UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

**A decade of faecal pollution: Assessing faecal pollution trends in major South African Rivers.**

**Research Report**

**In fulfilment of the degree**

**MSc Environmental Science**

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## Declaration

I, Sibusiso Manxiwana, affirm that this research report is a product of my own efforts, and I have duly acknowledged all sources utilized. No portion of this research has been previously submitted for any other degree or qualification.

Signature of candidate

Date: 09 September 2024

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**Abstract**

Rivers represent crucial natural assets that sustain diverse socio-economic activities and ecosystems. However, these invaluable resources face imminent threats stemming from faecal contamination, which not only affects environmental integrity but also poses significant risks to public health. In South Africa, major rivers are grappling with escalating pollution levels and deteriorating water quality, primarily attributable to deficiencies in sanitation infrastructure, rapid population expansion, urbanization trends, and the inadequate treatment of municipal, industrial, and agricultural waste. This predicament is particularly alarming given the country's prevailing water scarcity and semi-arid climatic conditions. The present study constitutes a comprehensive examination aimed at critically assessing the microbial water quality status of eight prominent South African rivers spanning a decade-long timeframe from 2010 to 2019. These rivers encompass the Limpopo River, Olifants River, Inkomati River, Mzimvubu River, Breede River, Berg-Olifants River, Phongolo River, and Vaal River. Data utilized in this study were sourced from secondary repositories such as the National Microbial Monitoring Programme (NMMP) administered by the Department of Water and Sanitation (DWS). Rigorous statistical analyses were employed to elucidate temporal and seasonal variations in faecal contamination trends, alongside determining statistically significant mean concentrations of *Escherichia coli (E. coli)*. The outcomes of this investigation revealed pervasive faecal contamination across major South African rivers, with discernible escalations in *E. coli* levels recorded across multiple years. Furthermore, the analysis unveiled seasonal fluctuations in contamination levels, coinciding notably with precipitation patterns and land utilization practices.

**Keywords**: Faecal contamination, microbial water quality, South Africa, rivers, public health.

## Table of Contents

**List of Figures**

## List of Tables

## Acronyms

| | |
|---|---|
| Analysis of Variance | ANOVA |
| Cluster Analysis | CA |
| Colony Forming Unit | CFU |
| Council for Scientific and Industrial Research | CSIR |
| Central Water Quality | CWQ |
| Democratic Republic of Congo | DRC |
| Department of Water Affairs and Forestry | DWAF |
| Department of Water and Sanitation | DWS |
| *Enterococcus* | ETN |
| Factor Analysis | FA |
| Faecal Indicator Bacteria | FIB |
| Greed Drop | GD |
| Statistical Package for the Social Sciences. | IBM SPSS |
| Joint Monitoring Program | JMP |
| Kilometre | Km |
| National Microbial Monitoring Program | NMMP |
| National Water Act | NWA |
| National Water Resource Classification System | NWRCS |
| Millilitre | mL |
| Principal Component Analysis | PCA |
| Physicochemical Assessment Index | PAI |
| River Health Programme | RHP |
| South African National Standard | SANS |
| South African Press Association | SAPA |
| United Nations International Children's Emergency Fund | UNICEF |
| United States | US |
| World Health Organization | WHO |
| Water Service Act | WSA |
| Wastewater treatment facilities | WWTF |
| Wastewater treatment works | WWTWs |
| Statistical Software for Excel | XLSTAT |

# CHAPTER ONE: INTRODUCTION

## 1.1 Background of Study

Rivers, often referred to as the lifelines of terrestrial ecosystems, play a pivotal role in sustaining life on Earth by serving as crucial water bodies that cater to various needs such as agriculture, domestic consumption, and industrial activities (Mack *et al*., 2004; Pachepsky *et al*., 2012). These expansive natural networks not only shape landscapes but also support diverse ecosystems, fostering biodiversity and serving as habitats for numerous species including fish and mammals (Wohl, 2020). Additionally, rivers serve as vital conduits linking upland regions with coastal areas, facilitating the movement of sediments, nutrients, and organisms, thereby contributing significantly to ecological diversity. However, despite their critical importance, rivers face escalating threats, particularly from faecal pollution, as human activities intensify, industrialize, and urbanize (Pachepsky *et al.,* 2012).

The contamination of river systems is primarily attributed to the discharge of harmful effluents from various sources such as domestic sewage, human waste, and industrial activities, which introduce non-biodegradable pollutants into river ecosystems (Alm *et al*., 2003). Consequently, these pollutants disrupt the natural balance of river water quality, giving rise to a plethora of ecological and public health concerns. Wastewater effluents act as reservoirs for a wide array of contaminants, including pesticides, organic matter, and pathogens, posing significant risks to both ecosystems and human health (Garzio, 2010). The sources of faecal pollution in rivers underscore the intricate interplay between human activities, such as industrial discharges and urbanization, and natural ecosystems.

Evidence from the Joint Monitoring Program (JMP) conducted by the World Health Organization (WHO/UNICEF, 2017) reveals that numerous major rivers worldwide are heavily contaminated with faecal pollution, resulting in detrimental impacts on river ecosystems. This degradation is notably observed in the Danube River system, a vital water source in Europe, where industrial and agro-industrial effluents have significantly deteriorated water quality (Kirschner *et al*., 2017). Furthermore, the direct discharge of approximately 80% of untreated domestic sewage into rivers globally exacerbates the problem, with a significant proportion of rivers, such as 46% in the United States, classified as poor due to faecal contamination (USEPA, 2017). Consequently, this poses a significant challenge when relying on river water as a source of freshwater supply. Efforts to mitigate faecal pollution in rivers are imperative to safeguard both ecosystems and human well-being in the face of increasing anthropogenic pressures on these vital water bodies.

In the context of South Africa, faecal pollution in its rivers represents a pressing concern, compounded by the nation's limited availability of natural freshwater lakes. The reliance on surface water sources, primarily rivers, to sustain economic growth and accommodate a burgeoning population underscores the criticality of addressing water quality issues (Gqomfa *et al*., 2023). Notably, research conducted by Mbanga *et al*. (2020) underscores the accelerating degradation of major South African rivers, with the Olifants River cited as notably impacted by faecal contamination, closely followed by the Umgeni and Vaal Rivers (Marie and Lin, 2018). The compromised water quality not only imperils the livelihoods of communities reliant on river water but also impacts the health of aquatic ecosystems (Mbanga *et al*., 2020).

Land use activities, including agriculture, mining, and urbanization, exert significant pressure on river systems, as evidenced by the direct discharge of waste into water bodies (Pachepsky *et al.,* 2012). This multifaceted pollution, comprising faecal matter, heightened salinity, and toxic contaminants, underscores the gravity of the situation (Oberholster and Ashton, 2008). Given prevailing pollution trends and South Africa's expanding population, concerns arise regarding the sustainability of freshwater resources to meet escalating demands (Mbanga *et al*., 2020).

In response to the imperative of addressing river water pollution, South Africa has instituted several initiatives and policies aimed at enhancing water resource management and pollution control measures. Prominent among these are the National Microbial Monitoring Programme (NMMP), the Water Services Act 108 of 1997 and the National Water Act (Act 36 of 1998), serving as legislative frameworks for water resource governance (Edokpayi *et al*., 2020). Despite these legislative interventions, the persistent deterioration of river water quality persists as a formidable challenge, impeding the nation's capacity to ensure the provision of safe and adequate water resources (DWS, 2017).

Elevated concerns regarding river water quality underscore the necessity for robust monitoring mechanisms aimed at data collection, information dissemination, and informed decision-making. While efforts have been directed toward assessing and addressing river conditions, further emphasis is required to integrate water quality monitoring into comprehensive water resource management strategies. Closing the knowledge gap about the water quality status of diverse river systems across South Africa necessitates leveraging reliable and relevant time-series data acquired through water quality monitoring programs. This strategic approach is indispensable for informed policy formulation and sustainable water resource management.

## 1.2 Problem Statement

Over the years, the South African government has been proposing to solve the country's growing water pollution crisis. Local governments (i.e. municipalities) have formulated corresponding pollution control measures. The formulation of these measures should lead to a significant improvement in water quality standards in South Africa. However, reports by numerous studies have demonstrated that the country's major river systems are under severe pollution from various human activities such as wastewater treatment plants, effluents from mining, and agriculture runoff (Marie and Lin, 2018; Mbanga *et al.*, 2020). Additionally, Herbig. (2019) has shown that there has been a lack of implementation of pollution measures to reduce and mitigate pollution in major river systems. The current enforcement of pollution control is insufficient, water monitoring points are not fully covered, and the status of surface water quality is not published. Importantly, the government's ability to provide the country with safe drinking water has been questioned in recent years. Many communities across the country rely on rivers for a source of drinking water and communities have suffered from waterborne diseases such as cholera, dysentery, typhoid fever, and *Escherichia coli (E. coli)* infection (Pachepsky and Shelton, 2011). Considering the above, this research sought to investigate the microbial water quality status of South African major rivers. The findings of the study would provide a reference for future river water quality management strategies in South Africa.

### 1.3 Aims and Objectives.

The primary aim of this study is to assess faecal pollution trends in major South African river systems by analyzing historical data spanning a decade from 2010 to 2019. To fulfill this overarching goal, the study outlines the following specific objectives:

- To assess the temporal patterns of faecal contamination, specifically *Escherichia coli (E. coli),* within eight major river systems in South Africa. These rivers include the Limpopo River, Olifants River, Inkomati River, Mzimvubu River, Breede River, Berg Olifants River, Pongolo River, and Vaal River.
- Use one-way Analysis of Variance (ANOVA) to identify statistically significant disparities in mean *E. coli* concentrations across different years and among the specified rivers.
- Assess the impact of seasonal variations on *E. coli* contamination using Seasonal Mann-Kendall and Sen's slope analysis.

## 1.4 Structure of Study

Chapter One offers a comprehensive background to the study, delineating the pertinent issues influencing river water quality. It introduces the study's aims, objectives, and problem statement, setting the foundation for the subsequent analysis. Chapter Two delves into the selected study area, focusing on South Africa. This chapter provides a detailed description of the major rivers chosen for analysis, including their geographical locations and directional flow, alongside an examination of activities impacting their water quality. Chapter Three conducts a thorough review of existing literature, encompassing both global and local perspectives on river water quality. It investigates factors contributing to the degradation of water quality, highlighting the ramifications of waterborne diseases on human health and the environment. Additionally, this chapter outlines water quality guidelines and regulatory frameworks aimed at managing water resources. Chapter Four elucidates the methodologies employed to address the aims and objectives outlined in the research. It expounds on the acquisition and preparation of the dataset utilized for analysis. Chapter Five presents the findings derived from the analysis of the collected data. Chapter Six furnishes a detailed discourse on the results obtained. Chapter seven encapsulates the conclusion, summarizing the primary findings of the study while acknowledging its limitations and offering recommendations for future research.

# CHAPTER TWO: DESCRIPTION OF THE STUDY AREA

## 2.1 Study Area

South Africa, situated on the southern extremity of the African continent, is delineated by the Atlantic Ocean to the west and the Indian Ocean to the south and east. It shares land borders with Zimbabwe, Namibia, Botswana, Eswatini, and Mozambique, exhibiting a diverse topography that encompasses expansive savannahs across the central plateau, coastal stretches along the Indian and Atlantic Oceans, and rugged mountain ranges reaching altitudes of approximately 3050 meters above sea level (Lennard, 2019). Administratively, South Africa is divided into nine provinces: Mpumalanga, Gauteng, Limpopo, KwaZulu-Natal, Eastern Cape, Western Cape, Northern Cape, and North West (Palmer and Ainslie, 2002). Climatically, the country is classified into four distinct zones. The interior regions predominantly experience semi-arid conditions characterized by cold winters and hot summers with minimal precipitation. Conversely, the southwestern parts exhibit a Mediterranean climate typified by dry summers and wet winters (SAWS, 2018). Northeastern territories, such as Limpopo, Mpumalanga, and KwaZulu-Natal, feature subtropical climates marked by humid, sweltering summers and relatively mild, dry winters (Reason *et al*., 2006). Despite the diverse climatic patterns, the average annual rainfall across South Africa is approximately 450 mm, considerably lower than the global average of 860 mm per year. Moreover, there exists regional variation in rainfall distribution, with a discernible decrease from eastern to western regions. Consequently, the country grapples with scarcity and inequitable distribution of surface water resources, further compounded by limited freshwater availability (NDA, 2001).

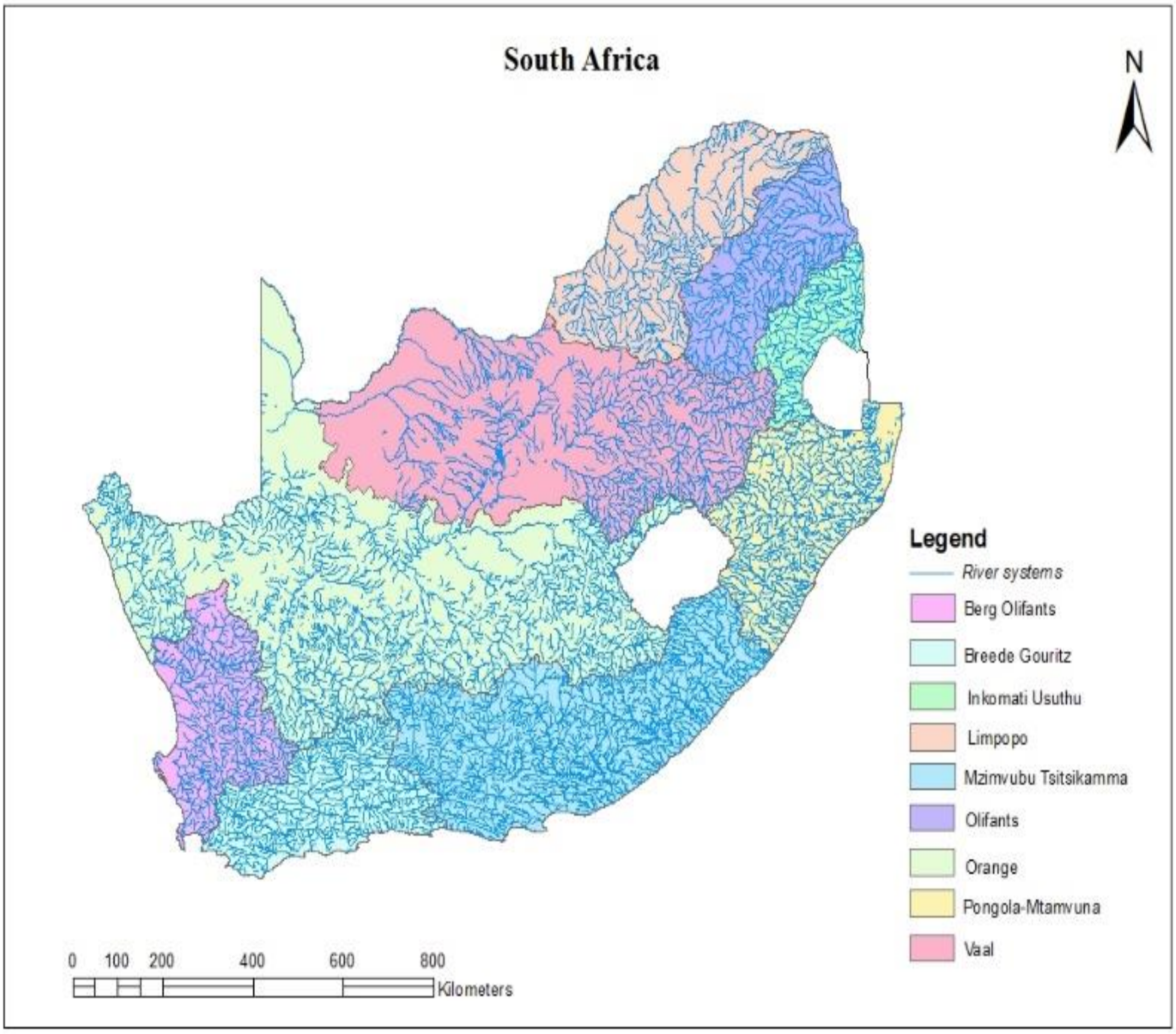



*Figure 1. The study area map showing the major South African river systems and their catchment areas.*

## 2.2 Description of major South African river systems.
### 2.2.1 Limpopo River

The Limpopo River is a transboundary waterway that separates South Africa, Mozambique, Zimbabwe, and Botswana (Mosase *et al*., 2019). The river rises in the Witwatersrand Mountain range near Polokwane and flows easterly then northeasterly thus forming a border between Botswana and South Africa. It then continues its course passing through Zimbabwe and Mozambique before draining into the Indian Ocean. The river covers an area of approximately 1750 km and its drainage basin covers an extensive area (Mosase *et al*., 2019). The Limpopo River and its surrounding areas are home to a range of wildlife including various species such as crocodiles and fish. Furthermore, the river is an important water source for agriculture and other livelihood activities supporting local communities. Unfortunately, anthropogenic activities such as mining and agriculture are a threat to the water quality and overall health of the ecosystem. Additionally, the river also faces challenges of water scarcity particularly during dry seasons due to drought (du Plessis, 2019).

### 2.2.2 Olifants River

The Olifants River, span an expansive area of approximately 850 km. Originating in the Mpumalanga province, it meanders northward from Witbank Dam before converging eastward within the precincts of Kruger National Park (Heath *et al*., 2010). Downstream of Loskop Dam, the river is augmented by the confluence of Elands and Moses rivers. Eventually, the Olifants River merges with the Limpopo River, culminating in its discharge into the Indian Ocean. It is noteworthy that the Olifants River has undergone extensive damming activities to cater to the water demands of smaller agricultural enterprises and mining operations (Nkhonjera, 2017). In terms of land use, agriculture assumes a pivotal role along the riverbanks, underlining its significance as an agricultural hub. However, despite its importance, the Olifants River faces a myriad of threats, rendering it one of the most imperilled river systems in the country. The degradation of its water quality can be attributed to a confluence of factors including agricultural practices, mining operations, and industrial activities (De Villiers and Mkwelo, 2009; Nkhonjera, 2017).

### 2.2.3 Inkomati River

The Inkomati River rises in the high plateau of Swaziland and Mpumalanga draining between Limpopo in the north and Pongolo in the south (Waalewijn *et al*., 2005; DWA, 2013). The river winds its way through the Lowveld of Mpumalanga and is fed by numerous tributaries in its course, including the Sabie River and Crocodile River. It is approximately 480 km long with 63% draining in South Africa and 5% draining in Eswatini (DWA, 2013). The river flows through the coastal region of Mozambique and enters the Indian Ocean in Maputo. This river is one of the most important rivers in the country because it is a destination for tourism. Moreover, it is an important water source for human settlements, mining, manufacturing industries and nature conservation. Reservoirs and dams have been constructed in this river for hydrological power generation, and irrigation. However, due to the increasing demand for water supply, the Inkomati River is facing various challenges including habitat destruction, over-extraction of water and water pollution (Saraiva Okello *et al*., 2015).

### 2.2.4 Mzimvubu River

The Mzimvubu River originates in the middle of rural Transkei in the Eastern Cape. It is approximately 200 km long and drained by steep tributaries rising from the eastern escarpment of the Drakensberg mountains (Madikizela and Dye, 2003). Along its course, the river is joined by four major tributaries (i.e. Kinira, Tina, Tsitsa and uMzintlava rivers). The river system is nationally prioritized because it is one of the few remaining near-natural rivers (Freshwater Health Index, 2022). Importantly, the river is also one of the less engineered rivers for example, it is uninterrupted by dams thus still maintaining its natural flow (Tau, 2013). The river finally discharges into the Indian Ocean in Port St Johns. There is a growing concern about the water quality in this water body due to various anthropogenic activities such as inappropriate location of rural settlements, overgrazing and land degradation (Freshwater Health Index, 2022).

### 2.2.5 Breede River

The Breede River, approximately 322 km long, originates from the Skurweberg mountains in the Western Cape (River Health Programme, 2004). The river drains between Infanta and Witsand estuary. Various tributaries join Breede in the upper reaches, the Holsloot, Wit, and Molenaars rivers and further joined by Hex River near Worcester. In the middle reach below the Greater Brandvlei Dam, the Breede River is then joined by Kingna River that confluence with the Riviersondered River. The lower reach of Breede is also joined by its main tributary, the Buffeljagsrivier and finally discharges into the Atlantic Ocean near the town of Infanta (Cullis *et al.*, 2018). The state of water quality in the headwaters of Breede River is good but becomes poor downstream because of irrigation return flows entering the river. Intensive urban and agricultural development pose serious water quality problems in this river. These include increased nutrient enrichment, salinity, and microbiological water quality (DWA, 2010)

### 2.2.6 Berg-Olifants River

The Berg-Olifants River, one of the most important rivers in the country has its upper reaches supplying the City of Cape Town with freshwater (Cullis *et al.*, 2019). The middle reaches also support regional towns for irrigation or agricultural fields as well as meeting the water demands in regional towns (Gorgens and de Clercq, 2005). The Berg River rises in the Groot Drakenstein mountains flowing northward through the lower reaches of Paarl, Gouda, and Wellington. It is approximately 300 km long and drains a catchment of 9000 km$^2$ (River Health Programme, 2004). Before reaching the sea, the Berg River is joined by the Klein Berg on the west coast of Helena Bay. About six major perennial tributaries including Wemmershoek and Franschhoek also join the Berg River. The increasing rate of informal settlements and urbanisation along the course of the river has resulted in major concerns regarding the impacted water quality from surface runoff (Cullis *et al*., 2019).

### 2.2.7 Pongolo River

The Pongolo River is an important watercourse and the tributary of the Maputo River. It originates from the northeastern part of KwaZulu-Natal near Utreicht and flows easterly where it is dammed at Pongolapoort (Jaganyi *et al*., 2009). The river covers approximately 245 km and crosses the Lebombo Mountains meandering through wetlands and hills (Birkhead *et al*., 2018). As it progresses through the region, numerous tributaries contribute to its flow. It then flows north towards Mozambique and joins the Maputo River (DWA, 2009). The Pongolo River is known for its rich biodiversity supporting a diverse array of animal species such as hippos, fish, and crocodiles. Not only does it support biodiversity, but it also plays a vital role in sustaining local communities by providing water resources for agriculture. Activities along the Pongolo River include cultivation in fertile areas along the riverbanks as well as livestock farming. However, like many other rivers, the Pongolo faces challenges such as habitat degradation, pollution and the impacts of climate change (Smit *et al*., 2016).

### 2.2.8 Vaal River

The second largest river in South Africa, the Vaal River commonly referred to as the hardworking horse is a valued river system as it provides water for Gauteng and the surrounding water management areas (DWAF, 2004). The river crosses several provinces namely Northern Cape, Free State, Mpumalanga, and Gauteng. It is about 1200 km long and starts in the eastern highveld plains in Mpumalanga then flows westward without waterfalls thus broadening into a larger river. The Vaal River is a major river but is still a tributary of the Orange River which has its confluence at Douglas in the Northern Cape (Wepener *et al*., 2011).

