 14% of the Dusi Marathon participants experienced diarrhea as a result of exposure to the uMsunduzi River. Moreover, the 2019 Dusi survey figures revealed that 60% of participants had gastrointestinal illnesses after the Dusi Canoe Marathon. The consistently high rate of water-related infection among canoeists who participate in the annual marathon was reinforced by all of these studies.

Similarly, the findings of this study confirm that the risk of water-related infection for canoeists was not restricted to 2016, 2017, and 2019 Dusi Canoe Marathon, but has been a

significant factor in the sport in the catchment for many years. This issue has been apparent since 2006, as Oliver established.
In keeping with the results of this research, the elevated levels of *E. coli*, *Cryptosporidium spp*., and *Pseudomonas aeruginosa* detected in the uMsunduzi River before, during, and following the Dusi canoe marathon are correlated with an increased frequency of diarrhea among the participants. Water quality analysis corroborates the relationship between water-related illness and major pollution events when the river is polluted with sewage or industrial waste after large rainfall occurrences (Ngowi, 2022). The uMsunduzi River is severely and routinely impacted by these events, placing people at great risk of illness. In addition, the timing and intensity of these episodes are unpredictable, since they are strongly influenced on natural rain events and the unreported activities of the industry within the watershed. As a consequence, warning systems and mitigation attempts are restricted in their scope.

This was underlined in the 2022 Dusi Canoe Marathon, when storm events occurred in early and mid-January 2022, influencing the water quality in particular areas of the river system as reported by Umngeni water (2022). The contamination had a lasting impact on the canoeists, as observed in this study through the analysis of water quality and the cross-sectional survey. The effects were still apparent for several days after the marathon.

The conclusions drawn from the microbial water quality analysis and the Dusi Canoe Marathon survey conducted as part of this research have unequivocally demonstrated that utilizing the uMsunduzi River for recreational and professional canoeing activities exposes participants to a significant risk of contracting waterborne illnesses. An important determinant in minimizing the risk of illness associated with the watershed is the promotion of improved governance of catchments across all levels of land use, ranging from agriculture to infrastructure development. To effectively address the issue of disease in the river, both the public and the municipalities must provide support to the joint endeavours of health departments and relevant organizations, such as the DUCT and the Region Monitoring Forums.

7.3 Probabilistic microbial risk assessment for recreational use of the uMsunduzi River.

The levels of *E.coli*, *Pseudomonas aeruginosa*, and *Cryptosporidium spp*. in the uMsunduzi River exceeded the recommended values set by DWAF(1996) and WHO(2003) for surface water used for recreational purposes. Consequently, the utilization of this river for recreational activities may result in gastrointestinal infections for both the local population and individuals engaging in water sports (Chapter 3).

The study's probabilistic assessment reveals that the median values of the combined gastrointestinal risks from both bacteria at all investigated sites were below the established benchmark of 0.036 and 0.05 (USEPA 2012; WHO 2016). Nevertheless, the analysis of *Cryptosporidium spp.* benchmarks revealed that the overall gastrointestinal infection risk at all sites surpassed the previously mentioned benchmarks (Chapter 5). The significant risk of infection linked to *Cryptosporidium spp*. was anticipated due to the proximity of the uMsunduzi catchment to numerous cattle farms. As discussed in chapter 3, *Cryptosporidium spp.* oocysts are commonly found in cattle excreted in their faces.

The growth of the agricultural and livestock industries has had a significant impact on the environment, leading to the depletion of natural resources and increased pollution (Yitbarek 2019). *Cryptosporidiosis* has been observed in various livestock animals, including cattle, pigs, goats, sheep, horses, and poultry like chickens and ducks (Ryan *et al.* 2014; Robertson *et al*. 2014). The presence of cattle, and potentially grazing animals, has a notable impact on the release of *Cryptosporidium* oocysts into the environment. These oocysts are known to be the cause of waterborne diarrhea outbreaks in humans across the globe. The presence of various species of *Cryptosporidium*, including *C. hominis*, in cattle raises concerns about the potential public health risks associated with this association (Hatam-Nahavandi *et al*. 2019).