Many industries and communities rely on the Vaal River for water supply. However, over the years degradation and pollution from industries and metropolitan cities has caused serious water pollution (McCarthy *et al*., 2007). Microbiological pollution, salination and eutrophication are the major problems affecting the state of water quality in the river (du Plessis, 2019). Thus, this has resulted in the Vaal River being regarded as the most polluted river in the country (Jordaan and Benzuidenhout, 2013).

## 2.3 Justification of the study area

The selection of the study area for investigation is justified by the increasing evidence of faecal contamination in South African rivers as indicated by various reports (DWAF, 2010; DWS, 2017; Gqomfa *et al*., 2023). Traditionally, rivers have been perceived as naturally clean sources of water that require minimum treatment for human consumption. However, the burgeoning impact of anthropogenic activities such as agricultural runoff, urbanization and sewage discharges has led to significant altercations in the quality of river water. These changes have repercussions on microbial dynamics within these aquatic ecosystems. Moreover, the expansion of human settlements in South African catchment areas, as documented in previous studies (Potgieter *et al*., 2020; Gqomfa *et al.,* 2023), has further escalated the risk of faecal contamination in rivers. This traditional threat compounds the existing challenges posed by pollutants like fertilizers and livestock runoff. Consequently, there is an urgent need for an in-depth analysis of microbial data to discern the patterns and trends of faecal contamination across major river systems in South Africa. Such analysis would establish a foundational framework for identifying highly contaminated areas, thus facilitating informed resource allocation and ensuring regulatory adherence.

# CHAPTER THREE: OVERVIEW OF LITERATURE

## 3.1 River water quality issues: a global perspective.

River water quality issues are a global concern in the 21st century due to the significant impact of urbanization and industrialization on ecological environments (Goto and Yang, 2011). The rapid economic development witnessed worldwide has led to a surge in domestic sewage and industrial wastewater discharge, resulting in heightened pollution loads within river systems (Hasan *et al*., 2019). Particularly affected are rivers traversing urban areas which often bear the heavy burden of faecal contamination, posing increased infection risks to humans through the introduction of various pathogens (Goto and Yang, 2011; Paule-Mercado *et al*., 2016). Such faecal contamination not only threatens the suitability of river water for domestic use but also raises concerns about its broader ecological implications (Ercumen *et al*., 2017).

To elucidate the dynamics of river water quality, studies have been conducted in various regions such as the Fuji River basin in Japan (Lee *et al*., 2001; Adams *et al*., 2001; Simeonova *et al*., 2003) and the Phongolo River in South Africa (de Necke *et al*., 2020). In the Fuji River, an extensive dataset spanning eight years (1995-2002) was analyzed. Employing multivariate statistical techniques like cluster analysis (CA), factor analysis (FA), and principal component analysis (PCA), researchers aimed to discern spatial and temporal variations resulting from both natural processes and human activities (Shrestha and Kazama, 2007). Through CA, distinct pollution gradients were identified, delineating areas into less polluted, moderately polluted and highly polluted regions, each influenced by specific sources of contamination. In less polluted areas, factors such as domestic waste and soil erosion, exacerbated by seasonal variations, played a significant role in faecal concentration. Conversely, in moderately polluted regions, agricultural activities and seasonal fluctuations were implicated, while highly polluted areas were characterized by industrial effluents and wastewater from treatment plants (Lee *et* *al*., 2001; Adams *et al*., 2001; Simeonova *et al*. 2003). Such analysis provides important insights into the complex interplay between anthropogenic activities and river water quality. In the Pongolo River, de Necke *et al*. (2020) assessed the long-term water quality of the middle and lower Phongolo River and determined the trends and potential changes in water quality variables from the 1970 to 2017. Using multivariate statistical analyses and Physicochemical Assessment Index (PAI), the study revealed that water quality has degraded in the middle and lowe Phongolo River due to nutrient inputs and increased salinity from surrounding irrigation activities.

In India, there has been a concerning trend of deterioration observed in major river systems over time. Murugan *et al*. (2012) conducted a study focusing on the Tirumanimulta River, revealing it to be among the most severely polluted rivers in the country. The investigation highlighted multiple sources of faecal contamination contributing to the compromised water quality, including effluent discharge from healthcare facilities and eateries, as well as direct input of agricultural runoff into the river system. The study underscored a rapid escalation in faecal coliform levels over the years, surpassing recommended thresholds. Similarly, Skariyachan *et al*. (2015) conducted research assessing the faecal status of the Cauvery River from 2011 to 2012. The Cauvery River serves as a vital source of drinking water for nearby urban and rural settlements in Karnataka, India. However, it faces contamination from industrial discharge along its banks and sewage effluents from healthcare facilities. The accumulation of domestic and industrial waste has fostered the proliferation of pathogenic microorganisms, leading to the spread of waterborne diseases such as typhoid fever and cholera (Skariyachan *et al.*, 2015).

In Lebanon, Dagher *et al*. (2021) evaluated the water quality of major rivers by quantifying faecal indicator bacteria densities, specifically *Escherichia coli* and faecal coliforms. Their findings indicated high levels of contamination, with 96.2% and 95.5% of river water samples contaminated with *E. coli* and faecal coliforms, respectively. Particularly elevated contamination levels were observed in the midstream sections of the Ortwein and Beirut rivers, and downstream of the Bared and Kabir rivers. These rivers traverse areas characterized by densely populated refugee camps lacking proper sanitation infrastructure, leading to heightened human activities impacting water quality. Notably, samples collected from upstream locations exhibited lower levels of *E. coli* and faecal coliforms compared to midstream and downstream samples, indicating relatively reduced faecal pollution owing to diminished human activities in upstream areas (Dagher *et al*., 2021).

In South America, the ongoing illegal disposal of sewage into nearby river systems is a major concern (Voloshenko-Rossin *et al.,* 2015). This continuous discharge of effluents causes microbial proliferation and contamination of rivers across the region. In 2015, Voloshenko-Rossin *et al. (*2015) assessed the organic pollutants in the San Pedro Guay in Ecuador, while Lambamba *et al.* (2020) evaluated the microbial status of 18 river systems in the region of Quito. Their findings showed that major rivers in Ecuador were considered the most contaminated with faecal matter (Voloshenko-Rossin *et al.,* 2015; Lambamba *et al.,* 2020). Furthermore, Vinueza *et al.* (2021) also carried the research in which their study analysed the water quality in 12 major rivers across the Amazonia, Coastal and Andean regions. The water quality was analysed through the bacteriological indicators (i.e. total coliforms and *Escherichia coli*). The author's findings showed that *E. coli* and total coliform counts were above the concentrations recommended by the Brazilian and Ecuador guidelines. With Andean and Coastal River systems are considered to be the most contaminated while the Amazonia River system showed lower counts of faecal pollution. Similarly, the contamination of river water was also caused by a lack of wastewater treatment plants which is a big challenge in Ecuador. This is further exacerbated by overpopulation in urban areas with no adequate sanitation infrastructure (Vinueza *et al*., 2021).

Like in many other continents, faecal pollution poses a significant threat to African river systems. Due to the continent's rapid urbanization and inadequate waste management, many rivers have been widely contaminated with faecal matter (Hansen, 2015; Nansubuga *et al*., 2016). Many countries in Africa have limited access to sanitation facilities, as a result, this has led to open defecation and release of human waste into rivers. Furthermore, the rapid urbanization across major cities has put a strain on the existing sanitation systems thus causing insufficient sewage treatment (Hansen, 2015).

In Egypt, the Nile River is the main source of water for humans and animal usage. Unfortunately, its water quality has been deteriorating over the years because of heavy loads from industries, and agricultural and domestic waste (Al-Afify *et al*., 2018). El-Khayat *et al*. (2021) conducted bacteriological and chemical pollution in the Nile River in the Great Cairo region in Egypt from 2019 to 2020. Their study found that all samples from the sites were biologically contaminated with coliform bacteria while chemical pollution was reported to be within the maximum permissible levels. Their research demonstrated that the Nile River is polluted. Similar studies were also undertaken by reporting the evaluation of bacteriological quality in the Nile River at the Damietta branch. They found that maximum counts of faecal coliforms were recorded during summer while winter showed low counts. They also found that there was an increase in faecal indicator counts from upstream to downstream. Elhdad. (2019) stressed that the presence of faecal constituents in the Nile River is due to direct contamination caused by human activities. For example, people dispose of their domestic and agricultural waste into the river.

Nigeria is grappling with the challenges of faecal pollution within its river systems, presenting a grave threat to both public health and the environment (Abdulkadir *et al.,* 2020). The proliferation of faecal pollution in Nigeria can be attributed to various factors. A significant portion of Nigeria's population lacks access to adequate sanitation facilities, and numerous cities and towns lack functional wastewater treatment facilities (WWTFs), leading to the direct discharge of untreated sewage into waterways (Eyankware and Ephraim, 2021). Numerous scholarly studies have documented alarming levels of faecal contamination in Nigeria's major rivers (Fawole *et al.,* 2011; Olufeagba *et al.,* 2012; Abdulkadir *et al.,* 2020). Fawole *et al.*, (2011) investigated the influence of seasonal variations on the water quality of the Ogun River at Abeokuta, a critical water source serving millions of people in Lagos and Ogun States. Their findings revealed elevated levels of faecal coliforms during rainy seasons, attributed to increased runoff. Similarly, Olufeagba *et al.* (2012) evaluated the bacteriological quality of the Ogun River, finding that faecal coliform counts surpassed both the World Health Organization (WHO) and Nigeria's water quality standards. These findings were corroborated by Akindele *et al.* (2018), who observed a persistent rise in faecal contamination levels in the Ogun River. Further investigations were conducted on the Niger River, Nigeria's largest river. Okonkwo. (2012) noted a significant increase in faecal contamination over time, exceeding recommended limits in Nigeria. Abdulkadir *et al.* (2020) similarly documented heightened levels of faecal coliforms and other pollutants in the Niger River, attributing this increase to inadequate sanitation facilities.

Like many other developing countries, the Democratic Republic of Congo (DRC) has also shown extremely elevated levels of faecal pollution in many of its urban rivers. The study by Kilunga *et al*. (2016) assessed the impact of hospital and urban wastewater on bacteriological contamination in Kinshasa urban rivers. The authors quantified faecal indicator bacteria (FIB) including *Enterococcus (ENT)* and *E. coli* from 2014 to 2015. The results suggested that effluents without pretreatment from a hospital showed significantly increased levels of *ENT* and *E. coli.* The situation was exacerbated by multiple diffuse pollution sources such as uncontrolled landfills, and unregulated discharge of effluents and excreta into the urban environment of Kinshasa. Further studies were undertaken in Bukavu City by Bisimwa *et al.*

(2022). The scholars determined the occurrence of FIB in the Bukavu urban rivers as well as assessed the effects of physicochemical variables fluctuations on the abundance of FIB from 2017 to 2019. The results indicate that Bukavu urban rivers like Kinshasa posed prominent levels of faecal contamination, and the FIB abundance varied significantly from upstream to downstream across the rivers (Bisimwa *et al.,* 2022).

**3.2 River water quality issues: a local perspective.**

The degradation of river water quality presents significant challenges at the local level, particularly in South Africa, where numerous studies have underscored the deterioration of rivers (Potgieter *et al*., 2020; Makuwa *et al*., 2020; Phungela *et al*., 2022). The Olifants River, Vaal River and Tugela River have been identified as among the most highly polluted river systems in the country (Sibanda *et al*., 2015). Faecal contamination stemming from both point and non-point sources stands out as a major concern. Point sources, including untreated sewage and industrial discharges, contribute substantial amounts of pathogens and organic matter into rivers (Sibanda *et al*., 2015). Inadequately maintained sewage systems exacerbate the problem, with leaks, blockages, and overflows releasing raw sewage directly into water bodies (CSIR, 2010; Green Drop Report, 2022).

Non-point sources of faecal contamination, comprising diffuse inputs from various activities such as human settlements, agriculture, livestock and wildlife pose significant challenges in control and quantification (Holcomb and Stewart, 2020). Agricultural practices, especially improper handling of livestock waste, can introduce pathogens into rivers through runoff (Jose-Figueras and Borrengo, 2010). Improper management of animal waste, compounded by ineffective use of manure as fertilizers, further exacerbates faecal contamination when washed into the rivers by rainwater (Abia *et al*., 2015a).

### 3.3 Overview of microbial water quality research in South African rivers.

Research on microbial water quality in South African rivers underscores important concerns regarding contamination and its implications on public health and environmental integrity. With approximately 77% of South Africa's water sourced from rivers, the quality of this vital resource has paramount importance (Verster and Bouwman, 2020). Pathogens introduced into river systems through faecal contamination pose a substantial risk, with findings from studies highlighting elevated pollution levels in various regions. For example, in the Eastern Cape, the Keiskammahoek River exhibited heightened faecal pollution, primarily attributed to sewage treatment plant discharges (Fatoki *et al*., 2003; Okoh and Sibanda, 2015). Similarly, preliminary assessments of the Chunes River in Limpopo revealed poor water quality due to faecal contamination, rendering it unsuitable for domestic use (Germs *et al.*, 2004).

Assessment Programs, such as the South African River Health Programme (RHP), utilize *E. coli* as an indicator of faecal contamination. Results from monitoring efforts indicated alarming levels of contamination in various rivers across different provinces. For example, the Palmeit and Umgeni Rivers in KwaZulu-Natal recorded *E. coli* counts exceeding 1 million colony-forming units per 100mL (cfu/100mL), while the Mayville and Bellair streams exhibited levels above 100,000 cfu/100mL due to poor sanitation infrastructure (Bezuidenhout *et al*., 2002). Studies further highlighted severe pollution incidents, such as the Plankenburg River in the Western Cape Province, where *E. coli* levels reached exceptionally high concentrations of 12 million cfu/100mL (Barnes and Tylor, 2004; Alegbeleye *et al*., 2016). The Berg River also faced contamination with faecal counts ranging from 36 to 17 million cfu/100mL, attributed to sewage spills and organic waste from nearby informal settlements (Paulse *et al*., 2007, 2009; Cullis *et al*., 2019).

These findings underscore the urgent need for effective management strategies to mitigate faecal contamination in South African rivers. Failure in municipality wastewater treatment works (WWTWs) and challenges posed by informal settlements emerged as significant contributors to river pollution. Addressing these issues is imperative to safeguard human health and preserve the ecological integrity of South Africa's River systems.

## 3.4 Environmental and Health implications of faecal pollution

### 3.4.1 Environmental implications

Contamination of water bodies due to the introduction of faecal matter into the environment is one of the primary environmental concerns (Sibanda *et al*., 2015). When untreated or partially treated sewage enters rivers, it introduces harmful pathogens such as *E. coli*. This bacterium poses significant threats to the ecosystems and can have long-lasting effects that affect terrestrial and aquatic environments (Adefisoye and Okoh, 2017). Contaminated water with faecal matter can have severe impacts on amphibians, fish, and other aquatic organisms due to exposure to harmful strains of *E. coli* thus leading to the disruptions in the food chain. Additionally, *E. coli* can survive in water for prolonged periods thus leading to widespread diseases if the water is consumed. Importantly, this bacterium when found in natural habitats can serve as an indicator of ecosystem stress (Edokpayi *et al*., 2017). Various activities such as agriculture practices are one of the major contributors to the environmental presence of faecal matter. For example, agriculture runoff from farms carrying animal waste can introduce *E. coli* into rivers. Consequently, using contaminated water for irrigation can contribute to the spread of this bacterium to crops, leading to foodborne disease (DWAF, 1996; WHO, 2011). Furthermore, the use of antibiotics in veterinary medicine also contributed to the emergence of antibiotic-resistant strains of *E. coli*. These strains persistent in the environment can lead to a potential transfer of resistance genes to other bacteria. As a result, this makes it difficult to treat and poses a threat to both the environment and human health (Adefisoye and Okoh, 2017).

### 3.4.2 Human Health Implications.

The health implications of drinking water contamination vary significantly between developing and industrialized nations. In developed countries, emphasis is placed on maintaining water supply infrastructure and monitoring faecal contamination in river streams to ensure water safety (Li *et al*., 2021). Conversely, in developing nations, many river streams remain exposed to untreated sewage discharge (WHO, 2008; Li *et al*., 2021). This lack of sanitation infrastructure contributes to the staggering statistic that over 785 million people worldwide still lack access to proper sanitation, with a pronounced disparity between urban and rural areas (Liu and Bridget, 2020). Consequently, over 1.6 million deaths per year are attributed to the consumption of contaminated water, with children being disproportionately affected, as evidenced by the fact that 50% of childhood deaths occur in the developing world (Juryl and Vaux Jr, 2007; Majeed and Munir, 2020). The primary health risks associated with drinking contaminated river water in developing nations stem from bacteria, viruses, and protozoa transmitted through faecal contamination (WHO, 2008; Li *et al*., 2021).

According to reports from the World Health Organization (WHO), the period from 1998 to 2001 witnessed several infectious disease outbreaks, with cholera emerging as the most prevalent disease (WHO, 2002; Ashbolt, 2004). Research conducted in Bangladesh revealed alarming rates of infection among children, with 75% found to be infected with *Vibrio cholera*, *Escherichia coli*, and *Cryptosporidium* (Albert *et al.*, 1999). In North America, gastrointestinal diseases attributed to microbial contamination of water contribute to 15-30 per cent of deaths, underscoring the global impact of waterborne pathogens on public health (Payment *et al*., 1997; Tauxe, 2002). These findings underscore the urgent need for improved water sanitation infrastructure and comprehensive public health measures to mitigate the adverse health effects associated with drinking water contamination, particularly in regions with inadequate sanitation facilities.

### 3.5 The significance of microbiological indicators

To assess the quality of river water, microbiological indicators are a useful tool used to detect the presence of microorganisms, particularly bacteria. The indicators are widely used in various water quality monitoring programs due to their cost-effectiveness and are simple to measure the presence of pathogens that may result in waterborne diseases. Commonly used microbial indicators include Total Coliform (TC) and *Escherichia coli*

#### 3.5.1 *Escherichia coli.*

*Escherichia coli* commonly known as *E. coli* is also a type of coliform bacteria that is used to determine faecal pollution in water sources. These bacteria can be found in animal faeces as well as human digestive systems. They are not considered pathogenic, however, since they are found within pathogenic organisms, their presence is used as an indicator of harmful organisms in water sources. Therefore, *E. coli* must not be detected in water used for human consumption (Edberg *et al*., 2000).

#### 3.5.2 Total Coliforms

Total Coliforms (TC) are the bacteria that produce colonies and are found in the intestines of warm-blooded animals (DWAF, 1996). These bacteria include faecal and other bacteria that are found in non-faecal sources and the soil (USEPA, 2001). Total Coliform bacteria are generally used as a practical indicator of hygienic water quality. They are essential for monitoring purposes such as domestic and recreational use. In South Africa, the concentration of TC should remain below 5 counts per 100 m/L for water consumption (DWAF, 1996).

### 3.6 Water Quality Management Framework

#### 3.6.1 Regulatory framework

The conceptualization of water governance has undergone significant evolution, recognizing that challenges associated with water transcend national borders. Consequently, addressing these challenges necessitates a collaborative approach, as water issues extend beyond territorial confines (Cooley *et al.*, 2013). The management of transboundary rivers exemplifies such complexities, exemplified by rivers like the Limpopo, traversing multiple countries such as Zimbabwe, Botswana, and Mozambique. The management of these shared water bodies poses considerable challenges owing to the diverse water quality concerns, obligations, and national priorities of involved nations (UN-WWAP, 2012).

Following 1994, South Africa initiated a series of reforms in its water policies and institutions in response to the escalating water crisis and the recognition of citizens' rights to access clean and safe water. Central to this overhaul was the enactment of the National Water Act (NWA) (No. 36 of 1998), serving as the cornerstone legislation for the governance of the nation's water resources. Widely regarded as pioneering legislation globally, the NWA delineates strategies for the sustainable utilization and management of water resources within the country (Rossouw *et al*., 2005). Its primary objective lies in the facilitation of equitable and responsible water resource management nationwide.

Moreover, the NWA emphasizes the imperative of safeguarding the country's water resources, emphasizing their judicious use, management, control, and conservation. Recognizing the socio-economic imperatives, the Act also underscores the necessity of distinct ecological protection of rivers (Nomquphu *et al*., 2007). To operationalize these principles, several measures must be implemented to ensure river protection:

- Classification of rivers in accordance with the National Water Resource Classification System (NWRCS) to assign a management class to each river.
- Definition of water quality objectives for rivers to uphold their designated class.
- Assessment of water quality to meet both human consumption and ecological needs.

### 3.7 Water Quality Guidelines and Standards

#### 3.7.1 World Health Organization drinking water guidelines.

The water quality guidelines are an important aspect used to set limits or thresholds for water consumption to protect water users and water resources (WHO, 2011). They are used for developing and implementing water-related policies. Moreover, the guidelines and regulations are also used to stipulate the acceptable levels of particular water quality parameters (i.e. *E. coli* counts) as well as the suitability of specific uses (UNEP, 2016). When the limits have been exceeded it is an indication of environmental stressors and therefore, used to assess the quality of water.

The World Health Organization (WHO) water quality guidelines are used as the international reference points for water quality criteria. The criteria are based on various determinants that are used to characterize the quality of water. For example, there is a maximum level for the concentration of *E. coli* and other microbial determinants allowed in the water and would not cause any harm when the water is consumed continuously. However, there are determinants that have a minimum level allowed to ensure the sustenance and maintenance of ecological functions, for example, dissolved oxygen (DO). Although the WHO is used as a reference point, countries have the sovereign right to make their water quality guidelines and regulations (WHO, 2011).

### 3.7.2 Department of Water and Sanitation Water Quality Guidelines

South Africa like many other nations has established guidelines and regulations to inform quality requirements for water for various water purposes such as drinking water, protection of the aquatic system and recreation (DWA, 2013). The Department of Water and Sanitation (DWS) is primarily responsible for developing and implementing the water quality guidelines These guidelines are categorized in accordance with the water use for example, domestic, agricultural recreational and aquatic ecosystems (DWA, 2012). From these categories, some standards determine the fitness of use such as "ideal" which indicates that the water is 100% fit to be used, there is also "acceptable or tolerable" which means the water can be used for a limited period and lastly is the "completely unfit for use" which indicate the water cannot be used. In South Africa, the "ideal" fitness standard is the most desirable water quality target by the DWS (NWRS, 2004; CSIR, 2010; DWA, 2013) The DWS water quality standards are presented in Table 1 showing microbiological levels.