In developed nations, contaminated water resources serve as the main sources of *Cryptosporidium spp.* . Water pollution can occur in groundwater and surface waters that are used for drinking, recreation, and irrigation due to agricultural contamination sources. Contamination with *Cryptosporidium spp*. can occur in agricultural environments due to the presence of faecal material from both humans and animals (Golomazou *et al*. 2024: 170057). Based on the QMRA findings, it is evident that the populations relying on the uMsunduzi River face a considerable risk of infection. It is crucial to consistently monitor and manage microbial contamination in river systems. This study provides a valuable contribution to the existing literature on the microbial hazards associated with the uMsunduzi River.

7.4 Development of a risk reduction strategy for recreational use of the uMsunduzi River based on data obtained from the outcomes of objectives 1-3

Using the QMRA outcomes as an indicator, the objective of this research was to define risk reduction techniques and provide measures to lessen the likelihood that competitors in the Dusi canoe marathon may become ill. As per the risk characterization phase of the QMRA, the hazards indicated were microbial pollution of the uMsunduzi river and exposure to polluted surface water leading to illness among the participants of the Dusi Canoe Marathon. Two theoretical risk reduction strategies were proposed to address the challenge of uMsunduzi river experiencing heightened microbial contamination. The first strategy aimed to enhance water quality monitoring, while the second strategy targeted the reduction of non-point source pollution impacting the river and its catchment area. One risk mitigation strategy was proposed to reduce the occurrence of gastrointestinal resulting from future exposure to the uMsunduzi River.

The first risk reduction measure suggested that the existing water quality monitoring methods carried out by the regional municipality, the Dusi Canoe Marathon committee, Umngeni water, and regional forums should incorporate more micro-organisms into the currently screened common indicator organisms. Routine screening of *Cryptosporidium spp.* as a protozoa is recommended based on the findings of this research, which concluded that this specific organism constituted the highest risk of infection.

The second risk reduction strategy recommended routine maintenance be conducted on stormwater and sewer systems. Appropriate implementation of the wastewater risk abatement plan (W2RAP) is imperative. Considering that the wastewater treatment facility Darvill Wastewater Treatment Works is located along the uMsunduzi River and that its discharge occurs near the Pinetree Sample site. Pinetree's findings indicated a notable lack of compliance and inadequate treatment of the plant's effluents. The third risk reduction measure was subsequently divided into two components: medium measures and long-term measures, to reduce the risk of gastrointestinal illness in participants of the Dusi Canoe Marathon.

These methods included medical interventions, such as herbal and homeopathic therapies, traditionally used for many years. The safety and efficacy of these disciplines are well acknowledged (chapter 6). They are classified as complementary treatments, characterized by their minimal side effects. The methods proposed in this study were purely theoretical and have not been assessed for their effectiveness. It is recommended that these methods be considered for future research to enhance our understanding in the future.

7.5 CONCLUSION OF THE STUDY

The most significant outcomes derived from the objectives of the thesis are:
The concentration of microorganisms in surface water from the uMsunduzi river in Pietermaritzburg, KZN, exhibited variation both between and within the six sample points in the river. The concentration of microorganisms in the water samples from these six sample sites exceeded the guidelines set by the WHO and the DWAF for recreational use of surface water. As noted below, the risks associated with *Cryptosporidium spp.* infections were found to be higher than the acceptable risk level set by the WHO:

- The microorganisms commonly found in surface water in the uMsunduzi catchment area included *E. coli, Pseudomonas aeruginosa*, and *Cryptosporidium spp.*
- Throughout the three-month study period, there were fluctuations in the concentration of both bacteria and protozoa. This suggests a potential shift in infection prevalence or intensity within the populations under observation.

- The month of May 2022 had greater concentrations of all microorganisms.

Participants in the Dusi Canoe Marathon had a notable incidence of diarrhea. The incidence of diarrhea among the participants was influenced by several significant behavioural and environmental risk factors. The identified independent variables associated with increased risk were exposure to a water source (specifically uMsunduzi River) with elevated levels of indicator organisms and other pathogenic organisms, as well as accidental ingestion of this water source during recreational activities such as canoeing. Concluded below are the core findings of the study:

- The study included male participants (100%) who were predominantly of White ethnicity (100%). Most participants were between the ages of 25 and 39 (91%).
- 100% of the participants were exposed to the uMsunduzi River. A significant portion of respondents (46.2%) reported being exposed to the river for a duration of three days. The minimum number of days accounted for only 1.3% of the total, equivalent to a single day. The binomial test revealed that a significant proportion of individuals, 100%, ingested water from the river. Additionally, a significant proportion of individuals, 99%, reported illness after being exposed.
- A total of 70 respondents (89.7%) from the exposed cohort indicated that they had encountered at least one episode of diarrhea,
- In the bivariate analysis, a longer exposure time to the uMsunduzi River was found to be associated with an increased likelihood of participants experiencing diarrhea. However, this association was not of statistical significance [COR: 1.3, 95% CI (0.86 - 1.71)].
- The bivariate analysis revealed a significant association between exposure to the river and the incidence of diarrhea. The risk of diarrhea was 97% higher in those who were exposed to the river and accidentally ingested the water compared to those who did not [COR:139, 95% CI (42.1 – 459.1)].
- According to the COR, incidental ingestion of river water increases the risk of illness compared to not being exposed to the river. Therefore, the likelihood of a person being ill due to exposure and accidental ingestion of the river water is 139 times

higher compared to an individual who is not exposed to the river. The correlation was statistically significant, with a p-value of (<.001).

Recreational exposure to surface water was shown to be correlated to an increased risk of *Cryptosporidium spp.* infection among participants of the Dusi Canoe Marathon, in comparison to the group without exposure. As outlined below:

- The concentration of *Cryptosporidium spp.* oocysts in surface water used for recreation in KZN were found to exceed the recommended guideline value set by the WHO.
- The elevated concentration of *Cryptosporidium spp.* oocysts in the surface water and the subsequent rise in infection rates reveal a dose-response correlation between environmental exposure and infections.

The odds ratio for infection between the exposed Dusi Canoe Marathon participants and the non-exposed group suggests that the exposed Dusi Canoe Marathon participants were at a higher risk of infection than the non-exposed group, particularly when the surfaces water was accidentally ingested.

7.6 LIMITATIONS

- The study exclusively focused on participants of the Duzi marathon, excluding the broader community of the uMsunduzi catchment area. The generalizability of the findings to the broader population is limited, as marathon participants may not accurately represent the entire community.
- This investigation concentrated on the three primary pathogens (*E. coli, Pseudomonas aeruginosa,* and *Cryptosporidium parvum*) within the extensive and varied river microbiome. The limited focus may fail to encompass the complete spectrum of microbial hazards found in the uMsunduzi River, possibly neglecting other significant pathogens or environmental health concerns.

7.7 RECOMMENDATIONS

- Future research should expand the study population to encompass a broader spectrum of community members, thereby enhancing the understanding of microbial hazards and health risks associated with recreational water use in the uMsunduzi River.
- Future research should undertake a comprehensive analysis of the river's microbiome, including additional pathogens, indicators, and environmental factors to enhance the understanding of waterborne health risks.

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9. Appendices

Appendix 1: Ethics approval letter

DUT
DURBAN UNIVERSITY OF TECHNOLOGY
INYUVESI YEZOBUCHWEPHESHE

INSTITUTIONAL RESEARCH ETHICS COMMITTEE

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

29 May 2021

Dr Z P Mdluli
P.O. Box 1339
Tongaat
4400

Dear Dr Mdluli

A probabilistic and epidemiological assessment of health risks associated with recreational exposure to contaminated surface water: A case of the uMsunduzi River.
Ethical Clearance number IREC 021/21

The Institutional Research Ethics Committee acknowledges receipt of your gatekeeper permission letters.

Please note that FULL APPROVAL is granted to your research proposal. You may proceed with data collection.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC Standard Operating Procedures (SOP's).

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Yours Sincerely

Prof J K Adam
Chairperson: IREC

Appendix 2a: Letter of request for approval to uMsunduzi municipality

Letter of request for gatekeeper permission to the General Manager of uMsunduzi Municipality.