**Table 1. Water quality standards for microbiological indicators from WHO, SANS 241 and DWS.**

| Guideline/Standard Determinants | Unit | WHO | SANS 241 | DWS Domestic | DWS Agriculture | DWS Livestock & Watering | Aquatic ecosystems |
|---|---|---|---|---|---|---|---|
| **Microbiological determinants** | | | | | | | |
| **Faecal coliform** | Cfu/100mL | 0 | 0 | 0 | 0-10000 | NA | NA |
| ***E. coli*** | Cfu/100mL | 0.0 | 0.0 | NA | 0-10 | 0-200.0 | NA |

NA= Not Available

### 3.7.3 Discharge limits of effluents into rivers.

Under the National Water Act (No. 36 of 1998), it is legally required in South Africa for all treated effluent to be discharged into public water bodies or the ocean (DWA, 2015). Before this effluent can be released, it must undergo a final disinfection process to partially eliminate disease-causing organisms. The effectiveness of disinfection, typically achieved through the use of chlorine, depends on factors like disinfectant concentration, contact time, and the properties of the wastewater (Oweseni *et al*., 2017). Currently, most wastewater treatment facilities in South Africa use chlorine or chlorine compounds for disinfection (Dungeni *et al*., 2010). The effluents discharged into rivers must meet regulatory standards, ensuring that they contain no more than 0.25 mg/L of free chlorine residual, with a more lenient limit of 0.1 mg/ℓ in some cases. These levels are necessary to reduce faecal coliform bacteria in the effluent to the required standard of zero cfu/100 mL, or to a special limit of 1,000 cfu/100 mL (DWA, 2013).

## 3.8 Conclusion.

The literature review has provided a comprehensive analysis of the existing body of research regarding faecal pollution in rivers. Faecal pollution is not only a pressing issue on a local scale but, it is a global challenge affecting both developed and developing countries. The literature has highlighted the presence of contaminants of faecal origin in rivers, indicating the urgent need for effective river water quality management strategies. The findings from numerous studies have shown the pervasive nature of faecal contamination in South African rivers, caused by various environmental factors and human activities. Industrialization, inadequate sanitation infrastructure, agricultural runoff, urbanization and informal settlements emerged as primary drivers of faecal pollution in rivers. Furthermore, faecal pollution presents substantial and multifaced challenges to both human and river ecosystem health. Elevated levels of faecal indicator bacteria such as *E. coli* pose significant risks to communities that rely on the rivers for domestic purposes. Addressing these multifaced issues needs a holistic approach involving different stakeholders and sectors. Although there is progress in understanding and mitigating faecal pollution in South African rivers, there is a knowledge gap and future research priorities. Longitudinal research that tracks spatial and temporal trends in *E. coli* pollution levels in South African major rivers. Many of the studies have only prioritized small tributaries that flow in urban areas. There must be interdisciplinary research to integrate the socio-economic contributors of faecal pollution. Moreover, there is an urgent need for knowledge exchange platforms and stakeholder collaboration to promote a culture of collective action and shared responsibility towards river water quality management. By encouraging community participation and leveraging scientific expertise, it is possible to mitigate faecal pollution in South Africa.

# CHAPTER FOUR: METHODOLOGY

## 4.1 Data Acquisition

To accomplish the goals and objectives outlined in the study, secondary data about the faecal concentration of *E. coli* was obtained from the National Microbial Monitoring Programme (NMMP). This initiative was established by the Department of Water and Sanitation (DWS) in South Africa to centralize microbial water quality data collection across the country. Under this programme, the DWS oversees the collection and analysis of water samples at prioritized sampling sites within river systems, with all resulting samples and data archived within the Central Water Quality (CWQ) database. Detection of faecal concentrations at prioritized sampling sites was facilitated through the utilization of the Colilert 18 system, which offers NMMP coordinators quantitative insights into faecal contamination levels following an 18-hour incubation period (DWS, 2017). Notably, the NMMP maintains a centralized repository of microbial water quality data, with public access to this information. For this study, microbial water quality data spanning from January 2010 to December 2019 was obtained for each respective river. *E. coli* was employed as the microbial indicator for faecal pollution within the specified rivers, constituting the primary microbiological parameter of interest.

### 4.1.1 Data Analysis

In this study, two distinct statistical analyses were performed utilizing IBM SPSS version 28.0.1.1, GraphPad Prism version 10.1.2, and XLSTAT version 2021.4. Firstly, univariate statistical analysis, Analysis of Variance (ANOVA), was executed to ascertain whether there existed statistically significant disparities in mean levels of *E. coli* across the years spanning from 2010 to 2019, as well as among different rivers. The null hypothesis (H0) posited by ANOVA suggests no statistical difference across the years, while the alternative hypothesis (Ha) postulates that at least one of the years exhibits a statistically distinct mean from the overall mean across the years (Kim, 2014). After detecting significant differences, Tukey's post hoc test was applied to pinpoint specific years and rivers manifesting noteworthy discrepancies at a probability threshold of p-value <0.05. Conversely, if the p-value surpasses 0.05, it implies the absence of significant disparities between variables at a 95% confidence interval (Kim, 2014).

Additionally, a Seasonal Mann-Kendall test was conducted to evaluate the monotonic (either increasing or decreasing) trend within the dataset throughout the designated study period (Hashim *et al*., 2021). The Mann-Kendall test shows the significance level, which is determined by the probability value p-value of 0.05, (Chen *et al*., 2022).

- If p-value <0.05, there is evidence of a monotonic (increasing or decreasing) trend.
- If p-value >0.05, there is no significant trend over time.

Upon identifying the trend, Sen's slope is subsequently employed to discern its direction, thereby determining whether the trend is ascending or descending. A positive Sen's slope signifies an increasing trend, whereas a negative slope value indicates a declining trend (Hashim *et al*., 2021).

Descriptive statistics was also performed to provide a summary of the dataset. This helps to gain a better understanding of patterns and distributions within the dataset. The summary of the data includes measures such as central tendency (mean) data dispersion (minimum and maximum) standard deviation and range (Mishra *et al*., 2019).

At this point, it should be noted that the results of one-way ANOVA and Mann-Kendall analysis are applied with great caution. This is due to the data being characterized by non-normality and violating the assumptions required for temporal trend analysis. Hirsch *et al*, (1991) stated that when assumptions are required for temporal trends analysis, the method tends to lose its power to detect trends. As a result, it was paramount to perform logarithmic transformation commonly known as (log transformation) on the raw dataset. This is a widely accepted method in water quality studies used to reduce skewness and enable valid results (Mustapha *et al*., 2012). For this reason, the dataset used in this study was log-transformed to base 10 (log10). This method is commonly used to compress the y-axis when plotting graphs. The data used in this study comprised of data points with an enormous range from zero to hundreds of millions, thus smaller values get overwhelmed by larger values. Therefore, log transformation helps to make the visualization of the data clearer and easier to interpret.

# CHAPTER FIVE: RESULTS

## 5.1 Introduction

Statistical analyses were conducted in this study to identify faecal pollution trends in the specified South African rivers. This chapter is a presentation of the results from One-way ANOVA and the Seasonal Mann-Kendall test on *E. coli* over the study period. The one-way ANOVA results for the comparison of means between years and the comparison of means between rivers are present in the Appendices. The Mann-Kendal Statistics and respective p values (probability), the Sen's slope and its respective p values are presented in Table 10. The descriptive statistics of each river are presented in Tables 2 to 9. Plots from the ANOVA analysis are presented in Figures 2 to 5 and plots from the Mann-Kendall are presented in Figures 6 to 13.

## 5.2 Analysis of *E. coli* in the rivers.

### 5.2.1 Limpopo River.

In the Limpopo River (Table 2), it was observed that in most years, the minimum *E. coli* levels recorded was 0, suggesting that there were instances of undetectable levels of contamination. However, in 2014, the minimum value was 681 cfu/100mL, indicating some minimal contamination event. Furthermore, the maximum *E. coli* levels varied considerably across the years, ranging from 1,728 cfu/100mL in 2012 to an exceptionally high level of 7,283,933 cfu/100mL in 2018. This shows considerable variability and potentially worsening contamination over the years. The range of *E. coli* also varied from year to year, indicating fluctuations in contamination over time. In 2010, the range was exceptionally high at 980,210 cfu/100mL, while in 2012 it was much lower at 1,728 cfu/100mL. The mean *E. coli* levels ranged from 531.3 cfu/100mL in 2012 to 2,881,839 cfu/100mL in 2018. This indicates that on average, *E. coli* contamination has been increasing exponentially over the years. Higher standard deviation was observed in 2010 and 2018, indicating that there was great variability in *E. coli* levels within those years, suggesting inconsistent levels of *E. coli.*

**Table 2. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Limpopo River spanning from 2010 to 2018. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 0 | 980210 | 980210 | 82937 | 282572 |
| 2011 | 0 | 300160 | 300160 | 39702 | 85156 |
| 2012 | 0 | 1728 | 1728 | 531,3 | 728,1 |
| 2013 | 0 | 4898 | 4898 | 960,9 | 1590 |
| 2014 | 681 | 115656 | 114975 | 12593 | 32490 |
| 2015 | 0 | 8240 | 8240 | 1877 | 2561 |
| 2016 | 0 | 1288161 | 1288161 | 109525 | 371241 |
| 2017 | 0 | 24958 | 24958 | 2763 | 7170 |
| 2018 | 0 | 7283933 | 7283933 | 2881839 | 2885764 |

### 5.2.2 Olifants River.

In the Olifants River (Table 3), minimum *E. coli* concentration fluctuated across the years, with some years having undetected contamination (2016, 2017 and 2018) and others having increased minimum levels (2010, 2011, 2012, 2013, 2014 and 2015). The maximum *E. coli* concentration varied from year to year, ranging from a few thousand to several million cfu per 100 millilitres. This variability is confirmed by a wide range within each year. Some years exhibited a wide range (i.e. 2011, 2013, 2014, 2016, 2017 and 2018), while others have a narrower range (i.e. 2010, 2012 and 2015). Conversely, mean *E. coli* levels also varied considerably ranging from 1,716 cfu/100mL in 2012 to 1,326,511 cfu/100mL in 2016. Higher standard deviation was also noted in several years like 2011, 2013, 2016 and 2017 which suggested fluctuations in *E. coli* levels while other years had lower standard deviations indicating more consistent *E. coli* pollution.

**Table 2. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Olifants River spanning from 2010 to 2018. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 13 | 7426 | 7413 | 2945 | 2535 |
| 2011 | 301 | 600388 | 600087 | 168326 | 198643 |
| 2012 | 20 | 16194 | 16174 | 1716 | 4575 |
| 2013 | 198722 | 4752449 | 4553727 | 1007041 | 1328398 |
| 2014 | 2424 | 3660834 | 3658410 | 554427 | 1068953 |
| 2015 | 44 | 3076558 | 3076514 | 502251 | 952750 |
| 2016 | 0 | 4898903 | 4898903 | 1326511 | 1509008 |
| 2017 | 0 | 3873556 | 3873556 | 1184191 | 1246492 |
| 2018 | 0 | 3448000 | 3448000 | 737713 | 1142644 |

### 5.2.3 Inkomati River.

The analysis revealed notable variations in *E. coli* concentrations over the study period in the Inkomati River (Table 4). The minimum *E. coli* levels ranged from 0 in different years (2011, 2014, 2017, 2018 and 2019) while other years (2010, 2012, 2015 and 2016) detected levels ranging from 4 cfu/100mL to 275 cfu/100mL. Maximum *E. coli* concentrations ranged from 3,312 cfu/100mL in 2019 to 49,390 cfu/100mL in 2015. Wide ranges, indicative of substantial variability within each year, were observed, ranging from 21,710 cfu/100mL in 2010 and 49,265 cfu/100mL in 2015. Mean *E. coli* concentrations fluctuated from 639.1 cfu/100mL in 2019 to 8,271 cfu/100mL in 2015, with standard deviations ranging from 775.1 cfu/100mL in 2018 to 15,263 cfu/100mL in 2015. Years with particularly high maximum values and wide ranges, such as 2010, 2015 and 2016 might be of particular concern as they indicated instances of potentially high contamination levels. Conversely, years with high standard deviations such as 2015 suggest periods of high *E. coli* variability.

**Table 3. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Inkomati River spanning from 2010 to 2019. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 275 | 21985 | 21710 | 5364 | 6384 |
| 2011 | 0 | 5807 | 5807 | 2282 | 1698 |
| 2012 | 3 | 7978 | 7975 | 3133 | 2992 |
| 2013 | 0 | 12709 | 12709 | 1791 | 3507 |
| 2014 | 0 | 16780 | 16780 | 1729 | 4779 |
| 2015 | 125 | 49390 | 49265 | 8271 | 15263 |
| 2016 | 4 | 13948 | 13944 | 2421 | 4069 |
| 2017 | 0 | 11865 | 11865 | 1760 | 3342 |
| 2018 | 0 | 2400 | 2400 | 741,1 | 775,1 |
| 2019 | 0 | 3312 | 3312 | 639,1 | 909,4 |

### 5.2.4 Mzimvubu River.

The analysis of *E. coli* concentrations in the Mzimvubu River from 2010 to 2017 revealed notable patterns and fluctuations in showing varying pollution levels over time (Table 5). Examination of minimum and maximum concentrations illustrated significant variability, minimum concentration consistently remained at 0 cfu/100mL for most years except for sporadic instances in 2011 and 2012. Conversely, the maximum concentration ranged from 740,670 cfu/100mL in 2011 to 1,540 cfu/100mL in 2017, suggesting pronounced fluctuations in pollution levels across the study period. The range also demonstrated considerable variability from year to year, underscoring the dynamic nature of pollution levels throughout the study period. On the other hand, mean *E. coli* concentrations revealed substantial differences across years, with 2011 exhibiting the highest mean level of 237,629 cfu/100mL and 2017 showing the lowest at 194.2 cfu/100mL, indicating varying pollution intensities. Standard deviation further elucidated this variability, with 2011 exhibiting high standard deviation around the mean at 232,708 cfu/100mL and 2017 showing comparatively less variability of 442.5 cfu/100mL. The data showed that pollution levels, as indicated by *E. coli* levels, were generally high in 2011 and 2012 compared to other years, with 2011 having the highest mean concentration and widest range. In contrast, 2013 and 2017 appeared to have comparatively lower pollution levels as indicated by their lower mean levels and smaller ranges.

**Table 4. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Mzimvubu River spanning from 2010 to 2017. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 0 | 185387 | 185387 | 20115 | 58089 |
| 2011 | 165 | 740670 | 740505 | 237629 | 232708 |
| 2012 | 221 | 274300 | 274079 | 100345 | 119267 |
| 2013 | 0 | 2623 | 2623 | 451,3 | 854,1 |
| 2014 | 0 | 50000 | 50000 | 4935 | 14962 |
| 2015 | 0 | 5400 | 5400 | 531,7 | 1550 |
| 2016 | 0 | 3500 | 3500 | 809,8 | 1195 |
| 2017 | 0 | 1540 | 1540 | 194,2 | 442,5 |

### 5.2.5 *E. coli* trends in the Limpopo, Olifants, Inkomati and Mzimvubu River.

Faecal contamination levels exhibited notable variations across multiple rivers, as evidenced by the data presented in Figure 2. In the Limpopo River (Figure 2. a), *E. coli* concentrations demonstrated an initial increase of 980,210 cfu/mL (log10= 2.99) in 2010, followed by a decline of 1,728 cfu/mL (log10= 0.23) in 2012. A substantial increase of 115,656 cfu/mL (log10= 3.84) was noted in 2014, succeeded by a minor increase of 8,240 cfu/mL (log10= 3.91) in 2016, and subsequent fluctuations until a rapid escalation reaching 7,283,93 cfu/mL (log10= 5.86) in 2018. Similarly, in the Olifants River (Figure 2. b), *E. coli* contamination fluctuated greatly during the study period. With *E. coli* levels ranging from 7,426 cfu/mL (log10= 3.87) in 2010 to 3,448,000 cfu/mL (log10= 6.53) in 2018. This simultaneous increase in contamination levels in 2018 was a noteworthy trend observed in both the Limpopo and Olifants Rivers. Conversely, the Inkomati River (Figure 2. c) exhibited declining *E. coli* concentrations of 21,985 cfu/mL (log10= 3.34) in 2010 to 7,978 cfu/mL (log10= 2.90) in 2012, followed by a moderate increase of 12,709 cfu/mL (log10= 3.90) in 2013 and relatively stable levels until 2018, with a subsequent decrease of 3,312 cfu/mL (log10= 3.55) in 2019. Moreover, the Mzimvubu River (Figure 2.d) displayed fluctuations throughout the study period, with elevated *E. coli* levels reaching 740,670 cfu/mL (log10= 5.86) in 2011 followed by a sharp decrease of 2,623 cfu/mL (log10= 2.41) in 2013. A spike in contamination occurred in 2014 reaching 50,000 cfu/mL (log10= 4.69), succeeded by a sudden drop of 5,400 cfu/mL (log10= 3.73) in 2015, while *E. coli* levels decreased from 3,500 cfu/mL (log10= 3.54) to 1,540 cfu/mL (log10= 3.18) in 2016 and 2017 respectively. These observations underscore the dynamic nature of faecal contamination in these river systems, necessitating continued monitoring and management efforts to safeguard water quality and public health.

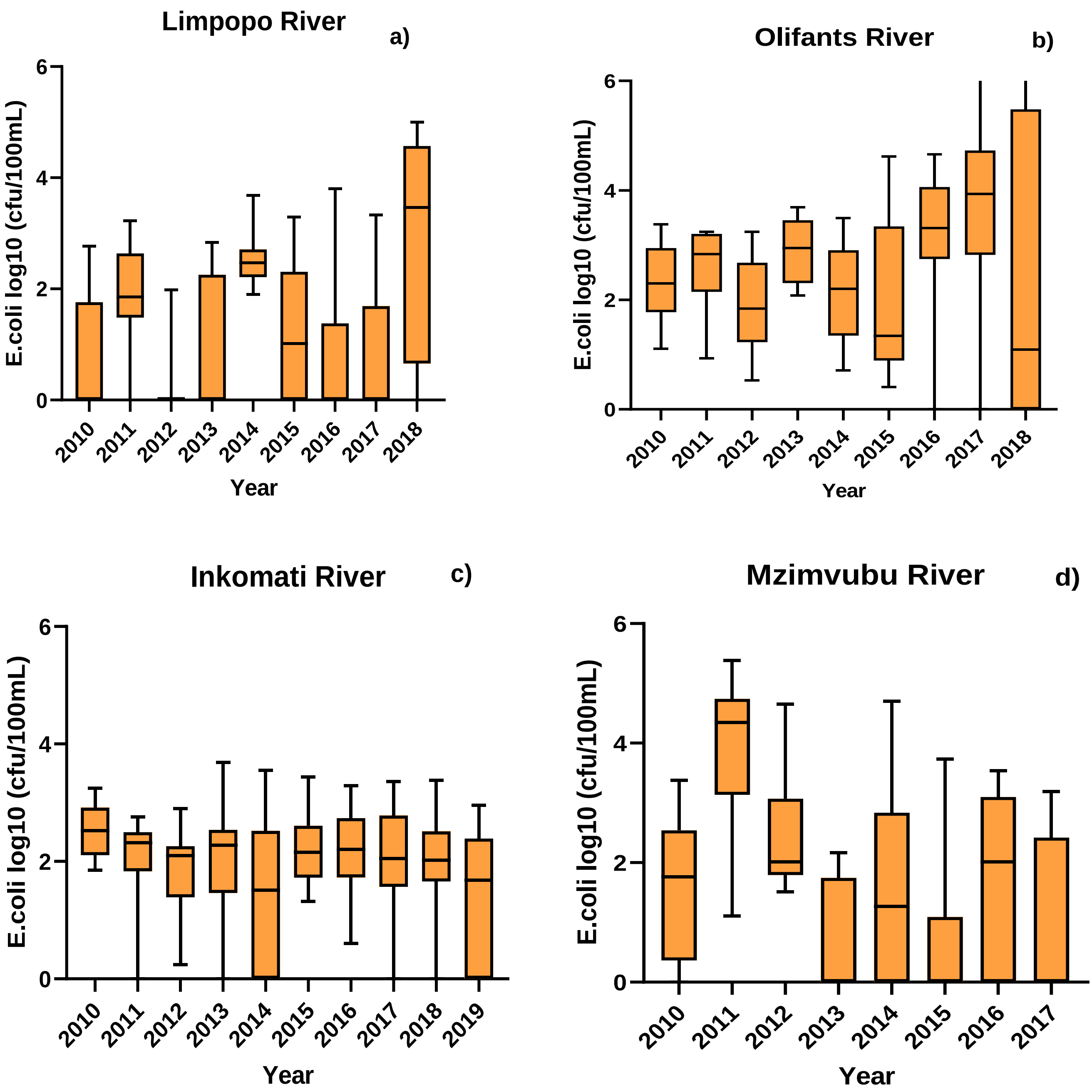



*Figure 2, Box plots demonstrating log tranformed E. coli concentrations in the Limpopo River (a) from 2010 – 2018; Olifants River (b) from 2010 – 2018; Inkomati River (c) from 2010 – 2019 and Mzimvubu River (d) from from 2010 – 2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to to a tenfold increase in the actual concentration.*

### 5.2.6 Breede River.

The summary statistics of *E. coli* concentrations in the Breede River spanning from 2010 to 2019 revealed notable variability (Table 6). The minimum *E. coli* levels ranged from 0 cfu/100mL to 205 cfu/100mL in 2010, indicating that in some years, there were undetected *E. coli* levels. Maximum *E. coli* varied significantly ranging from 280 cfu/100mL in 2014 to 264,787 cfu/100mL in 2013, showing considerable fluctuations in pollution levels across the years. Conversely, the range was quite large, indicating substantial variability in *E. coli* concentrations within each year, ranging from 280 cfu/100mL in 2014 to 264,787 cfu/100mL in 2014. Additionally, the mean *E. coli* concentrations, varied between years ranging from a low 40.67 cfu/100mL in 2014 to a high 28,725 cfu/100mL in 2013, reflecting differences in overall pollution levels. High standard deviations further emphasize the variability in *E. coli* levels within each year. The years 2010, 2012 and 2013 stood out with exceptionally high maximum and mean *E. coli* levels, suggesting potential pollution events during those years. The analysis highlights the variability and potential pollution events in the Breede River over the years.