P.O. Box 1339
Tongaat
4400
[Date]

Municipal General Manager
UMsunduzi Municipality
Private Bag X321
Pietermaritzburg 3200

Request for Permission to Conduct Research

Dear Mr. Felix Nxumalo

My name is Dr Zinhle Pearl Mdluli, a PhD: Health Sciences student at the Durban University of Technology. The research I wish to conduct for my doctoral thesis is: **A probabilistic and epidemiological assessment of health risks associated with recreational exposure to contaminated surface water: A case of the uMsunduzi river**.

I am hereby seeking your consent to carry out this study in uMsunduzi river and ask for your written permission in this regard.

I have provided you with a copy of my proposal which includes copies of the data letter of information, consent and collection tools to be used in the research process, as well as a copy of the ethics clearance, which I received from the Institutional Research Ethics Committee (IREC).

If you require any further information, please do not hesitate to contact my supervisor, Prof M.N. Sibiya, email: nokuthulas@dut.ac.za

Thank you for your time and consideration in this matter.

Yours sincerely,

…………………………………….
Dr Zinhle Pearl Mdluli
Durban University of Technology
Cell:074 9624 928
Email: zinhlepearlmdluli@gmail.com

Appendix 2b: Approval letter from uMsunduzi municipality

The Msunduzi Municipality
OFFICE OF THE MUNICIPAL MANAGER

Private Bag X 321
Pietermaritzburg
3200
(033) 392 2882

City Hall, Chief Albert Luthuli Street
Pietermaritzburg
3201
www.msunduzi.gov.za

CITY OF CHOICE
PIETERMARITZBURG
MSUNDUZI

Enq: M C Jackson **Tel. 033 392 2882** **E-mail: madeleine.jackson@msunduzi.gov.za**

Dr Zinhle Pearl Mdluli
P.O. Box 1339
Tongaat
4400

15 March 2021

Per Email: zinhlepearlmdluli@gmail.com

RESEARCH CONSENT LETTER - A PROBABILISTIC AND EPIDEMIOLOGICAL ASSESSMENT OF HEALTH RISKS ASSOCIATED WITH RECREATIONAL EXPOSURE TO CONTAMINATED SURFACE WATER: A CASE OF THE UMSUNDUZI RIVER.

Your correspondence regarding the above has reference.

Please be advised that you hereby granted permission to conduct your research within Msunduzi Municipality, subject to the following conditions:

(i) Correspondence from your institution confirming:
  a. Ethical clearance;
  b. That you are a registered student, with the relevant student number;
  c. Qualification that you are studying towards;
  d. Area of research and research topic.

(ii) Ensure that the Office of the City Manager is informed when you commence your research in the municipality.

(iii) You will forward a copy of the completed research report to the Office of the Municipal Manager, c/o Ms. Madeleine Jackson as per the above contact details;

(iv) None of the information and/or findings obtained during the research project will be used to construe the Municipality in a negative light and/or against the Municipality in any court of law.

(v) The municipality will not be responsible and expected to provide resources for your study such as transport, research assistants, etc.

(vi) Permission must be obtained from the municipality prior to any publication or paper that will be published or presented containing municipal information.

I trust the above is in order

Sincerely,

MADELEINE JACKSON
SENIOR MANAGER: OFFICE OF CITY MANAGER

OFFICE OF THE CITY MANAGER

Telephone/uCingo: 033 3922882
Facsimile/iFeksi: 0868047309

Private Bag / Isikhwama: X321
Pietermaritzburg/ePietermaritzburg 3200

Appendix 3a: Letter of request for approval to Duzi Canoe Marathon Association

Letter of request for gatekeeper permission to the Dusi Umgeni Conservation Trust

P.O. Box 1339
Tongaat
4400
[Date]

The Manager
Dusi Umgeni Conservation Trust
240 West Street
Pietermaritzburg
3200

Request for Permission to Conduct Research

Dear Madam/Sir

My name is Dr Zinhle Pearl Mdluli, a PhD: Health Sciences student at the Durban University of Technology. The research I wish to conduct for my doctoral thesis is: **A probabilistic and epidemiological assessment of health risks associated with recreational exposure to contaminated surface water: A case of the uMsunduzi river**.

I am hereby seeking your consent to carry out this study in uMsunduzi river and ask for your written permission in this regard.

I have provided you with a copy of my proposal which includes copies of the data letter of information, consent and collection tools to be used in the research process, as well as a copy of the ethics clearance, which I received from the Institutional Research Ethics Committee (IREC).

If you require any further information, please do not hesitate to contact my supervisor, Prof M.N. Sibiya, email: nokuthulas@dut.ac.za

Thank you for your time and consideration in this matter.

Yours sincerely,

…………………………………….
Dr Zinhle Pearl Mdluli
Durban University of Technology
Cell:074 9624 928
Email: zinhlepearlmdluli@gmail.com

Appendix 3b: Approval letter from Duzi Canoe Marathon Association letter

t/a NCC Events

The Dusi Canoe Marathon
KwaZulu-Natal Canoe Club
Tel: 087 133 1237
Fax: 086 666 0244, 033 0421525
Email: info@dusi.co.za
www.dusi.co.za
www.natalcc.co.za

26 May 2021

Email: zinhlepearlmdluli@gmail.com
Student no. 21109951

Dr. Zinhle Pearl Mdluli
P.O.Box 1339
Tongaat
4400

Dear Dr Mdluli

RESEARCH CONTENT LETTER
A probabilistic and epidemiological assessment of health risks associated with recreational exposure to contaminated surface water: A case of the uMsunduzi river

This letter serves to advise that Dr Z P Mdluli has been given permission to conduct her research and survey relating to the Dusi Canoe Marathon, as detailed below.

As advised by Dr Mdluli, the study is an epidemiological study, that aims to assess the microbes found in the Msunduzi river and then try to correlate it to the illnesses experienced by the Dusi participants, such illnesses include diarrhoea, skin infections and so forth. So therefore she would have to do a survey in order to achieve this. The survey would involve the Dusi participants. Results from the study would help the participants, because as part of the study she will come up with preventative methods for the Dusi participants to use to prevent illness .

Safety measures as stipulated by the department of health will be adhered to as precautionary measures. The researcher conducting the interviews will be required to wear protective material that includes a facial mask and gloves. Sanitizers and sterilized pens will be provided for the participants. Sanitizers will be used by the participants to disinfect theirs hands before making contact with the pens or the questionnaires. No participant will be issued a questionnaire without wearing a mask. 1-meter distance will be kept between the participants and researcher whilst conducting the interview. All completed questionnaires will be put into a box after completion. Questionnaires will then be taken by the researcher back to the Durban University of technology for storage overnight and will be analysed the following day.

Yours faithfully

DOUG GOW
CHAIRMAN NATAL CANOE CLUB

Appendix 4: Letter of information

DUT DURBAN UNIVERSITY OF TECHNOLOGY INSTITUTIONAL RESEARCH ETHICS COMMITTEE

Dear Participant

Thank you for agreeing to consider participating in this study.

Title of the Research Study: A probabilistic and epidemiological assessment of health risks associated with recreational exposure to contaminated surface water: A case of the uMsunduzi river.

Principal Investigator/s/researcher: Dr Zinhle Pearl Mdluli, PhD: Health Sciences Candidate.

Co-Investigator/s/supervisor/s: Prof. M.N. Sibiya D Tech: Nursing (Supervisor), Dr Isaac Dennis Amoah, PhD: Health Sciences (Co-supervisor), Dr S.K.K. Pillai PhD: Biosciences (Collaborator).

Brief Introduction and Purpose of the Study: Waterborne diseases encompass illness resulting from both direct and indirect exposure to contaminated water. This can be a result of consumption or by skin exposure during bathing or recreational water use. This study will, provide an assessment of the microbial water quality and possible risk of infection for recreational use of the uMsunduzi river, provide evidence of a link between exposure and infections and propose possible interventions that could be implemented to reduce the risks of infections.