**Table 5. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Breede River spanning from 2010 to 2019. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 205 | 27674 | 27469 | 7426 | 10497 |
| 2011 | 0 | 31063 | 31063 | 9526 | 11735 |
| 2012 | 133 | 28548 | 28415 | 8196 | 10926 |
| 2013 | 0 | 264787 | 264787 | 28725 | 75467 |
| 2014 | 0 | 280 | 280 | 40,67 | 82,99 |
| 2018 | 0 | 4500 | 4500 | 385,3 | 1296 |
| 2019 | 0 | 2170 | 2170 | 419,3 | 660,3 |

### 5.2.7 Berg-Olifants River.

In the Berg-Olifants River (Table 7), it was observed that the data showed notable variations in *E. coli* concentrations from 2010 to 2019. Notably, the minimum *E. coli* vary across years, ranging from 0 cfu/100mL to 63,175 cfu/100mL in 2012, with some years showing no *E. coli* detected (2013, 2014, 2016, 2018 and 2019). The maximum levels fluctuated widely, reaching exceptionally high *E. coli* levels during the years (2011, 2012, 2018 and 2019). The range of *E. coli* was considerable, ranging from 57.89 cfu/100mL in 2016 to 3,258,420 cfu/100mL in 2019. In years with higher maximum *E. coli*, the ranges were also larger. Furthermore, the mean levels also varied from year to year, ranging from a low of 17.45 cfu/100mL in 2016 to a high of 1,830,109 cfu/100mL in 2019. Standard deviations are relatively high, indicating substantial variability. Years like 2018 and 2019 showed exceptionally high mean and maximum *E. coli* levels, indicating potentially severe pollution episodes. It is noteworthy that in some years, there were instances where no *E. coli* was detected, this could indicate either low pollution levels or limitations in detection methods.

**Table 6. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Berg-Olifants River spanning from 2010 to 2019. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 5088 | 2378968 | 2373880 | 733057 | 919935 |
| 2011 | 6919 | 2980681 | 2973762 | 1153895 | 1167066 |
| 2012 | 63175 | 3102178 | 3039003 | 1093068 | 1114627 |
| 2013 | 0 | 2284383 | 2284383 | 694658 | 875922 |
| 2014 | 0 | 268 | 268 | 43,25 | 101,1 |
| 2016 | 0 | 192 | 192 | 17,45 | 57,89 |
| 2018 | 0 | 7400064 | 7400064 | 740026 | 2340099 |
| 2019 | 0 | 9000540 | 9000540 | 1830109 | 3258420 |

### 5.2.8 Phongolo River.

In the Phongolo River, the analysis of *E. coli* from 2010 to 2019 showed notable variations indicative of microbial water quality dynamics in this water body. Across the analysed period, the minimum and maximum levels demonstrated a wide range of contamination levels, suggesting significant variability in *E. coli* concentrations. Notably, the year 2015 recorded an exceptionally high maximum concentration of 2,447,712 cfu/100ml, indicative of a potential data anomaly or a particular event leading to extreme contamination levels in that year. It was further noted that there was significant variability in *E. coli* levels across the years, as indicated by wide ranges and varying standard deviations. Moreover, the mean *E. coli* fluctuated from year to year suggesting that they might be factors influencing contamination levels that vary over time.

**Table 7. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Phongolo River spanning from 2010 to 2017. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 860 | 29181 | 28321 | 8696 | 8974 |
| 2011 | 654 | 73006 | 72352 | 8482 | 20579 |
| 2012 | 1433 | 27650 | 26217 | 9412 | 7534 |
| 2013 | 1046 | 77412 | 76366 | 26774 | 23989 |
| 2014 | 5668 | 322259 | 316591 | 52400 | 93529 |
| 2015 | 0 | 2447712 | 2447712 | 216797 | 702755 |
| 2016 | 2303 | 19873 | 17570 | 7406 | 4711 |
| 2017 | 0 | 28866 | 28866 | 4119 | 9011 |

### 5.2.9 Vaal River.

Table 2 shows a comprehensive overview of *E. coli* contamination in the Vaal River from 2010 to 2017. Across the years, the minimum and maximum levels showed a wide range of contamination levels with instances of 0 cfu/100mL concentrations in several years (i.e. 2011, 2014, 2015 and 2015). Particularly striking is the year 2017, wherein the maximum *E. coli* level reached an exceedingly 6,024,395 cfu/100mL indicating a potential outlier or exceptional contamination event. Furthermore, years such as 2011, 2013 and 2017 indicated substantial variability ranging between 2,089,548 cfu/100mL and 6,024,395 cfu/100mL. Elevated mean concentrations ranging from 269,139 cfu/100mL and 1, 013,516 cfu/100mL, such as those observed in 2011 and 207, suggested overall higher contamination levels during those periods. Additionally, Years with notable higher standard deviations such as 2011 and 2017, suggest pronounced fluctuations and potential sources of variability in microbial water quality.

**Table 8. Descriptive statistics of *E. coli* concentrations (measured in cfu/100/mL) in the Vaal River spanning from 2010 to 2017. It outlines the lowest, highest, range, average, and standard deviation of *E. coli* levels for each respective year.**

| Year | Minimum | Maximum | Range | Mean | Std. Deviation |
|---|---|---|---|---|---|
| 2010 | 7339 | 350066 | 342727 | 102291 | 113477 |
| 2011 | 0 | 2089548 | 2089548 | 269139 | 582615 |
| 2012 | 3887 | 303610 | 299723 | 74842 | 100479 |
| 2013 | 7152 | 2416189 | 2409037 | 271303 | 700855 |
| 2014 | 0 | 2400000 | 2400000 | 289315 | 674925 |
| 2015 | 0 | 839 | 839 | 191 | 271,7 |
| 2016 | 12 | 383140 | 383128 | 102666 | 145940 |
| 2017 | 0 | 6024395 | 6024395 | 1013516 | 2107253 |

### 5.2.10 *E. coli* trends in the Breede, Berg-Olifants, Phongolo and Vaal Rivers.

In the Breede River (Figure 3. a), *Escherichia coli* contamination levels remained relatively stable from 2010 to 2012 but showed an increase of 26,4787 cfu/mL (log10= 4.42) in 2013, followed by a drastic decline of 280 cfu/mL (log10= 2.44) in 2014. There was a slight resurgence in contamination in 2018 and 2019. Similarly, the Berg-Olifants River (Figure 3. b) exhibited stable *E. coli* concentrations from 2010 to 2012, with considerable variability noted in 2013, followed by decline of 268 cfu/mL (log10= 2.42) in 2014 and 192 cfu/mL (log10= 2.28) in 2016. Notably, *E. coli* levels experienced an exponential increase of 740,0064 cfu/mL (log10= 6.86) in 2018 and 900,0540 cfu/mL (log10= 6.95) in 2019. Additionally, the Phongolo River (Figure 3. c) displayed elevated *E. coli* levels from 29,181 cfu/mL (log10= 4.46) in 2010 to 322,259 cfu/mL (log10= 5.50) in 2014, with 2015 exhibiting exceptionally high contamination levels reaching 2,447,712 cfu/mL (log10= 6.38) while 2016 and 2017 showed a decrease of 19,873 cfu/mL (log10= 4.29) and 28,866 cfu/mL (log10= 4.26) respectively. Conversely, the Vaal River (Figure 3.d) demonstrated an increase of 350,066 cfu/mL (log10= 4.54) in 2010 followed by consistent *E. coli* concentrations until 2013. Notably, *E. coli* levels peaked rapidly in 2014 reaching 2,400,000 cfu/mL (log10= 6.38) followed by a sudden decrease of 839 cfu/mL (log10= 2.92) in 2015 and increased from 383,140 cfu/mL (log10= 4.58) in 2016 to 6,024,395 cfu/mL (log10= 4.45) in 2017. These findings suggest complex dynamics in faecal contamination levels across these river systems, influenced by various environmental and anthropogenic factors. Continued monitoring and management efforts are crucial to mitigate risks to water quality and public health in these regions.

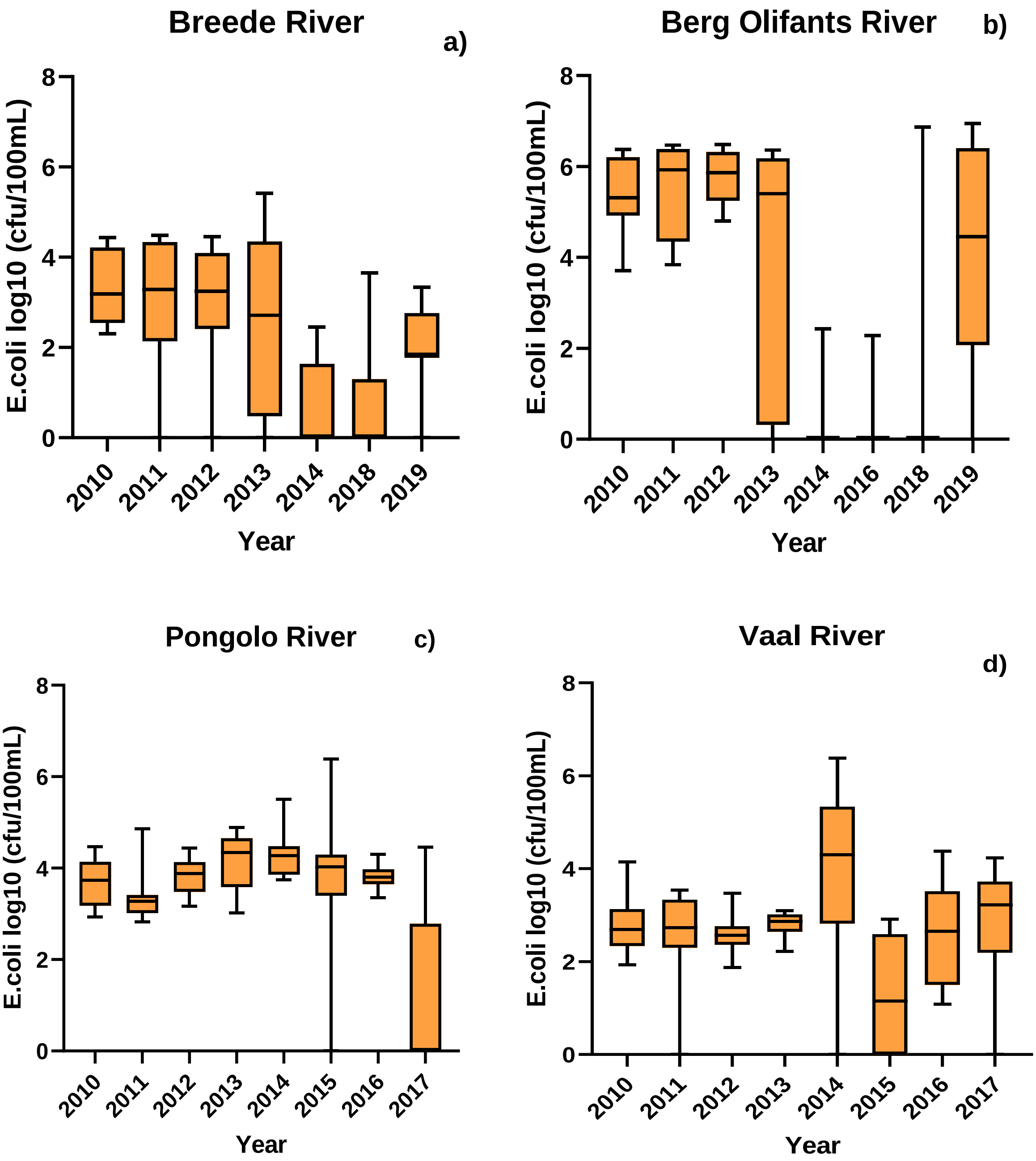



*Figure 3, Box plots demonstrating log tranformed E. coli concentrations in the Breede River (a) from 2010 – 2019; Berg Olifants River (b) from 2010 – 2019; Phongo River (c) from 2010 – 2017 and Vaal River (d) from from 2010 – 2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration.*

### 5.3 post-hoc comparisons of mean *E. coli* concentrations between years.

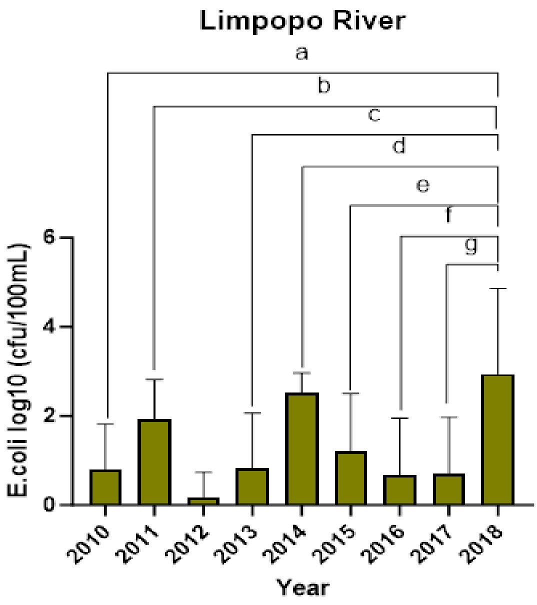
An analysis of variance (ANOVA) was conducted to ascertain variations in the mean concentration of *Escherichia coli* within the Limpopo River across different years (refer to Appendix A). The findings of the ANOVA revealed statistically significant differences (p-value < 0.05) in mean *E. coli* levels among the various years under consideration. Similarly, in the Olifants River (refer to Appendix B), mean *E. coli* levels exhibited significant disparities (p-value < 0.05) across different years. Conversely, within the Inkomati River (refer to Appendix C), there were no statistically significant differences (p-value > 0.05) observed in mean *E. coli* levels among the years analyzed. However, in the case of the Mzimvubu River, mean *E. coli* levels displayed significant variability (p-value < 0.05) across the years (refer to Appendix D). Subsequent post-hoc comparisons utilizing Tukey's test were performed to discern specific differences between individual years across the rivers, providing additional insights into temporal variations in *E. coli* contamination levels within these aquatic ecosystems.

#### 5.3.1 Analysis of Tukey's test in the Limpopo River.

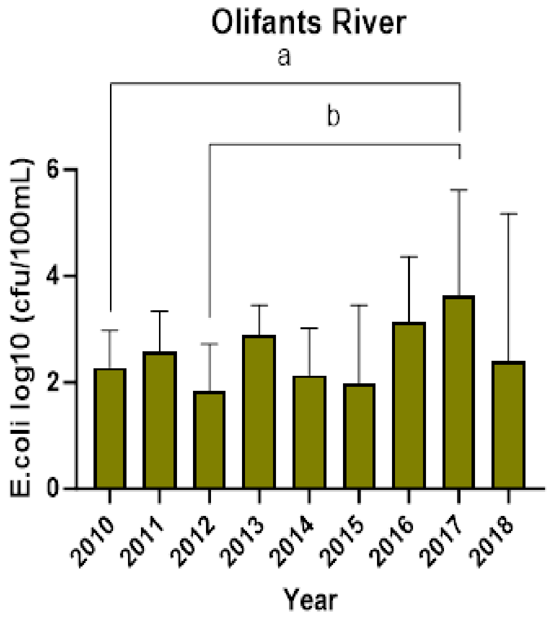
The results of multiple comparisons as indicated in (Figure 4. A), showed that mean *E. coli* levels varied significantly between 2010 and 2018 ranging from 39,702 (log10= 0.79) to 2,881,839 cfu/100mL (log10= 2.94). Similarly, disparities were observed between 2011 and 2018 with mean *E. coli* ranging from 82,937 (log10= 1.91) to 2,881,839 cfu/100mL (log10= 2.94). Conversely, mean *E. coli* concentration varied in 2013 and 2018 ranging from 960,9 (log10= 0.82) to 2,881,839 cfu/100mL (log10= 2.94). Furthermore, 2014 and 2018 showed significant mean concentrations of *E. coli* ranging from 12,593 (log10= 2.50) to 2,881,839 cfu/100mL (log10= 2.94). A significant variation was identified between 2015 and 2018 with mean *E. coli* ranging from 1,877 (log10= 1.21) to 2,881,839 cfu/100mL (log10= 2.94). Also, between 2016 and 2018; 2017 and 2018, there were significant differences observed ranging from 109,525 (log10= 0.66) to 2,881,839 cfu/100mL (log10= 2.94), 2,763 (log10= 0.69) to 2,881,839 cfu/100mL (log10= 2.94) respectively.

**5.3.2 Analysis of Tukey's test in the Olifants, Inkomati and Mzimvubu Rivers.**

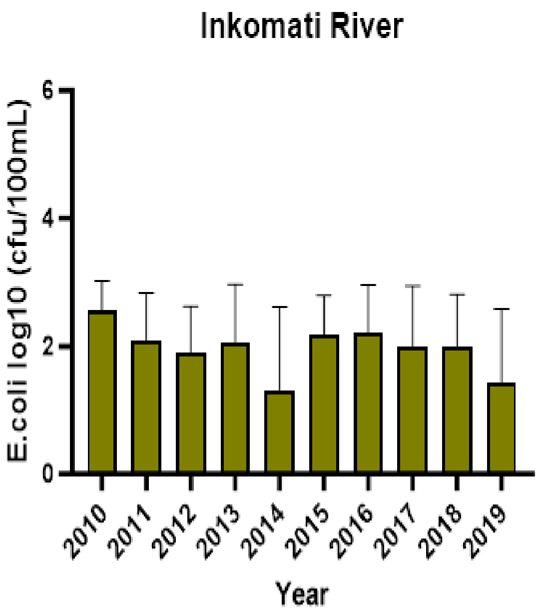
The results indicated a statistically significant difference in mean *E. coli* levels across different years in the Olifants River (Figure 4. B). Specifically, a significant difference was observed between 2010 and 2017, with mean *E. coli* counts ranging from 2,945 (log10 = 2.27) to 1,184,191 cfu/100mL (log10 = 3.62). Additionally, there was a significant difference between 2012 and 2017, with mean concentrations of 1,716 (log10 = 1.84) and 1,184,191 cfu/100mL (log10 = 3.62), respectively. In contrast, the ANOVA analysis in the Inkomati River (Figure 6. C) did not reveal a significant difference between the years, but the mean *E. coli* ranged from 8,272 (log10= 2.35) to 741.1 cfu/100mL (log10= 1.87). Conversely, the results from the Mzimvubu River (Figure 6. D), displayed a significant difference in *E. coli* concentration between 2010 and 2011, with mean *E. coli* ranging from 7,426 (log10= 1.36) to 237,629 cfu/100mL (log10= 4.30). 2011 and 2012 also showed significantly different mean *E. coli* levels ranging between 100,345 (log10= 2.28) and 237,629 cfu/100mL (log10= 4.30). It was further noted that 2011 and 2013 were significantly different. Even 2011 and 2014, 2011 and 2015, 2011 and 2016, and 2011 and 2017, were all significantly different from each other. The mean *E. coli* levels varied within these years, ranging from 237629 cfu/100mL (log10 = 3.93) to 194.2 cfu/100mL (log10 = 0.86).



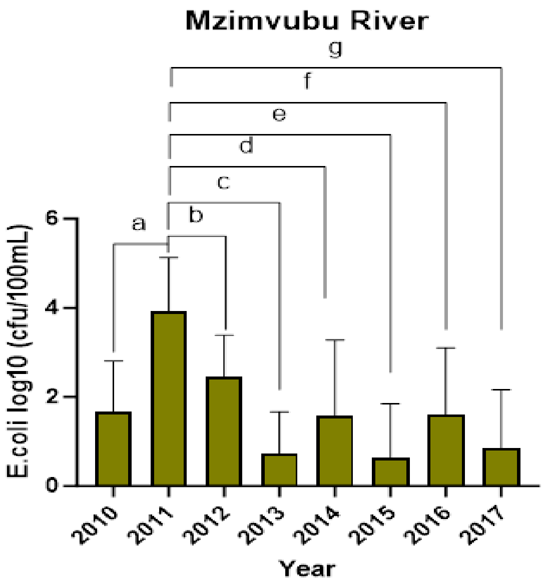

A




B




C




D

*Figure 4, Bar graphs showing statistically significant mean E. coli levels between years in the Limpopo River A from 2010 - 2018; Olifants River B from 2010 - 2019; Inkomati River C from 2010-2019; and Mzimvubu River D from 2010-2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Brackets indicate significant differences (p<0.05) as determined by one-way ANOVA.*

### 5.3.3 Analysis of Tukey's test in the Breede River.

Statistical analysis of *E. coli* in the Breede River (figure 5. A) showed significant differences in significance (p-value <0.05) across years. The post hoc test revealed a significant difference between 2010 and 2014 with mean levels ranging from 40.67 (log10 = 0.72) to 7,426 cfu/100mL (log10 = 3.28). Again, mean levels were found to be significantly different between 2010 and 2018 and the registered mean *E. coli* ranged from 385.3 (log10= 0.60) to 7,426 cfu/100mL (log10 = 3.28). It was further noticed that there was a significant difference between 2011, 2014 and 2018 with mean levels ranging between 40.67 (log10 = 0.72), 385.3 (log10= 0.60) and 9,526 cfu/100mL (log10 = 3.10) respectively. Also, 2013, 2014 and 2018 indicated significantly different mean levels of *E. coli* ranging between 40.67 cfu/100mL (log10= 0.72), 385.3 (log10= 0.60) and 28,725 cfu/100mL (log10= 2.58). Some of the years (i.e. 2012 and 2019) did not show any significant differences (p> 0.05). Overall, the Breede River results demonstrated that *E. coli* concentrations varied significantly throughout the study period.

### 5.3.4 Analysis of Tukey's test in the Berg-Olifants, Pongolo and Vaal Rivers.

The ANOVA results in the Berg-Olifants River (Figure 5. B) indicated statistically significant differences (p< 0.05) across years. According to Tukey's test, it was concluded that there were noticeable significant differences between 2010, 2014, 2016 and 2018 with their mean levels ranging between 740,026 (log10= 5.76) 733,057 (log10 = 5.40), 17.45 (log10= 0.19), 43.25 cfu/100mL (log10= 0.40). Similarly, significant differences were also identified between 2011, 2014, 2016 and 2018 with the mean *E. coli* concentrations ranging between 1,153,895 (log10= 5.50) to 740,026 cfu/100mL (log10= 5.76). Additionally, 2012 was also significantly different from 2014, 2016 and 2018 with mean levels recorded ranging from 1,093,066 cfu/100mL (log10= 5.76) to 740,026 cfu/100mL (log10= 0.76). It was further noted that 2013 was also significantly different between 2014, 2016 and 2018 with the mean ranging from 740,026 (log10 = 0.76) and 694,658 cfu/100mL (log10= 3.68). It was also observed that mean levels varied significantly between 2014, 2016, 2018 and 2019. With mean concentrations ranging between 1,830,109 (log10= 4.23) and 43. 25 cfu/100mL (log10 = 0.40). It was noted that the years (2014 and 2018) have shown low *E. coli* levels in both Breede and Berg Olifants Rivers. Moreover, there were no statistically significant differences ($p > 0.05$) in *E. coli* levels in the Pongolo (Figure 5. C) as well as the Vaal River throughout the study period (figure 5.D).

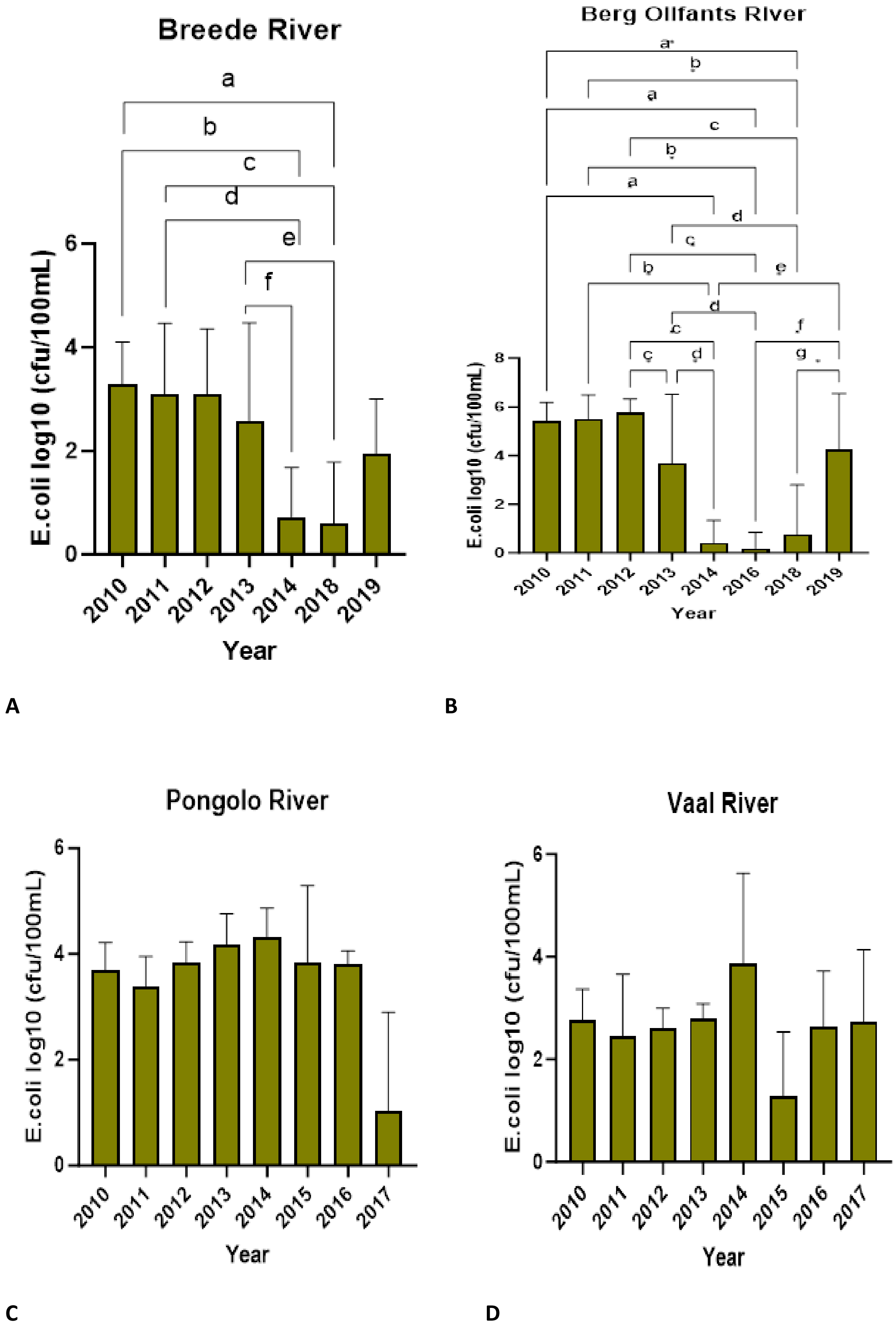



*Figure 5. Bar graphs showing statistically significant mean E. coli levels between years in the Breede River (A) from 2010 - 2019; Berg Olifants River (B) from 2010 - 2019; Pongolo*

***River(C) from 2010- 2017; and Vaal River (D) from 2010-2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Brackets indicate significant differences (p<0.05) as determined by one-way ANOVA.***

### 5.4 post-hoc comparisons of mean *E. coli* between rivers.

The comparison of mean *E. coli* concentrations among various rivers revealed significant disparities (refer to Appendix I). Post hoc analysis unveiled substantial differences in mean *E. coli* levels within the Limpopo River when compared with the Inkomati, Mzimvubu, Breede, Pongolo, and Vaal rivers ($p < 0.05$), with mean concentrations ranging between 153,113 and 1,259 cfu/100mL. Interestingly, no significant disparity emerged between the Limpopo and Olifants River ($p > 0.05$), yet the Olifants River exhibited marked discrepancies from all other rivers ($p < 0.05$), with mean *E. coli* levels ranging between 133,460 and 1,259 cfu/100mL. Similarly, the Inkomati River showcased significant differences in mean *E. coli* concentration compared to the Limpopo, Olifants, and Berg Olifants rivers ($p < 0.05$), with mean levels spanning from 153,113 to 1,259 cfu/100mL. Conversely, no statistically significant differences were discerned between the Inkomati and Mzimvubu, Breede, Pongolo, and Vaal rivers ($p > 0.05$). Within the Mzimvubu River, no significant differences were observed with all other rivers ($p > 0.05$), except for the Limpopo and Olifants Rivers, which exhibited significance in comparison to the Mzimvubu ($p < 0.05$). Mean *E. coli* levels among the Inkomati, Breede, Berg Olifants, Pongolo, and Vaal rivers ranged between 45,516 and 1,259 cfu/100mL. Particularly, the Pongolo River demonstrated significant differences compared to the Limpopo, Olifants, and Berg Olifants rivers ($p < 0.05$), with mean levels ranging between 153,113 and 78,930.75 cfu/100mL. Nevertheless, no significant differences were identified between the Pongolo River and the Inkomati, Mzimvubu, Breede, and Vaal rivers ($p > 0.05$). Similarly, the Vaal River exhibited significant differences compared to the Limpopo, Olifants, and Berg Olifants rivers ($p < 0.05$), with mean levels ranging between 153,113 and 78,930.75 cfu/100mL. Moreover, no significant difference was observed between the Vaal River and the Inkomati, Mzimvubu, Breede, and Pongolo rivers ($p > 0.05$).

**5.5 The influence of seasonal variations on *E. coli* contamination.**

The analysis of seasonal trends employing the Mann-Kendall test and Sen's Slope estimator yielded discernible patterns across the selected rivers, as summarized in Table 10. Notably, significant increasing trends were identified in the autumn and winter seasons for the Olifants River, supported by $p<0.05$ and positive Sen's Slope values. Conversely, no significant trend was detected during the summer and spring seasons in this river. The Inkomati River displayed a notable decreasing trend during the summer season, denoted by $p=0.032$ and a negative Sen's Slope. No significant trends were discerned in the other seasons, as indicated by $p>0.05$. Similarly, the Mzimvubu River exhibited significant decreasing trends during the autumn and winter seasons, with $p<0.05$ and negative Sen's Slope values. Moreover, no significant trends were observed in the summer and spring seasons for this river. Notably, several other rivers, including the Limpopo, Breede, Berg-Olifants, Phongolo, and Vaal, did not display any significant trends $p>0.05$. These findings provide valuable insights into the temporal dynamics of water quality parameters across diverse river systems, emphasizing the importance of considering seasonal variations in environmental monitoring and management practices.

**Table 10. Presents seasonal trend analyses employing the Mann-Kendall test and Sen's Slope estimator. A p-value below 0.05 indicates the presence of a trend in the dataset, while a p-value exceeding 0.05 suggests no discernible trend. The Kendall Tau statistic indicates the direction of the trend, with positive values indicating an upward trend and negative values indicating a downward trend. Sen's Slope provides insights into the magnitude of the trend, where positive values signify an increasing trend and negative values signify a decreasing trend. Bold numbers highlight instances where a trend has been detected in the dataset.**

| River | Season | Mann Kendall Test | | | Trend decision |
|---|---|---|---|---|---|
| | | Kendal Tau | p-value | Sen's Slope | |
| Limpopo | summer | 0.145 | 0.670 | 0.007 | No trend |
| | autumn | 0.167 | 0.602 | 0.197 | No trend |
| | winter | -0.141 | 0.675 | -0.057 | No trend |
| | spring | 0.087 | 0.831 | 0.017 | No trend |
| Olifants | summer | 0.111 | 0.754 | 0,082 | No trend |
| | autumn | 0.611 | **0.029** | 0,089 | Increasing |
| | winter | 0.556 | **0.048** | 0,274 | Increasing |
| | spring | 0.111 | 0.754 | 0,082 | No trend |
| Inkomati | summer | -0.556 | **0.032** | -0,180 | Decreasing |
| | autumn | -0.422 | 0.107 | -0,113 | No trend |
| | winter | -0.156 | 0.592 | -0,045 | No trend |
| | spring | -0.289 | 0.283 | -0,104 | No trend |
| Mzimvubu | summer | -0.567 | 0.075 | -0,347 | No trend |
| | autumn | -0.643 | **0.035** | -0,518 | Decreasing |
| | winter | -0.714 | **0.019** | -0,254 | Decreasing |
| | spring | -0.189 | 0.610 | -0,414 | No trend |
| Breede | summer | -0.293 | 0.448 | -0,282 | No trend |
| | autumn | -0.524 | 0.133 | -0,268 | No trend |
| | winter | -0.488 | 0.172 | -0,344 | No trend |
| | spring | -0.390 | 0.288 | -0,481 | No trend |
| Berg Olifants | summer | -0.416 | 0.203 | -0,788 | No trend |
| | autumn | -0.327 | 0.319 | -0,059 | No trend |
| | winter | -0.113 | 0.799 | -0,074 | No trend |
| | spring | -0.327 | 0.319 | -0,085 | No trend |
| Pongolo | summer | 0.000 | 1.000 | 0,009 | No trend |
| | autumn | 0.214 | 0.536 | 0,254 | No trend |
| | winter | -0.357 | 0.266 | -0,098 | No trend |
| | spring | -0.286 | 0.386 | -0,074 | No trend |
| Vaal | summer | -0.429 | 0.174 | -0,348 | No trend |
| | autumn | -0.071 | 0.902 | -0,153 | No trend |
| | winter | -0.071 | 0.902 | -0,030 | No trend |
| | spring | 0.500 | 0.108 | 0,214 | No trend |

### 5.5.1 Seasonal trends in the Limpopo River.

During the study period, the summer season in the Limpopo River exhibited fluctuations without a clearly defined trend (Figure 6. a). *E. coli* concentrations experienced a decline from 2010 to 2012, followed by an increase until 2013, stabilizing thereafter, and declining again in 2015. A notable increase was observed in 2017, succeeded by a rapid decrease. Despite the absence of a distinct trend, the positive Sen's slope in Table 10 implies relatively stable *E. coli* levels during the summer period. Similarly, the autumn season showed an undefined trend characterized by fluctuations (Figure 6. b). *E. coli* counts increased from 2011 to 2013, moderately increased in 2014, then sharply declined in 2016, followed by a substantial increase in 2018. Correspondingly, positive Sen's Slope values in Table 10 suggest a moderate increasing trend during autumn. Moreover, the winter and spring seasons displayed fluctuations in *E. coli* concentrations. In winter (Figure 6. c), *E. coli* levels slightly increased in 2011 before sharply declining in 2013, rising again in 2014, and stabilizing until 2016. This was succeeded by a sudden decrease in 2017 and a sharp increase in 2018. Similarly, in spring (Figure 6.d), *E. coli* levels slightly increased in 2011, then fluctuated significantly thereafter, with the highest peak noted in 2016. Interestingly, Sen's slope values suggest a stable decreasing trend during winter and a stable increasing trend in spring.

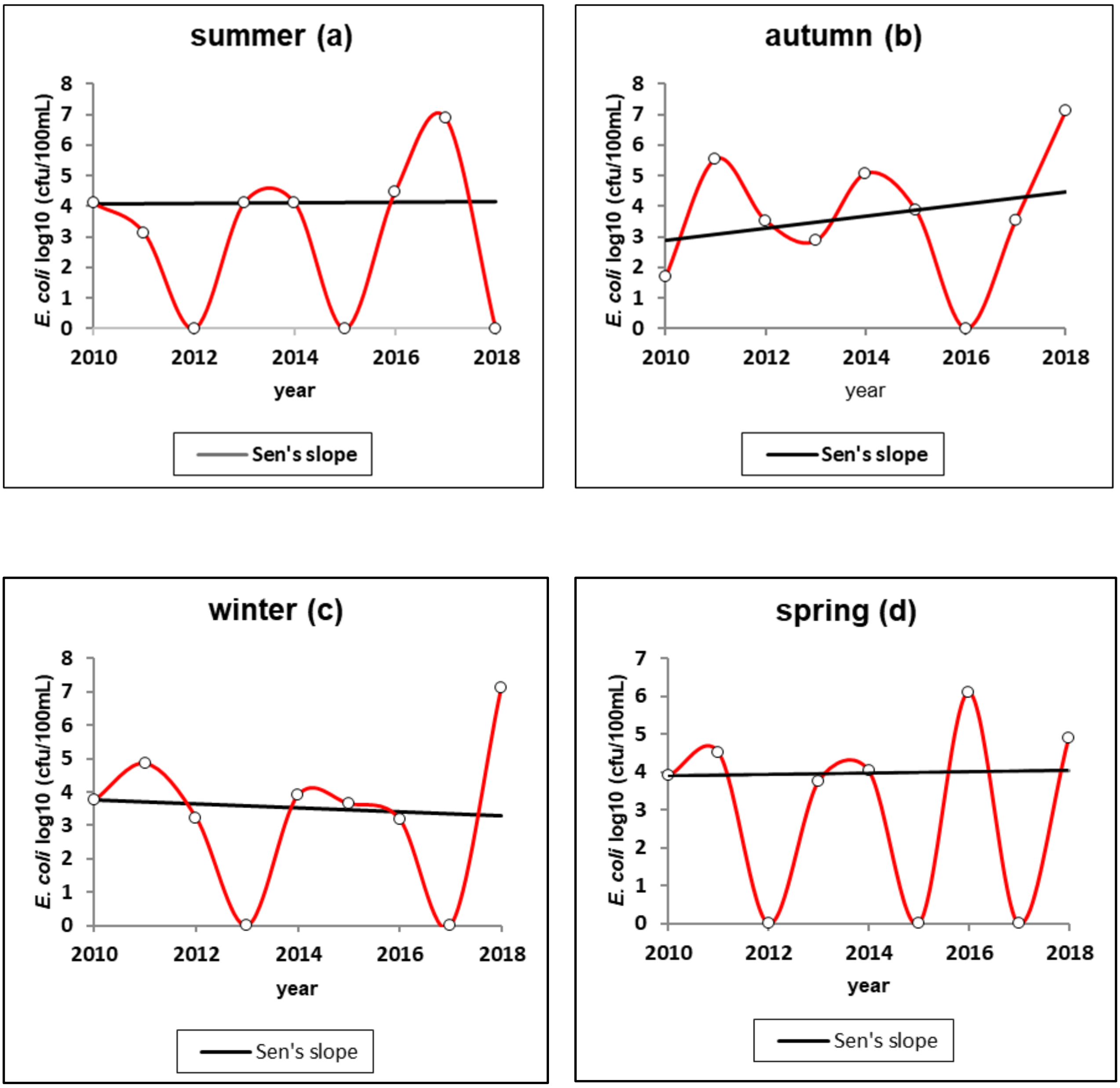



*Figure 6. Seasonal trends of E. coli in the Limpopo River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2018. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.2 Seasonal trends in the Olifants River

In the Olifants River, the summer season (Figure 7. a) did not exhibit a discernible trend, as indicated by the Mann-Kendall test. However, positive Sen's slope values from Table 10 suggest a general increase in *E. coli* concentrations over the years. Notably, there was a pronounced increase observed from 2010 to 2013, followed by a decline in 2014, subsequent stability until 2015, and a rapid decrease in 2018. During autumn (Figure 7. b), *E. coli* concentrations displayed an increasing trend in the Olifants River. The concentrations increased from 2011 to 2013, stabilized until 2014, experienced a slight decline in 2015, followed by an increase in 2016, and remained relatively stable until 2018. Similarly, the winter season (Figure 7. c), revealed an increasing trend over the study period. *E. coli* concentrations showed slight increases but were marked by a rapid decrease in 2012, followed by a sharp increase in 2013, and stability until 2018. The spring season (Figure 7.d) exhibited fluctuations over the years, resembling the summer season, with both seasons showing a substantial decline in 2018.

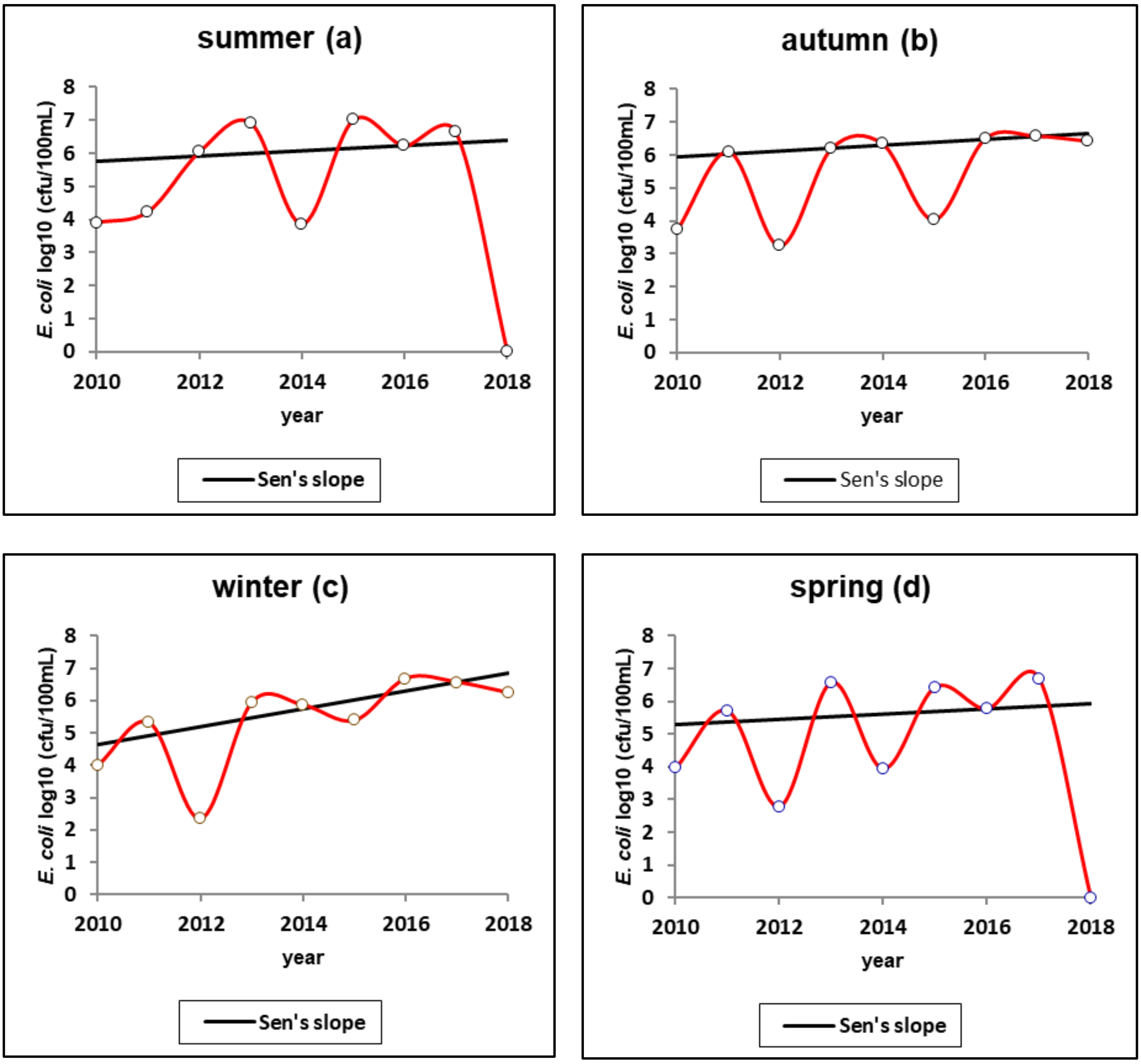



*Figure 7. Seasonal trends of E. coli in the Olifants River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2018. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.3 Seasonal trends in Inkomati River

In the analysis of seasonal trends within the Inkomati River, distinct patterns in *E. coli* concentrations were observed across different seasons, as illustrated in Figure 8. During the summer season (Figure 8. a), a discernible decreasing trend was detected, notwithstanding notable fluctuations in contamination levels. Notable instances include a decrease in 2013, succeeded by a slight increase in 2015, followed by a sharp decline in 2018. The observed decreasing trend aligns with the positive Sen's Slope presented in Table 10. Conversely, the autumn period (Figure 8. b) exhibited fluctuations without a clear trend of increase or decrease, although a pronounced increase was observed in 2015, followed by a sustained *E. coli* concentration thereafter. Despite the absence of a defined trend during this season, the positive Sen's slope suggests a decrease in *E. coli* over the study period. Similarly, both the winter and spring seasons displayed fluctuations without distinct trends. In the winter season (Figure 8. c), *E. coli* levels remained relatively constant from 2010 to 2012 before experiencing a substantial decline in 2014, followed by fluctuations in contamination levels throughout 2016 and 2019. Conversely, during the spring season (Figure 8.d), a moderate decrease in *E. coli* concentrations was observed until 2013, followed by an increase in 2014 and stable levels thereafter, with a subsequent sharp decrease noted in 2018. These results show the dynamic nature of *E. coli* concentrations within the Inkomati River across different seasons, characterized by fluctuations and occasional spikes in contamination levels. While certain seasons exhibited discernible trends, others displayed fluctuations without a clear trajectory.