Outline of the Procedures: You are kindly requested to complete the questionnaire. A trained research assistant will administer this questionnaire. Completion of the questionnaire will take you approximately 30 minutes to complete. A box will be made available for you to deposit the completed questionnaire.

Risks or Discomforts to the Participant: There is no anticipated risk nor discomfort.

Benefits: The study will help to know if water used for recreational purposes is safe. It will assist you to be aware of the causes of waterborne diseases and what can be potentially done to reduce the prevalence of waterborne diseases. The results will assist to propose possible interventions that could be implemented to reduce the risks of infections.

Reason/s why the Participant May Be Withdrawn from the Study: If you feel that you cannot continue answering the questions, you are free to withdraw from the study without any penalty.

Remuneration: You will not receive any remuneration of any kind for participating in this study.

Costs of the Study: You do not need to pay for participating in the study.
Confidentiality: Your name will not appear in the questionnaire; instead a code will be used to ensure confidentiality

Research-related Injury: There is no anticipated research-related injury by participating in the study.

Persons to Contact in the Event of Any Problems or Queries: If you have any questions, concerns or problems at any time about the study or the procedures feel free to contact researcher, Dr Mdluli on 074 9624 928, my supervisor, Prof Sibiya on 031-373 2284 or the Institutional Research Ethics Administrator on 031-373 2375. Complaints can be reported to the DVC: Research, Innovation and Engagement Prof S Moyo on 031-373 2577 or moyos@dut.ac.za

Appendix 5: Consent letter

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, Dr Zinhle Pearl Mdluli about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: ___________,
- I have also received, read and understood the above written information (Participant Letter of Information) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during the course of this research which may relate to my participation will be made available to me.

____________________ ____________ ________ ________________

Full Name of Participant **Date** **Time** **Signature / Right Thumbprint**

I, Zinhle Pearl Mdluli herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

________________ ____________ ________________

Full Name of Researcher **Date** **Signature**

________________ ____________ ________________

Full Name of Witness (If applicable) **Date** **Signature**

________________ ____________ ________________

Full Name of Legal Guardian (If applicable) **Date** **Signature**

Appendix 6: Diarrheal incidence sheet

DIARRHEAL INCIDENCE SHEET

PLEASE READ AND COMPLETE INFORMED CONSENT FORM BEFORE FILLING IN THIS DATA SHEET

INSTRUCTIONS:

- ✓ Write an X in the appropriate box if it applies to your symptoms
- ✓ Please use the spaces provided to specify your answer
- ✓ Compulsory Information *

Date: *	2020	-		-		(YYYY-MM-DD)		
First Name								
Last Name:								
Age: *								
Gender: *	Male		Female					
Race: *	Black		Coloured		White		Asian/ Indian	

Please answer all the following questions.

- STOOL CHARACTERISTICS: *

1. Number of bowel motions a day

0-2	
3-4	
5-6	
7-8	
9-10	

2. Presence of loose /watery stool

Yes		No	

3. Presence of blood in the stool

Yes		No	

4. Presence of pus in the stool

Yes		No	

5. Presence of mucus in the stool

Yes		No	

6. Incontinence/ urgency

Yes		No	

- DURATION OF SYMPTOMS: *

7. Onset of symptoms

7.1	Before Marathon	
7.2	After Marathon	

8. Duration of symptoms, please indicate the no. of days/ hours e.g. 2 days 4 hours

8.1	No of days/hours	

- ASSOCIATED SYMPTOMS: *

9	Fever	
10	Nausea	
11	Vomiting	
12	Abdominal Pain	
13	Flatulence	
14	Bloating	
15	Skin Rashes/ Lesions	
16	Joint Pains/ Aches	

- PRESENT MEDICATIONS *

17	Antibiotics	
18	Laxatives	
19	NSAIDS	
20	Other	

If Other, please specify

Appendix 7: Questionnaire

Questionnaire

Please read and complete informed consent form before filing in this questionnaire.

Place of interview.

Date of interview.

1. Demographics

Please provide a tick in the appropriate box.