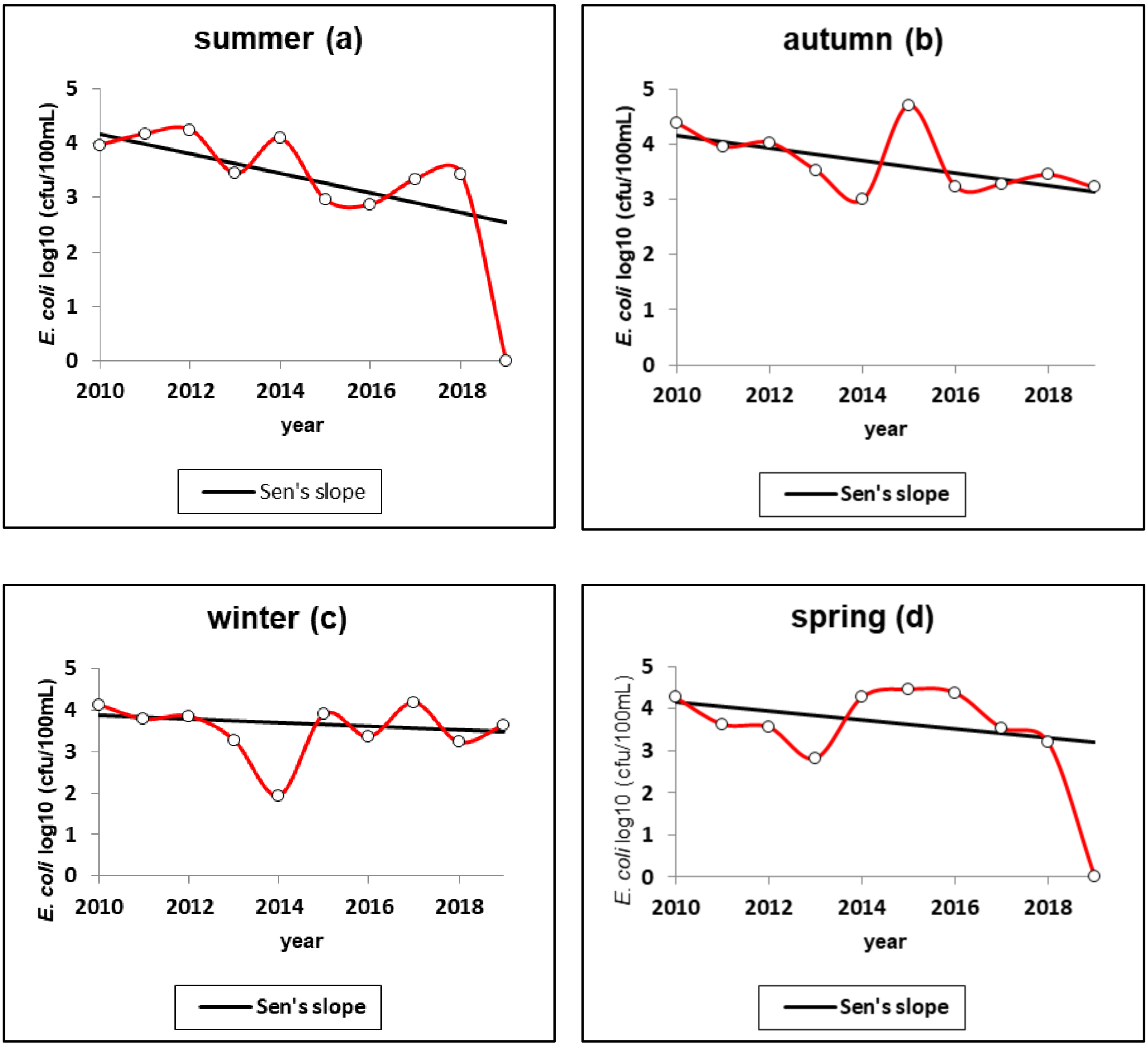



***Figure 8. Seasonal trends of E. coli in the Inkomati River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2019. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.***

### 5.5.4 Seasonal trends in the Mzimvubu River

The results of the Mann-Kendall test in *E. coli* concentrations within the Mzimvubu River revealed distinct patterns across different seasons, as depicted in Figure 9. In the summer season (Figure 9. a), the application of the Mann-Kendall test did not reveal any statistically significant trend. Despite the absence of a discernible trend, notable fluctuations in *E. coli* levels were observed throughout the study period. Specifically, an increase in contamination was noted in 2011, succeeded by a substantial decline in 2013, with stability maintained until 2014. Subsequent increases occurred in 2015, followed by stability until 2016, before another decline in 2017. The positive Sen's slope indicates an overall decreasing trend in *E. coli* concentrations during this interval, as delineated in Table 10. Conversely, a decreasing trend was identified during the autumn season (Figure 9. b). Initially, *E. coli* concentrations exhibited an increasing trajectory in 2011, gradually diminishing over the study period. Similarly, a decreasing trend was discerned during the winter season (Figure 9. c), characterized by an initial increase in 2011 and 2012, succeeded by a gradual decline extending until 2017. In contrast to other seasons, no discernible trend was detected during the spring season (Figure 9.d). Nevertheless, significant fluctuations were noted throughout the observation period. Following an initial increase in *E. coli* concentrations in 2011, a rapid decline was observed in 2013, with considerable oscillations, thereafter, marked by peaks in 2014 and 2016. The positive Sen's slope suggests an overall decreasing trend in *E. coli* levels during the spring season. These findings underscore the dynamic nature of *E. coli* concentrations within the Mzimvubu River across different seasons. While certain periods exhibited consistent trends of decreasing contamination, others displayed fluctuations without a clear trajectory.

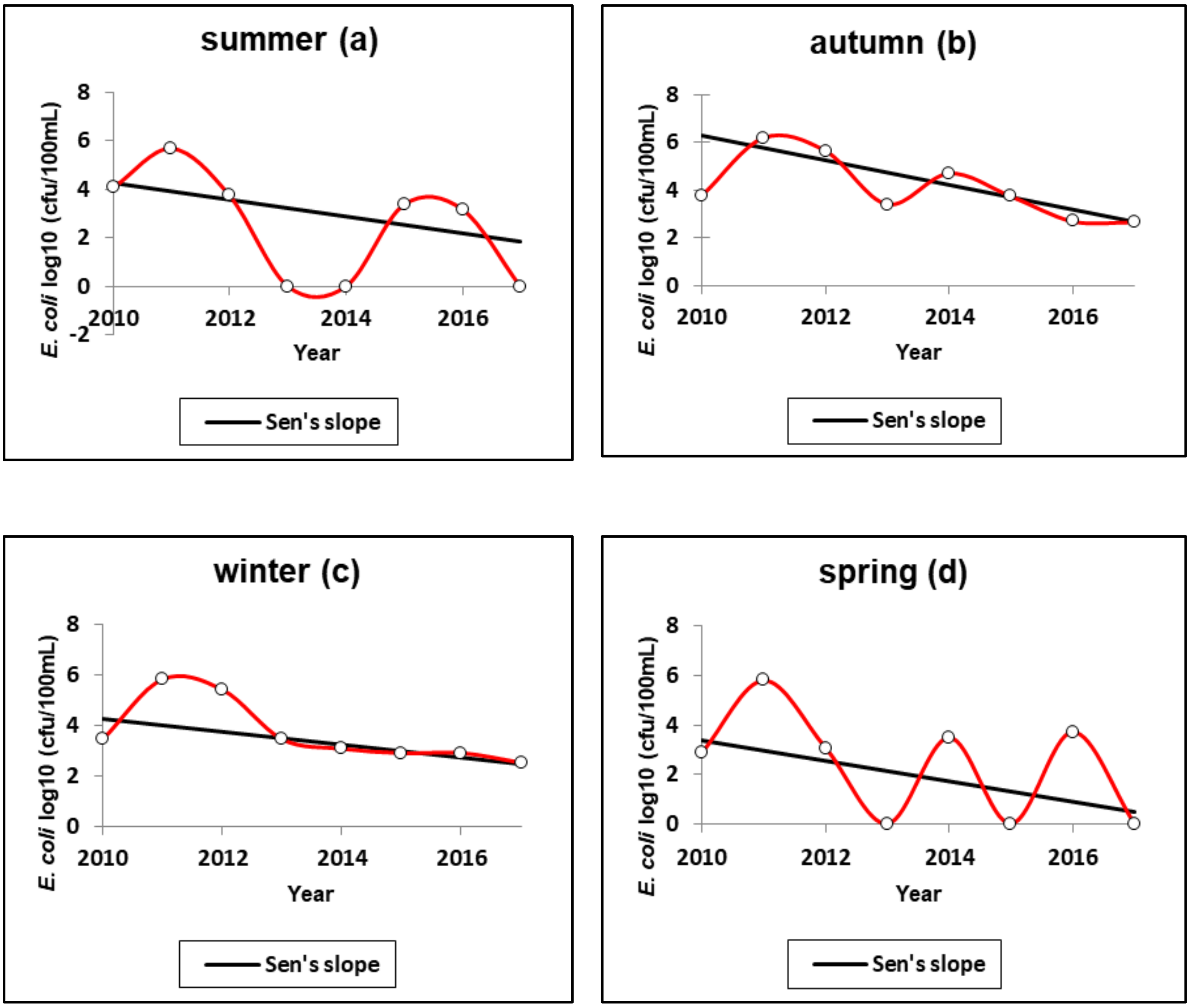



*Figure 9, Seasonal trends of E. coli in the Mzimvubu River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2016. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.5 Seasonal trends in the Breede River

In the Breede River, an analysis of seasonal trends in *E. coli* concentrations revealed notable dynamics across different periods, as depicted in Figure 10. During the summer season (Figure 10. a), no discernible trend was evident, as indicated by the findings in Table 10. Despite the absence of a clear trend, significant fluctuations were observed, notably characterized by a pronounced increase in 2012, followed by a substantial decline in 2013, with slight subsequent increases in 2014, maintaining constancy until 2018 before experiencing a decline in 2019. Notwithstanding the lack of a discernible trend, the negative Sen's slope suggests a decreasing trend in *E. coli* contamination during the summer season. Similarly, no trend was detected during the autumn season (Figure 10. b). Throughout this period, *E. coli* counts exhibited fluctuations, with peaks observed primarily in 2011 and 2013, while remaining relatively constant in other years, except for a notable increase in 2019. This pattern aligns with the negative Sen's slope, indicating a decrease in *E. coli* counts during the autumn period. Interestingly, both the winter and spring seasons (Figure 10. c and d) demonstrated comparable patterns of *E. coli* concentrations, with no evidence of a discernible trend, as delineated in Table 10. Noteworthy observations include elevated *E. coli* counts in 2012 and 2013, followed by a substantial decline in 2014, with consistent levels thereafter until a resurgence in 2019. The negative Sen's slope further corroborates an overall decreasing trend in *E. coli* concentration during both seasons. These findings underscore the dynamic nature of *E. coli* concentrations within the Breede River across different seasons, characterized by fluctuations and occasional spikes in contamination levels. While certain years exhibited notable increases or decreases in *E. coli* counts, overall trends suggest a decreasing trend in contamination levels, particularly during the autumn, winter, and spring seasons.

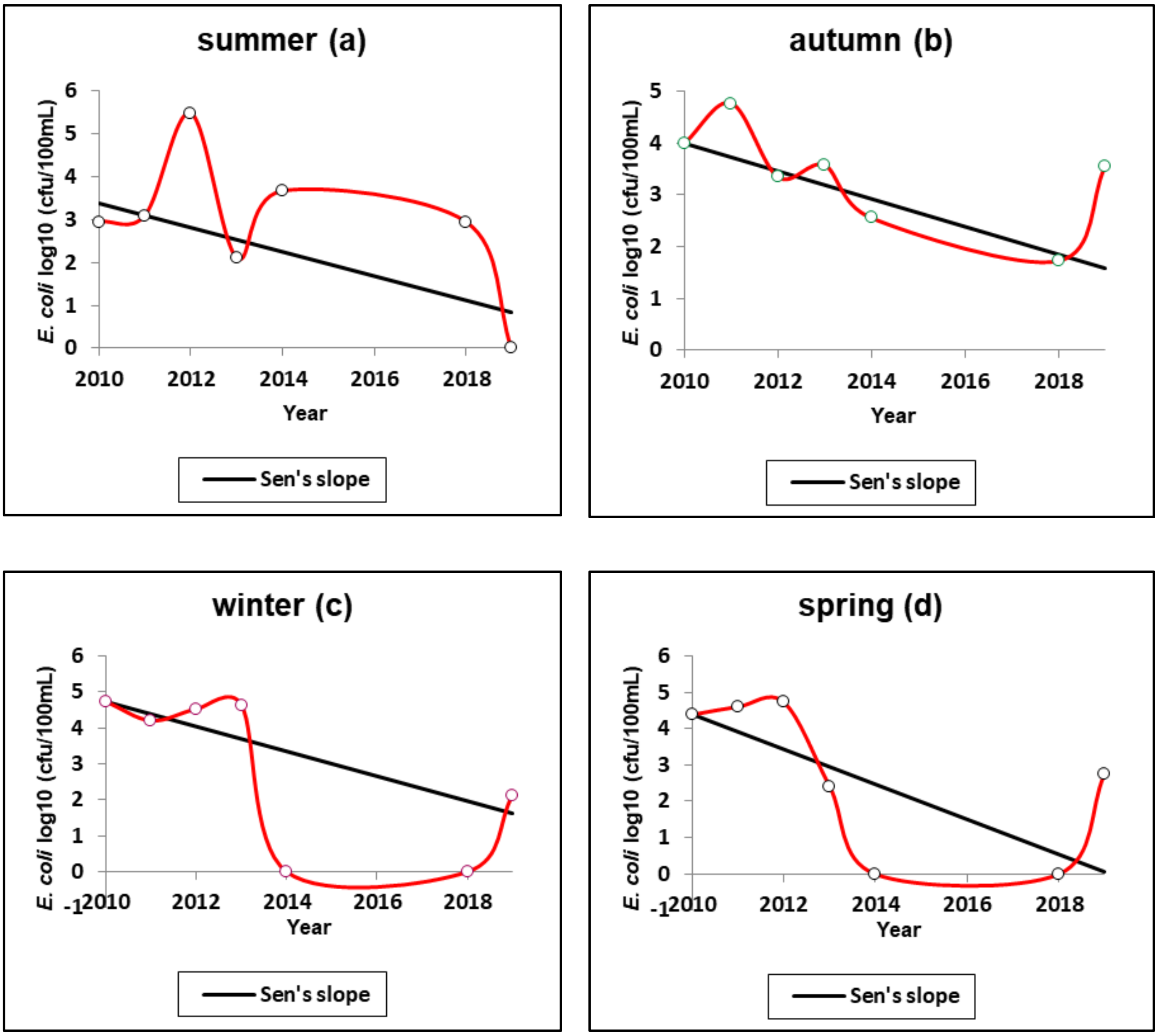



*Figure 10, Seasonal trends of E. coli in the Breede River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2019. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.6 Seasonal trends in the Berg-Olifants River

The results of the Mann-Kendall test as seen in Table 10 showed no trends identified in the summer (Figure 11. a) and autumn seasons of the Berg-Olifants River (Figure 11. b). It was noted that during the summer period, *E. coli* remained stable from 2010 to 2012 and declined drastically in 2014 where it remained stable until 2016. 2018 showed a pronounced increase in *E. coli* but a sudden decline occurred in 2019. During this season, Sen's slope indicates a significant increase in *E. coli* concentrations. The autumn season on the other hand showed constant *E. coli* concentrations from 2010 through to 2013. Notably, a rapid decrease occurred in 2014 and subsequent fluctuation until 2018, followed by a sharp increase in 2019. Unlike the summer period, the Sen's slope indicates constant *E. coli* concentrations during the autumn season. It was found that in the winter season (Figure 11. c) there was no significant trend detected by the Mann-Kendall test. In the summer season, *E. coli* concentration showed a moderate increase between 2010 and 2013 with a substantial decrease recorded in 2014 where it remained stable until 2018. This was followed by a sharp increase in 2019. Additionally, during the spring season (Figure 11.d), it was noted that between 2010 and 2012, *E. coli* remained constant. A drastic decline was, however, observed in 2013 and remained stable until 2016, followed by a pronounced increase in 2018. Interestingly, the Sen's slope suggests constant *E. coli* contamination over the years in both winter and spring seasons.

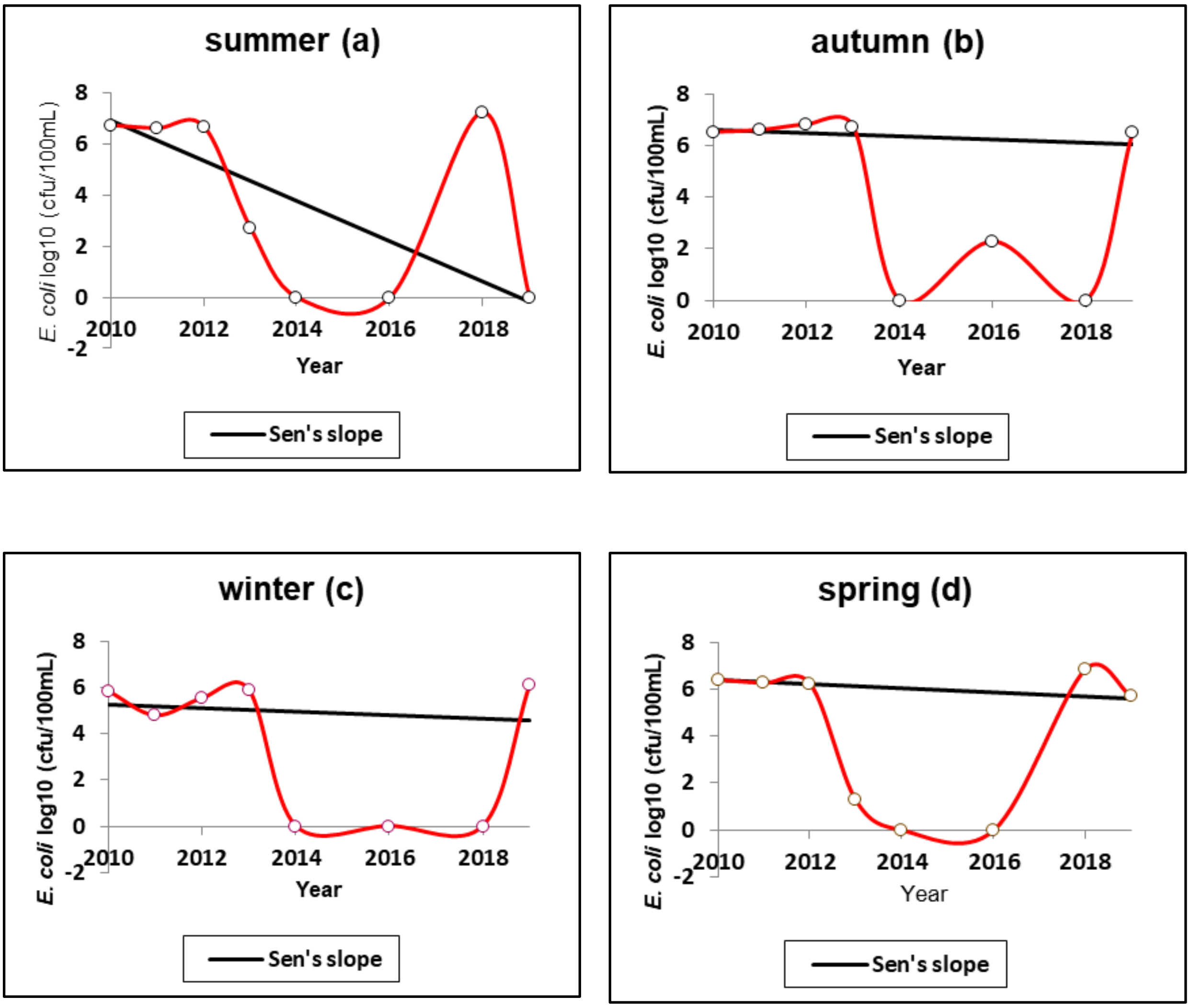



***Figure 11, Seasonal trends of E. coli in the Berg Olifants River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2019**. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.7 Seasonal trends in the Pongolo River

The Mann-Kendall test did not detect any significant trend in all the studied seasons of the Phongolo River as indicated in Table 10. However, there were noticeable fluctuations observed. In the summer season (Figure 12. a), *E. coli* was relatively stable between 2010 and 2014 with a slight decline in 2016 but a drastic decrease in *E. coli* was noted in 2017. According to Sen's slope, there is constant *E. coli* contamination during the summer season. It was further noted that there was no defined trend in the autumn season (Figure 12. b). However, Sen's slope suggests a moderate increase in *E. coli* levels during this period. *E. coli* concentrations progressively increased from 2010 through to 2014 but declined substantially thereafter. During the winter season (Figure 12. c), it was observed that *E. coli* concentration fluctuated moderately between 2010 and 2016 but decreased exponentially in 2017. Similarly, the spring season (Figure 12.d) also showed moderate fluctuations in *E. coli* levels between 2010 and 2013, while *E. coli* remained constant until 2016 before dropping rapidly. It is noteworthy that Sen's slope implies constant *E. coli* concentration in both winter and spring seasons.

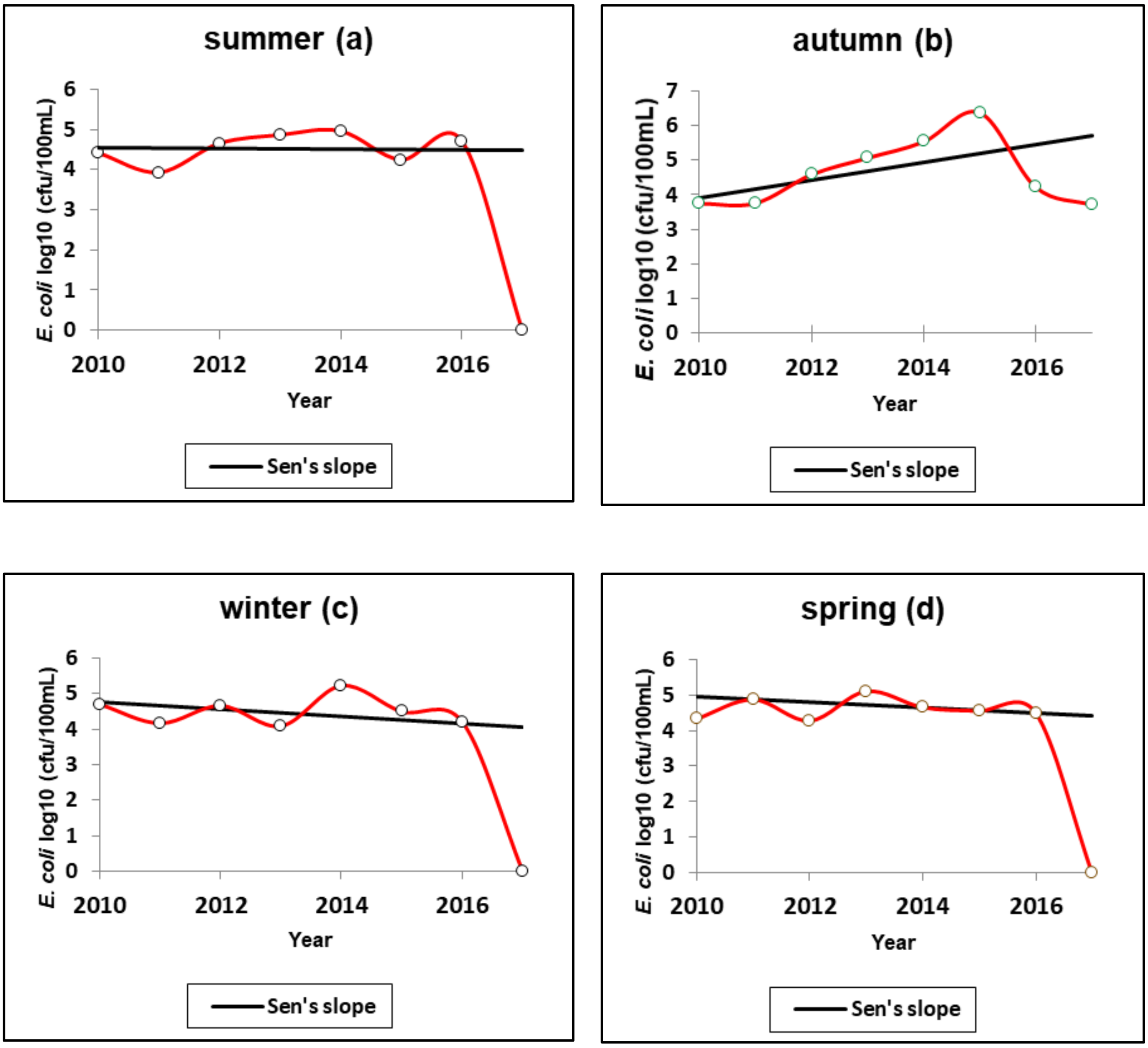



*Figure 12, Seasonal trends of E. coli in the Pongolo River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.*

### 5.5.8 Seasonal trends in the Vaal River.

The Mann-Kendall analysis as indicated in Table 10, showed that there was no significant trend detected during summer in the Vaal River (Figure 13. a). *E. coli* concentration was fluctuating with irregular peaks observed in 2012 and 2016 but a decline was noted in 2017. Although there was no trend detected, the negative Sen's sloped implies overall decreasing *E. coli* concentrations during the summer season in this waterbody. Additionally, the autumn season (Figure 13. b) also revealed that there was a trend identified. The contamination remained stable from 2010 to 2013 with a slight increase in 2015 but decreased significantly in 2016 before rising again from 2016 through to 2017. Like in summer, the negative Sen's slope also confirms a decreasing *E. coli* contamination during the winter-autumn period. Furthermore, the winter season (Figure 13. c) did not show any significant trend, however, notable fluctuations were noted. *E. coli* concentrations remained constant from 2010 to 2011 then followed by moderate fluctuating levels. A rapid decrease in *E. coli* concentration was noted in 2015 and a subsequent pronounced increase until 2017. Sen's slope suggests constant *E. coli* contamination throughout the study period. There was also no significant trend identified in the spring season (Figure 13.d), but a noticeable decline was observed in 2015. Whereas in the other years, *E. coli* remained relatively stable. Overall, Sen's slope indicates that there was a moderate increase in *E. coli* in the in-spring season.

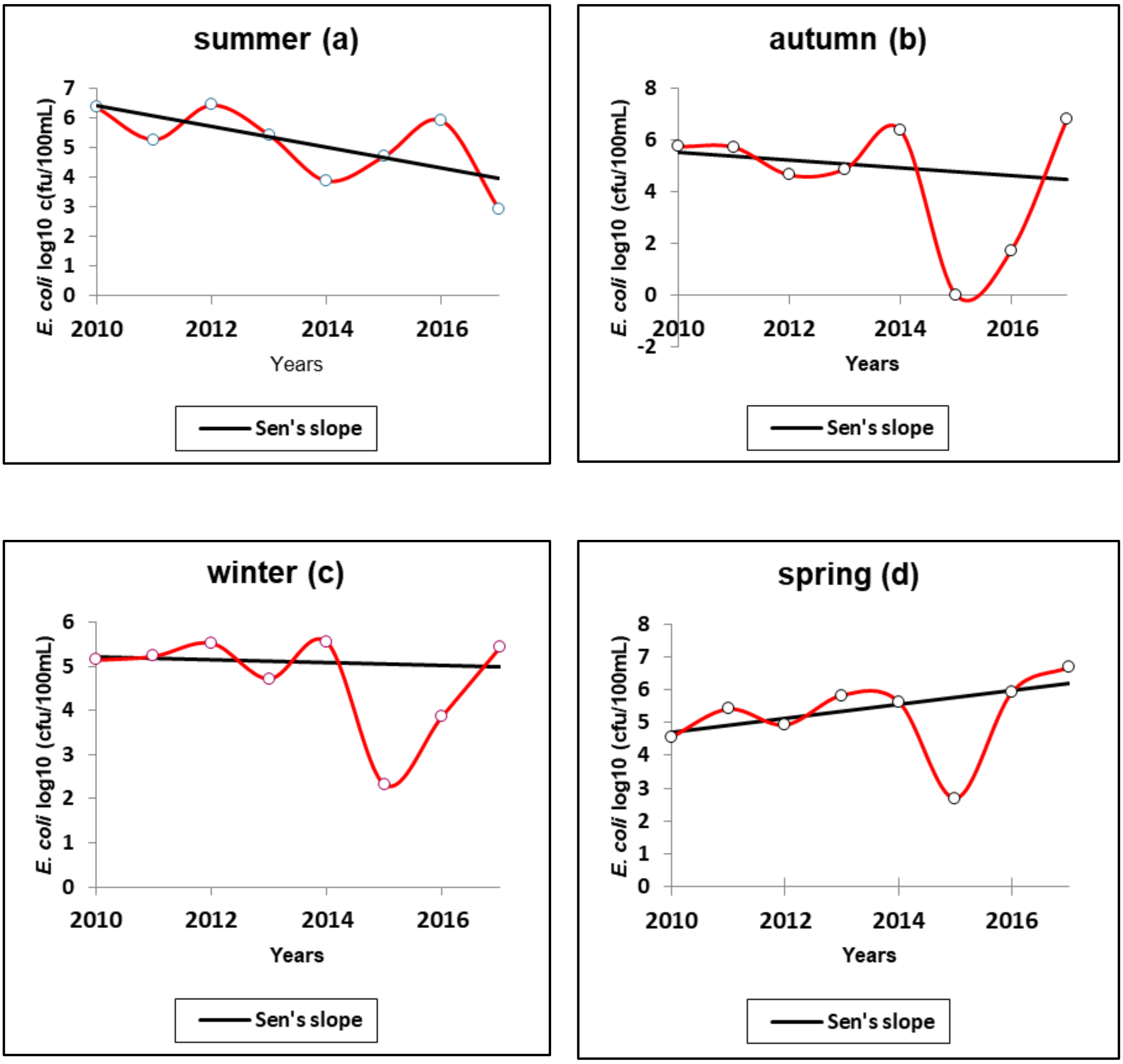



***Figure 13, Seasonal trend of E. coli in the Vaal River during the summer (a); autumn (b); winter (c) and spring (d) from 2010-2017. The data shown in the graph is log10 of cfu/100mL, meaning that one-unit increase in the concentration on the graph corresponds to a tenfold increase in the actual concentration. Black solid line is the Sen's slope estimator which show the magnitude and direction of E. coli levels.***

# CHAPTER SIX: DISCUSSION

## 6.1 Introduction

River water quality degradation is a prevalent issue globally, and South Africa is no exception to this trend. Research conducted by the Department of Water and Sanitation (DWA, 2012) underscores the deteriorating state of wastewater infrastructure systems in the country. The lack of upgrades to non-operational plants and insufficient refurbishment of sewer networks are identified as primary factors contributing to this decline. Assessments such as the Green Drop and Blue Drop reports, aimed at evaluating municipal services with a focus on promoting safe and healthy environments, have revealed widespread neglect of wastewater system maintenance among South African municipalities (Green Drop Report, 2022; Blue Drop Report 2023). Consequently, a significant volume of untreated wastewater is discharged directly into adjacent rivers (Mema, 2010; DWA, 2012; Makhari, 2016). It is essential to recognize that river water quality is influenced by a myriad of factors, encompassing both natural phenomena and human activities. Natural influences, including seasonal variations, interact with anthropogenic inputs such as industrial discharges, agricultural runoff, and urban activities (Palaniappan *et al*., 2010; WHO, 2011). The impact of these activities on river water quality varies depending on factors such as duration, frequency, and intensity, with densely populated areas typically experiencing more pronounced effects compared to sparsely populated regions (Chigor *et al*., 2013; Pirrie *et al*., 2013). Therefore, the water quality of the rivers under this study will be subject to the cumulative impacts of both natural processes and human interventions.

### 6.1.1 The state of faecal contamination in the Limpopo River

During the study period within the Limpopo River, a notable fluctuation in concentrations of *E. coli* was observed spanning from undetectable levels to exceedingly elevated concentrations. Particularly noteworthy were instances of exceptionally heightened *E. coli* levels, exceeding 7.2 million cfu/100mL in 2018, followed by levels surpassing 1 million cfu/100mL in 2016. Such pronounced elevations are indicative of specific episodic events or ongoing sources of pollution operative during those temporal intervals. Of notable concern are wastewater treatment works (WWTWs), acknowledged as among the principal sources of pollution within South Africa (Traore *et al*., 2016; Edokpayi *et al*., 2017; Sekgobela *et al*., 2023).

According to the Green Drop (GD) Report issued by the Department of Water and Sanitation (DWS), spanning the years 2009 to 2013, numerous municipalities along the Limpopo River basin attained an aggregate score of merely 15% in 2013, which further declined to 3% by 2021. These scores indicate non-compliance with the required standards, inadequate operational practices, and potentially poor technical and maintenance of WWTWs. The operational and compliance statuses of approximately 20 WWTWs were scrutinized, revealing that none achieved a GD score exceeding 90% for operational and compliance monitoring (Green Drop Report, 2022). This signifies a prevailing critical state wherein several treatment facilities operate beyond their intended capacity, consequently resulting in inadequately treated municipal wastewater discharged into proximal waterways, subsequently draining into the Limpopo River. Such circumstances likely contribute to the sustained elevation of *E. coli* levels within this water body.

Additionally, it was observed that mean *E. coli* concentrations within the Limpopo River displayed significant interannual variations. These variations divulge erratic fluctuations devoid of any discernible trend. Primarily instigated by anthropogenic activities encompassing informal settlements, agricultural practices within the Limpopo River catchment area, and compromised sewage infrastructure, the observed fluctuations in *E. coli* levels underscore the multifaceted nature of human-induced environmental impacts (Odiyo and Makungo, 2012; Traore *et al*., 2016).

Scholarly studies have underscored the influence of human and animal activities on river water quality (Chigor *et al*., 2013; Nyairo *et al*., 2015). Notably, informal settlements, often lacking adequate sanitation facilities, are predisposed to discharge inadequately treated effluent into water bodies during episodes of heavy rainfall, thereby exacerbating *E. coli* contamination (Odiyo and Makungo, 2012). Furthermore, the indiscriminate disposal of disposable diapers and other waste materials into riverine environments contributes not only to *E. coli* proliferation but also fosters the dissemination of other pathogenic bacteria detrimental to human health (WHO, 2014; Traore *et al*., 2016; Edokpayi *et al*., 2017). Recreational activities such as bathing and laundry conducted in proximity to the Limpopo River further compound faecal contamination concerns (Traore *et al*., 2016). Additionally, agricultural pursuits along the riverbanks, predominantly by subsistence farmers utilizing the adjacent savannah for cattle grazing, represent a significant source of faecal pollution. Studies have correlated the presence of livestock in the vicinity of rivers with heightened pathogen loads, as livestock excreta directly contaminate river water through deposition at the water's edge (Frank *et al*., 2009; Wilkes *et al*., 2013).

### 6.1.2 The influence of seasonal variations in the Limpopo River

The analysis of seasonal dynamics of *E. coli* concentrations within the Limpopo River indicated a nuanced interplay of environmental factors influencing microbial abundance over seasons. While overarching trends across the study duration were not consistently discernible, distinct patterns of elevation and decrease in *E. coli* levels were evident during specific seasons.

During the summer season, a decreasing trend in *E. coli* concentrations was identified. Notable reductions in *E. coli* prevalence were recorded in 2012, 2015, and 2018, counterbalanced by a marked escalation in 2017. These fluctuations coincide temporally with El Niño occurrences affecting South Africa between 2014 and 2016, and again in 2019 (SAWS, 2018; AGRISA, 2019). Characterized by elevated temperatures and arid conditions, El Niño-induced droughts have been documented to impact waterborne microbial dynamics (Lakhraj-Govender and Grab, 2019). During such periods of aridity, a decrease in faecal contamination is anticipated, attributed to diminished rainfall curtailing surface runoff, thereby reducing the influx of faecal matter from agricultural landscapes. Conversely, the notable spike in *E. coli* levels observed in 2017 can be attributed to sporadic precipitation events, augmenting surface runoff and facilitating the transportation of pollutants, including *E. coli*, from livestock operations and informal settlements into adjacent watercourses (Frank *et al*., 2009; Traore *et al*., 2016).

In contrast, the autumn season exhibited no discernible trend. Peaks in contamination were notably observed in 2011 and 2014, with a pronounced decline in 2016 followed by a sharp resurgence in 2018. Intriguingly, while *E. coli* levels abated during the summer of 2018, a marked upsurge was evident in the autumn of the same year. This deviation from anticipated patterns, as explained by Retamozo *et al*. (2010), wherein faecal counts typically peak during the wet season, may be attributable to heavy summer rainfall leading to pollutant dilution, thus precipitating the decline in 2018. Conversely, the escalation in autumn may be attributed to elevated temperatures which is the characteristic of the catchment region, fostering favourable conditions for *E. coli* proliferation (Edokpayi *et al.,* 2018).

The results of this study indicated a decline in *E. coli* levels in winter and an increase in spring. Despite the anticipated decline in *E. coli* levels during winter due to reduced precipitation, a notable increase was observed in winter 2018. The augmentation of *E. coli* concentrations during summer, autumn, and spring seasons may be attributed to various factors, including changes in rainfall patterns impacting microbial water quality. Elevated precipitation during these periods may intensify runoff, facilitating the conveyance of contaminants from surrounding lands into riverine systems, thereby fostering elevated *E. coli* concentrations. Furthermore, temperature fluctuations influence *E. coli* growth rates, with warmer conditions typically fostering bacterial proliferation, a phenomenon corroborated by extant literature (Sibanda *et al*., 2013; Edokpayi *et al*., 2015; Edokpayi *et al*., 2018). Conversely, diminished precipitation during winter precipitates reduced runoff, curtailing the influx of contaminated water into rivers, consequently leading to diminished *E. coli* levels. Additionally, colder temperatures during winter may impede bacterial growth, further contributing to lowered concentrations during this season (Sibanda *et al*., 2013).

### 6.1.3 The state of faecal contamination in the Olifants River

The findings of the study regarding faecal contamination in the Olifants River paint a concerning picture. This river waterbody is one of the important water resources in the Mpumalanga region. Persistent faecal contamination was observed over the years with several years (2012 to 2018) having recorded exceptionally high *E. coli* levels, suggesting severe contamination events during those years. Numerous studies have shown that the Olifants River is one of the most threatened rivers in South Africa. Incidents of crocodile and fish deaths within the river catchment have been reported for several years (WRC, 2011; Graffin *et al*., 2014). This is largely due to the discharge of poorly treated and untreated effluents from industries, mines, and sewage treatment plants in the upper reaches of the river. There are also active and abandoned coal mines that contribute nutrients to the river system creating conducive conditions for *E. coli* to thrive thereby leading to elevated levels (Ashton, 2007; Dabrowski *et al*., 2010). These findings are consistent with Le Roux *et al*. (2012) study that found most sampling sites located downstream of urban areas and intensive mining activities recorded high levels of *E. coli*.

The mean *E. coli* levels also varied greatly between the years 2010 and 2017; 2012 and 2017. The possible primary drivers of *E. coli* contamination during these years are intensive land use activities (i.e. citrus agriculture and game farming) as well as spills from WWTPs which are known to increase faecal indicator loads in rivers and streams (Le Roux *et al*., 2012). These non-point sources in many cases are sporadic contamination events (Loague and Corwin, 2005). According to the DWS State of the Rivers Report on the Olifants River system, the river ecosystem in this area is generally in poor to fair condition (State of Rivers Report, 2019). Most towns and smaller communities (i.e. towns in Mpumalanga Highveld) discharge untreated or partially treated industrial and domestic effluents into various Olifants tributaries (WRC, 2011; Le Roux *et al.*, 2012).

The Green Drop (GD) report, released by the Department of Water and Sanitation (DWS) stressed that elevated levels of waste come from municipality WWTPs (Green Drop Report, 2022). These plants are generally considered to be the primary source of pollution. The report lists most WWTPs along the Olifants River as being in poor or critical conditions, meaning that they release contaminated water. It also stressed in the report that the levels of pollutants received by the plants for treatment are the same as the levels of pollutants released by the plants into the nearby Olifants River. Eight WWTWs were assessed for operational and compliance monitoring status. All the sewage plants assessed did not pass the satisfactory GD

>90% for operation and only 2 of 8 achieved the GD >90% for compliance (Green Drop Report, 2022).

### 6.1.4 The influence of seasonal variations in the Olifants River

In the Olifants River, seasonal trends in *E. coli* revealed an interesting pattern across different seasons. Summer season did not show any significant trend but there was an overall stable increase over the years as per the Mann-Kendall test and Sen's slope. Notable fluctuations were observed including an increase from 2010 through to 2012 with a sudden decline in 2014 and an increase in 2015 and remained stable thereafter. Interestingly, a sharp decrease was observed in 2018 of particular interest is the rapid decline noted in 2018. As previously mentioned, that in 2018, South Africa experienced an El Nino event which caused low rainfall in many parts of the country including the Olifants River catchment between 2015/2016 and 2018/2019 (Copernicus, 2019). Studies have shown that reduced precipitation significantly contributes to the decrease of *E. coli* contamination because of reduced runoff (Ashton and Dabrowski, 2010; Dabrowski *et al*., 2014; Nkhonjera *et al*., 2021). Therefore, this could be the reason there was a sharp decline during the summer period.

Conversely, there was a significant increasing trend in *E. coli* concentration during the autumn season. The Olifants River catchment is characterized by intensive agriculture, and autumn is a critical time for farmers to apply fertilizers and manure in their fields in preparation in preparation for planting. During periodic rains in the catchment, these chemicals are washed into the nearby streams and rivers, thereby contributing to contamination of the receiving waterbody (Bollmohr, *et al*., 2008). Furthermore, the autumn season often coincides with the movement of livestock to different grazing areas or the intensification of animal farming which is also characteristic of the river catchment (Ashton and Dabrowski, 2010). The increased presence of animal waste, if not managed properly, can contribute to higher *E. coli* levels in the river (Ashton and Dabrowski, 2010).

Interestingly, the winter season exhibited a significant increasing trend over the study period. Oftentimes, faecal contamination in waterbodies tends to decrease during dry winters. However, in the Olifants River, this is not the case. This can be caused by several factors. During the winter period, there is reduced water flow which can lead to stagnation in the river allowing *E. coli* to proliferate more easily. Moreover, cold temperatures can slow down natural processes such as sunlight-mediated disinfection that would otherwise degrade *E. coli* (Griffin *et al*., 2014). Additionally, the river catchment experiences sporadic winter rainfall which can transport pollutants such as faeces from the surrounding land into the river (Dabrowski and De Klerk, 2013). These factors are likely the drivers of increased *E. coli* contamination in the Olifants River during winter. On the other hand, the spring season did not show any significant trend. However, the fluctuation observed was like that of summer with a sharp decrease again observed in 2018. This persistent decline in 2018 stems from multiple factors. The spring season brings rainfall, which results in higher water levels and faster flows in the river. This swift flow can help flush out *E. coli* from the water, decreasing the concentration.

### 6.1.5 The state of faecal contamination in the Inkomati River.

In the Olifants River, there were inconsistent *E. coli* levels across the years. Despite instances of zero *E. coli* readings, the variability observed suggests ongoing pollution challenges. This variability within each year underscores the dynamic nature of the pollution sources affecting the river. There was no statistical significance of mean *E. coli* levels between years. Moreover, there was no clear trend indicating whether *E. coli* levels increased or decreased over the study period. Like many other rivers, the Inkomati River is also affected by various pollution agents. As previously mentioned in Chapter 2 the river catchment area is characterized by extensive irrigated agriculture, mining, and manufacturing industries. Such activities are known to be major pollution sources that contribute to faecal contamination of rivers through runoff.

According to the Green Drop report undertaken in Mpumalanga from 76 wastewater treatment systems, it was found that all the municipalities had an average score of 36% in the 2010/2011 report. A total of 50 WWTWs were profiled and only seven of those 50 were authorized to operate. Only three WWTWs complied with the required standards and only four were known to operate within their capacity (IUCMA, 2014; du Plessis, 2019). Additionally, most of WWTWs was overloaded and overflowing with raw sewage flowing directly into nearby tributaries and finally into the Inkomati River (du Plessis, 2019). This could be one of the possible drivers behind elevated *E. coli* counts in the river.

### 6.1.6 The influence of seasonal variations in the Inkomati River.

The observed seasonal variations and trends in *E. coli* levels in the Inkomati River provide valuable insights into the dynamics of microbial contamination in this water body. During the summer season, a significant decreasing trend was observed. The summer season showed interestingly unexpected results. *E. coli* levels often tend to increase during the wet season due to factors like warmer temperatures. However, in this study, a significant decrease can be attributed to several factors. The summer season often coincides with a period of increased rainfall, resulting in higher river flow. Increased water flow can help to flush out *E. coli* thereby reducing their concentrations. Additionally, increased rainfall can also lead to greater dilution of *E. coli* (Riddell *et al*., 2020).

Unlike summer, the autumn season exhibited fluctuations without a clear increasing or decreasing trend, although there was a pronounced increase in 2015. However, despite the absence of a trend as there was an overall decreasing *E. coli* level. Conversely, both winter and spring seasons also displayed fluctuations without a defined trend. Nevertheless, similar to autumn, *E. coli* levels decreased during these seasons. The absence of clear trends in autumn, winter, and spring underscores the complexity of microbial pollution dynamics, potentially influenced by various environmental and anthropogenic factors.

The Autumn season in the Inkomati River catchment is characterized by less rainfall and lower temperatures compared to the summer season (Riddell *et al*., 2020). These conditions can lead to reduced runoff which is a common mechanism for transporting pollutants including *E. coli* into rivers and streams (Xie *et al*., 2022). Also, the autumn season marks the period of reduced agricultural operations such as irrigation, pesticides, and fertilizer application. With fewer agricultural operations, there may be a decreased runoff carrying agricultural pollutants (i.e. animal waste and fertilizers) from farmlands into the river thus contributing to lower *E. coli* levels (Tau *et al*., 2021). Furthermore, the observed decrease in the winter season can be attributed to cooler temperatures. *E. coli* thrives in warmer temperatures, however, in winter there are cooler temperatures which can slow down *E. coli* growth and reproduction. Additionally, colder temperatures can also increase die-off rates of existing *E. coli* in the water thereby reducing *E. coli* levels (Sibanda *et al*., 2013).