1.1.Gender	
Male	
Female	
1.2.Race	
Black	
White	
Indian/Asian	
Colored	
1.3.Age	
18-24	
25-39	
40-64	
65 years above	

2. Exposure assessment

2.1. Have you been exposed to the following in the last 4 weeks? Please provide a tick in the appropriate box

River	
Swimming facility e.g. pool	
Hot tub	
Water park	

2.2. Please indicate the name of the river, swimming facility or water park

2.3. How many times in the last 4 weeks were you exposed to the previous mentioned facility/facilities?

2.4. How long were you exposed in total in the last 4 weeks to the previous mentioned facility/facilities? example: hours/ days, please specify your answer

2.5. Did you swallow any of the water from the previous mentioned facility/facilities?

Yes	
No	

3. History of infection

3.1. Have you experienced any form of illness after exposure? Please provide a tick in the appropriate box.

Yes	

No	

<u>If answered YES to question 3.1</u>:

3.1.1 please specify the symptoms below

3.1.2 How long after exposure did you experience these symptoms? example: 1 hour later/ a day/ days later.

Appendix 8: Statisticians report

Gill Hendry B.Sc. (Hons), M.Sc. (Wits), PhD (UKZN)
Mathematical and Statistical Services

Cell: 083 300 9896
Email: gillhendrystats@gmail.com

9 November 2020

Re: Assistance with statistical aspects of the study

Please be advised that I am assisting Zinhle Pearl Mdluli (Student number 21109951), who is currently studying for a PhD: Health Sciences at DUT, with statistical aspects of her study. To date, I have assessed the data collection instruments and advised on the sampling.

Yours sincerely

Gill Hendry (Dr)

Appendix 9: Turnitin report

Turnitin Originality Report

Processed on: 14-Nov-2024 13:44 SAST
ID: 2519328485
Word Count: 39518
Submitted: 1

A PROBABILISTIC AND EPIDEMIOLOGICAL ASSESSMENT OF HEALTH RISKS ASSOCIATED WITH RECREATIONAL EXPOSURE TO CONTAMINATED SURFACE WATER: A CASE OF THE UMSUNDUZI RIVER By Zinhle Mdluli

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A PROBABILISTIC AND EPIDEMIOLOGICAL ASSESSMENT OF HEALTH RISKS ASSOCIATED WITH RECREATIONAL EXPOSURE TO CONTAMINATED SURFACE WATER : A CASE OF THE UMSUNDUZI RIVER Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy in Health Sciences in the Faculty of Health Sciences at the Durban University of Technology Zinhle Pearl Mdluli (21109951) Date Supervisor: Prof. Maureen Nokuthula Sibiya Co-supervisor: Prof. Isaac Denis Amoah Co-supervisor: Prof Sheena Kumari K Pillai Abstract Background Water quality has become a prominent global subject due to the challenges posed by water quality degradation and water scarcity. The release of poorly treated effluent from wastewater treatment facilities has emerged as a significant contributor to the deterioration of river water quality in South Africa, frequently exhibiting elevated levels of microorganisms. Aim The aim of this research study was to evaluate the microbial water quality of the uMsunduzi River and to establish a correlation between the exposure to uMsunduzi river and the incidence of diarrhoeal infections among the Dusi Marathon participants. In addition, the study conducted a theoretical assessment of the pre- and post-exposure risk reduction interventions that have the potential to safeguard participants of the canoe race. Methodology The study objectives were achieved by conducting a comprehensive evaluation of the water quality, microbial risk, and infection monitoring conducted before (January 2022), during (February 2022) and after (May 2022) the Dusi Canoe Marathon. Escherichia coli (E. coli), Pseudomonas aeruginosa, and Cryptosporidium spp. were the reference pathogens included in the study . The detection of the aforementioned microorganisms was conducted using the IDEXX Colilert-18 and Pseudolert detection and enumeration kits, for E. coli and Pseudomonas aeruginosa respectively. Furthermore, the research used digital droplet Polymerase Chain Reaction (ddPCR) to determine the presence or absence of Cryptosporidium spp. The association between river exposure and the incidence of diarrheal infections was established through the

Appendix 10: Certificate of Editing

Attach here once edited