### 6.1.7 The state of faecal contamination in the Mzimvubu River.

In the Mzimvubu River, the results demonstrated several important findings over the period from 2010 to 2017. It was observed that some years there was no *E. coli* detected with minimum values of zero, suggesting periods of relatively low contamination. However, in 2011 and 2012, minimum values were above the detection threshold indicating the presence of *E. coli* in the river during these years. Notably, 2011 stood out with particularly elevated contamination levels. These are surprising findings simply because the Mzimvubu River is considered one of the least modified river systems in the country (Mbele and Mbungazi, 2023). The elevated levels of *E. coli* can be caused by flooding that was recorded in 2012 affecting various parts of the Eastern Cape (Dalu *et al*., 2018). There are rural and peri-urban settlements in the upper reaches of the Mzimvubu River. During heavy rainfall organic waste from these settlements is transported into the river thus leading to elevated *E. coli* counts. Other studies have stressed that water quality in the Eastern Cape remains of poor quality (Momba *et al*., 2004). This situation was exacerbated by the municipality sewage systems that were in critical condition. The Blue Drop conducted in 2012 also showed that only a few of the Eastern Cape municipalities received the Blue Drop certificate (Blue Drop Report, 2012).

Average/mean *E. coli* levels varied considerable between years. The observed average/mean *E. coli* levels fluctuated over the study period. With elevated levels in 2011 followed by fluctuations through to 2017. The Mzimvubu River catchment along with its tributaries is mainly dominated by isolated patches of commercial farming and few municipal areas that are considered significant in terms of effluent discharge (Freshwater Health Index, 2022). Therefore, fluctuations in mean *E. coli* concentrations can be attributed to livestock grazing and waste from the surrounding farms.

**6.1.8 The influence of seasonal variations in the Mzimvubu River.**

The assessment of seasonal variations in *E. coli* levels in the Mzimvubu River revealed intriguing patterns. During the summer season, no significant trend was detected, as evidenced by a p-value $>0.075$ from the Mann-Kendall test. Nonetheless, the negative Kendall Tau and Sen's Slope suggested a decrease in *E. coli* levels during this period (Table 10). This finding contrasts with the typical expectation of increased *E. coli* levels in summer. The observed fluctuations indicate complex dynamics in microbial water quality, coinciding with a multi-year drought recorded from 2015 to 2020 in the Eastern Cape province (Archer *et al.*, 2022). Drought periods are associated with reduced precipitation and runoff into rivers, leading to decreased transportation of contaminants, including *E. coli*, from surrounding land into the river (Singh and Lin, 2015).

Conversely, a significant downward trend was detected in autumn and similarly in winter. These trends can be attributed to the cold winter seasons and reduced rainfall in the Eastern Cape, inhibiting *E. coli* growth and reproduction, as the bacteria thrive in warmer temperatures. This decrease in metabolic activity leads to a decline in *E. coli* levels in the river (Mamera, 2018). Additionally, coinciding with periods of drought, *E. coli* levels tend to decrease due to reduced input of faecal matter from agricultural runoff, which is a primary activity in the Mzimvubu River catchment (Mamera, 2018; Dalu *et al*., 2018). In contrast, no clear trend was identified during the spring. However, *E. coli* levels exhibited a decreasing trend over the study period. Similar to the summer season, the decreasing levels during spring could also be attributed to persistent droughts in the region.

### 6.1.9 The state of faecal contamination in the Breede River

In the Breede River, it was found that *E. coli* contamination displayed relatively stable levels, particularly during the years, 2010 through 2012. However, there were instances of high pollution levels in 2013 where exceptionally elevated *E. coli* levels were recorded. Such elevated levels show that there were specific pollution events that contributed during that period. These finds corroborate the research carried out by the Breede-Gouritz Catchment Management Agency (BGCMA) which assessed water quality in this catchment between 2010 and 2015. The study attributed significantly high values of faecal coliforms and *E. coli* to the irrigation return flow into the river and agricultural practices such as cereal crop cultivation and wine production (BGCMA, 2016). Previous studies have indicated that the state of water quality in the Breede River has been deteriorating since the 1960s (Kirchner, 1994; Murray *et al*., 1989; Cullis *et al*., 2018)

It was also observed that mean *E. coli* concentrations varied from year to year in the Breede River. This variability could be influenced by various factors such as sewage discharge, and human activities along the river basin. Generally, microbial water quality in the upper reaches of the Breede River and many of its tributaries is moderately good, however, it becomes progressively poorer in the lower reaches. This is due to rapid growth in urban and peri-urban areas and commercial farming (DWS, 2010; Cullis *et al*., 2018). Also, within the catchment area, informal settlements that lack adequate sanitation facilities contribute to the microbial life that ends up in the river (Gcanga *et al*., 2018). These findings are consistent with numerous studies that have reported exceptionally high levels of *E. coli* levels in some of the rivers in the Western Cape region. Paulse *et al*. (2009) reported increased *E. coli* levels reaching well over 3.5 million cfu/100 in the Plankeburg River. Ackermann. (2010) also reported *E. coli* counts of 160, 000 cfu/100mL in this river.

### 6.1.10 The influence of seasonal variations in the Breede River.

The assessment of faecal contamination in the Breede River revealed interesting seasonal trends in *E. coli* counts. In summer, while there was no significant trend, there was a notable decrease in *E. coli* counts. Although there was a spike in 2012, subsequent years showed a decline throughout the study period. The Breede River originates in the Western Cape region, which experiences winter rainfall (Cullis *et al*., 2018). This explains why *E. coli* counts showed a decline over the years. During the summer period, there is less rainfall to carry pollutants including *E. coli* into the river. Similarly, the autumn season did not exhibit a clear trend, but there were irregular peaks observed in 2011, 2013 and 2019. Although there was no clear trend, there was an overall decrease which can also be caused by dry conditions in the river catchment. Moreover, spikes in certain years stem from periodic rains that are common in the Western Cape. Winter and spring seasons showed parallel patterns in *E. coli*, with no significant trends. However. both seasons experienced stable *E. coli* counts in from 2010 through to 2012 followed by a substantial decline until 2018, with a subsequent increase in 2019. The findings coincide with the severe drought period that was experienced in the Western Cape region. The Cape Town water crisis which led to the city experiencing a “Day Zero” period started in 2015 and persisted until 2018 leading to extremely dry conditions (Ziervogel, 2019). This is likely the plausible reason for the drastic decline in *E. coli* contamination in the Breede River.

### 6.1.11 The state of faecal contamination in the Berg Olifants River

It is evident, as would be expected that the water quality in the Berg Olifants River has deteriorated over the years. Several years during the study period exhibited extremely high levels of *E. coli.* The highest *E. coli* levels were in 2019 reaching as high as 9,000,540 cfu/100mL and the lowest being 192 cfu/100mL. Such a considerable variability can be attributed to various anthropogenic activities in the river catchment. The river catchment is dominated by industries as well as other anthropogenic activities of the Cape Town Metropolitan area. These include intensive irrigated agriculture (wine production), extensive commercial agriculture, some industries related to food processing and packaging and livestock farming (Paulse *et al*., 2012). These activities are primary contributors to increased *E. coli* levels in this water body. A study by du Plessis and du Plessis. (2017) has stressed that the surface water quality of the Berg River is in a poor state, particularly in areas located around urban centres (i.e. Cape Town Metropolitan area) as well as downstream of cultivated areas. Furthermore, the study found that 14 WWTWs were not operating optimally (du Plessis and du Plessis, 2017).

It was observed that mean *E. coli* concentration also varied significantly between years. Such variability demonstrates that various factors are contributing to the deterioration of water quality in the Berg River. Although the catchment area of the Berg River is largely rural, it has experienced an increase in population and a substantial increase in urban development over the years (Lotter, 2010). As the population increases and urban areas expand, there is also an increase in domestic and industrial waste which is discharged directly into the river thereby contributing to elevated faecal pollution in the Berg River (Lotter, 2010).

### 6.1.12 The influence of season variations in the Berg Olifants River.

The assessment of seasonal variations on *E. coli* in the Berg Olifants River revealed intriguing patterns that underscore the complex interplay of environmental factors. There was a lack of significant trends in *E. coli* contamination during both summer and autumn seasons. Despite this, the observation of specific years within these seasons unveiled noteworthy fluctuations. In summer, *E. coli* counts remained relatively stable from 2010 to 2012 and then experienced a sudden decline until 2016, only to peak in 2018 before decreasing rapidly in 2019. This corroborates with trends observed in the Breede River. The Berg and Breede Rivers fall within the Western Cape region which experienced severe droughts in 2010/2011 as well as 2015 until 2018 (Dube *et al*., 2022). Drought is typically associated with reduced rainfall, with less water flowing into the river system, there is a reduced likelihood of contaminants being washed into the watercourse from surrounding land such as agricultural areas where *E. coli* can originate from livestock faeces or other sources. As a result, the overall input of *E. coli* in the Berg River decreases during drought conditions.

The analysis extended to winter and spring seasons, where again, no discernible trend emerged. However, closer inspection revealed distinct temporal patterns. In winter, *E. coli* pollution remained relatively stable until 2013, after which it decreased rapidly until 2018 before rising again in 2019. Meanwhile, spring exhibited stability, but a decline was noted in 2016, a peak in 2018 and a slight decrease in 2019. These findings underscore the dynamic nature of *E. coli* contamination in the Berg River, shaped by climatic influences. Likewise, the severe drought recorded in the Western Cape is the primary contributor to the decline observed in both the winter and spring seasons.

### 6.1.13 The state of faecal contamination in the Phongolo River

The Phongolo River, an important watercourse for the KwaZulu-Natal region has been subjected to alarming faecal pollution. The results obtained in this study showed that this water body has experienced heightened *E. coli* levels over the years. *E. coli* concentrations recorded over the study period ranged from a few thousand to well over 2.4 million cfu/100mL. Of particular concern is the 2015 year which recorded 2,447,712 cfu/10mL followed by 2014 with 322,259 cfu/100mL. Such elevated levels can be attributed to a diverse array of pollution sources impacting microbial water quality in the region. One important source could be wastewater treatment works operating beyond their capacity within the catchment area. Discharging untreated or partially treated wastewater into nearby streams leads to faecal contamination of the receiving water (Abia *et al.*, 2015b).

### 6.1.14 The influence of season variations in the Phongolo River.

During the summer period, an overall decrease in *E. coli* was noted. The decreasing *E. coli* concentration observed in the Phongolo River can be attributed to drought that was experienced in Kwa-Zulu Natal between 2015 and 2017 (Vetter *et al*., 2020). A long-term study conducted by Ndlovu and Demlie. (2020) has found that the northern part of the province including Phongolo district was hit by severe drought between 2015 and 2017. Drought years significantly impact *E. coli* concentration in rivers, typically resulting in a decline. Various studies have found that severe droughts can cause sections of rivers to dry up completely thus eliminating *E. coli* presence in those areas (Mishra and Singh, 2010; Vetter *et al*., 2020; Mishra *et al*., 2021). Of a particular importance is a sharp decline of *E. coli* in 2017. This sudden drop indicates a potential intervention or improvement in water quality management practices. This also can be due to implemented measures to reduce pollution sources. Furthermore, the Pongolapoort Dam can also be a contributing factor through various mechanis such as sediment trapping (Dube *et al*., 2015). Dams trap sediments that would otherwise be carried downstream. These sediments often contain *E. coli* attached to soil particles or organic matter. By trapping these sediments, dams effectively reduce the downstream transport of *E. coli* (de Necker *et al*., 2019)

**6.1.15 The state of faecal contamination in the Vaal River**

The Vaal River, like many other rivers, faces several microbial water quality challenges. The results of the study revealed substantial variability in *E. coli* concentrations in the Vaal River. Minimum and maximum concentrations varied considerably each year with some years (2011, 2013, 2014, 2017) exhibiting exceptionally high levels. These elevated levels could have been caused by wastewater from industries located in the upper reaches. Importantly, high *E. coli* levels coincide with a period of heavy rainfall that was recorded in many parts of Gauteng (Floodlist, 2017). Moreover, tributaries that drain the Vaal River pass through cultivated areas, highly populated areas, and mining areas (du Plessis, 2019). Studies have shown that in densely populated areas there is a lack of adequate sanitation infrastructure and improper disposal of solid waste which is a major issue in the Vaal region (Tempelhof, 2009; Wepener *et al*., 2011; du Plessis and du Plessis, 2017). Heavy rainfall can overwhelm aging sewage infrastructure leading to sewer overflows. The overflow releases poorly treated sewage into the river thus contributing to increased levels of faecal contamination. Organic waste from surrounding settlements can be washed off during heavy rainfall and worsen the existing sanitation challenges (du Plessis, 2021). As a result, flooding in these areas can lead to the mixing of sewage with surface water thereby increasing the risk of microbial contamination in the Vaal River.

The Vaal River has been previously considered a dumping site for farmers, industries, and surrounding towns (Dikio, 2010). As such, the elevated *E. coli* levels can be attributed to several pollution sources. Activities that threaten water resources in the Vaal River catchment include the steel industries which use water to cool steel products around Vereeniging and Vanderbijlpark areas. Wastewater from these industries is discharged back into the Vaal River and often it is partially treated (Wepener *et al*., 2011). Also, dysfunctional sanitation infrastructure in the local municipalities poses a threat as many of the treatment plants operate above their capacity, thus resulting in sewage spills (du Plessis, 2017).

The upper reaches of the Vaal River are largely altered by developments, while the middle reaches have few major developments with mining and agriculture being the primary activities (du Plessis, 2021). The lower areas are also less developed with only agriculture being the dominant land use. Various studies have shown that this waterboy passes through these heavily industrialized areas which have significantly contributed to the widespread deterioration of water quality (Dikio, 2010; CSIR, 2010; du Plessis, 2021). Furthermore, studies have shown that water quality issues in the Vaal River are largely caused by recent water hyacinth and lettuce. Although these invasive aquatic species do not directly contribute to faecal pollution, however, they cover surface water thereby creating stagnant conditions. These conditions create an inducive environment for bacterial growth including *E. coli* (Hill *et al*., 2020; Lukhele and Msagati, 2024). This explains the variability in *E. coli* levels observed over the years which shows that the Vaal River experiences faecal contamination from an array of pollution sources.

### 6.1.16 The influence of season variations in the Vaal River.

The Mann-Kendall analysis conducted for different seasons revealed varying trends in *E. coli* contamination levels in the Vaal River. During the summer season, an overall decrease in *E. coli* Was recorded and irregular peaks were observed in 2012 and 2016. The fluctuating patterns suggest multifaceted factors may have influenced *E. coli* contamination during summer. These include changes in rainfall and temperature fluctuations. Delpla *et al*. (2009) found that an increase in temperatures and heavy rainfall causes an increase in the concentration of water pollutants. However, it is noteworthy to state that there are two schools of thought regarding the influence of heavy rainfall on water pollution. The first one is that rainfall may dilute pollutants in the river due to increased water volumes, and the second is that during heavy rainfall, there is increased runoff which consequently leads to elevated faecal contamination in the receiving water resources (Delpla *et al*., 2009). As a result, *E. coli* contamination levels may fluctuate during the summer season.

fluctuation *E. coli* levels during autumn could be attributed to a combination of factors such as changes in rainfall patterns and changes in human activities within the river catchment or along the riverbanks. For example, the autumn season in South Africa marks the change from the wet season to the dry season (Koning *et al*., 2000). Therefore, changes in rainfall during this period can influence the flow of river water and the dilution of pollutants. Years with heavy rainfall events can result in increased runoff, carrying pollutants from the surrounding urban and rural areas into the river, while dry years may result in concentrated contaminants due to reduced dilution (Delpla *et al*., 2009). Such events explain the observed fluctuations during the autumn season in the Vaal River.

Both winter and spring seasons demonstrated similar patterns in *E. coli* contamination. Contamination levels were stable from 2010 to 2014, then declined in 2015 before increasing in 2016 and 2017. A moderate decrease in *E. coli* during winter and a moderate increase in spring likely stem from a complex interplay of anthropogenic and environmental factors. Changes in rainfall patterns during these periods may have played a role. For example, the winter season typically experiences reduced precipitation, while spring is often characterized by sporadic heavy rainfall events. These rainfall patterns impact dilution capacities in the river, with reduced rainfall leading to less dilution of *E. coli* during winter, whereas intermittent sporadic rains in spring can facilitate the mobilization of *E. coli* through surface runoff (Haji, 2011). Also, spring marks the beginning of planting season in many agricultural regions of the Vaal catchment area. The application of fertilizers and pesticides during this period contributed to elevated *E. coli* levels in 2017 through the deposition of animal manure on the fields which can be washed into the nearby tributaries and discharged into the Vaal main river (Dikio, 2010).

# CHAPTER SEVEN: Conclusion and Recommendations.

## 7.1 Concluding remarks.

This study aimed to assess faecal pollution trends in major South African river systems, focusing particularly on *E. coli* as a faecal indicator bacteria. The study has provided much-needed insights into the trends of faecal pollution in some of South Africa's most important river systems. The findings revealed concerning patterns of faecal contamination across all eight rivers studied, showcasing varying degrees of *E. coli* contamination over time. Elevated *E. coli* levels pose a significant threat to the microbial water quality of these rivers. These findings are in line with existing literature documenting widespread faecal contamination in many South African rivers.

Temporal analysis unveiled notable trends in *E. coli* contamination levels, with some rivers showing slight improvements while others experiencing alarming escalations in contamination levels. These increases are particularly prominent during periods of heightened environmental stressors such as floods and droughts. An in-depth assessment of potential *E. coli* pollution sources revealed a complex interplay of natural and anthropogenic factors contributing to faecal pollution in the studied rivers. Wastewater treatment works, and industrial discharges exacerbate the challenges of maintaining clean and safe river water. Additionally, localized sources of *E. coli* pollution such as informal settlements and livestock rearing exert impacts on nearby streams, emphasizing the importance of interventions at the community level.

The implications of this study extend far beyond the realms of environmental sciences. The findings not only underscore the intrinsic link between faecal pollution and the ecological integrity of rivers but also highlight the intricate interdependence between river ecosystems and human livelihoods. The first objective of the study was to assess the temporal patterns of *E. coli* within eight major river systems in South Africa. The study revealed that *E. coli* levels across the rivers increased greatly over the decade. These findings indicate that South African rivers have experienced worsening faecal contamination over time, which might be due to factors such as increased population density or inadequate sanitation. The second objective was to use ANOVA to identify statistically significant disparities in mean *E. coli* concentrations across different years and among the specified rivers. ANOVA analysis revealed that the mean *E. coli* concentrations differ significantly among the rivers. The significant disparity suggests that certain rivers are more heavily impacted by faecal contamination. This could highlight areas needing targeted intervention and resource allocation for pollution control. The last objective was to assess the impact of seasonal variations on *E. coli* contamination using Seasonal Mann-Kendall and Sen's slope analysis. Seasonal Mann-Kendall analysis showed an increase in *E. coli* contamination during the rainy season across all rivers. The increase in *E. coli* contamination during the rainy season could be attributed to runoff from agricultural lands, urban areas and effluents from WWTWs. This highlights the need for improved stormwater management and runoff control measures during the rainy season to reduce contamination. The study effectively meets its objectives by providing a detailed analysis of *E. coli* contamination trends, identifying significant disparities among rivers, and understanding seasonal impacts. These insights are crucial for informing policy reforms, enhancing sanitation infrastructure, and improving community practices to manage and reduce faecal pollution in South African rivers

Millions of individuals depend on rivers as a vital source of water for domestic use, recreation, and irrigation, forming the backbone of agricultural activities and sustaining communities across vast regions. Therefore, addressing faecal pollution is not merely an environmental concern but a critical imperative for safeguarding public health and ensuring the resilience of communities in the face of evolving environmental challenges. Considering these findings, addressing faecal contamination in South African rivers becomes imperative, necessitating a multifaceted approach integrating community engagement, technological innovations, and policy reforms. Strengthening regulatory frameworks for river water quality management, investing in sustainable sanitation infrastructure, and enforcing strict pollution measures are pivotal steps toward reducing *E. coli* contamination. Furthermore, enhancing wastewater practices and capabilities, promoting eco-friendly agricultural practices, and raising awareness about hygiene practices can alleviate the pressures of faecal pollution on river ecosystems.

In conclusion, this study underscores the urgent need for concerted efforts to combat faecal pollution in South Africa, mitigating severe impacts on river ecosystems and human health. The study's findings show the importance of the National Water Act as a critical tool in managing faecal pollution in South African rivers. By linking these findings to the legal obligations and frameworks established under the NWA, it becomes evident that addressing faecal contamination is not only a public health and environmental imperative but also a legal responsibility. The significant disparities in *E. coli* levels observed across different rivers, as identified in the study, may reflect inconsistencies in maintaining the ecological integrity of rivers. For example, the deterioration of water quality in the studied rivers over the past decade signals a potential failure to meet the legal requirements for water resource classification, necessitating a review of current management strategies. By enhancing understanding of faecal contamination sources and trends, this study lays the groundwork for informed decision-making in South Africa. Only through steadfast commitment and collaborative action can rivers be safeguarded as vital natural assets for current and future generations.

## 7.2 Recommendations

Faecal contamination continues to be a major challenge in South African rivers with elevated and persistent *E. coli* levels. The following recommendations are proposed to mitigate faecal pollution in South African major rivers:

- Upgrade and invest in wastewater treatment works (WWTWs) to increase their capacities and efficiency which can reduce the impact on the receiving waters.
- Improve already existing wetlands in each river catchment to help rivers cope with effluents from WWTWs, industries and agriculture. Wetlands are known to be useful in mitigating both point and non-point sources of pollution.
- Improve sanitation infrastructure in rural and peri-urban areas and promote reuse and recycling of wastewater initiatives to reduce faecal pollution from domestic sources.

## 7.3 Limitations of the study

This study utilized secondary data for its analysis, and as such, several limitations are applicable:

- The analysis of microbial water quality data primarily concentrated on *Escherichia coli (E. coli)* as a faecal bacteria indicator. While *E. coli* is commonly used as an indicator organism, the exclusion of other microbial contaminants may overlook the impacts associated with other different pathogens.
- Utilization of secondary datasets carries inherent risks of bias, as these datasets are often aggregated from multiple monitoring stations. This aggregation may obscure the heterogeneity in microbial water quality within individual rivers, leading to potential inaccuracies in presenting localized pollution sources or specific environmental conditions at different locations.

- The study assessed faecal contamination exclusively within the confines of eight major rivers. This limited scope may overlook potential variations in water quality dynamics within smaller tributaries thereby constraining the generalizability of findings to a broader geographical context.
- Some of the DWS monitoring stations were subjected to short monitoring periods and irregular data collection intervals. This sporadic data collection creates gaps within the overall dataset, potentially compromising the comprehensive understanding of temporal patterns and trends in faecal contamination.

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# Appendices

## Appendix A: post-hoc results of mean *E. coli* levels between years in the Limpopo River from 2010 to 2018.

| Tukey's multiple comparison | Mean Diff | 95,00% CI of ( | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -0,6904 | -1,998 to 0,6174 | No | ns | 0,7744 |
| 2010 vs. 2012 | 0,8945 | -0,9124 to 2,701 | No | ns | 0,8305 |
| 2010 vs. 2013 | -0,5952 | -2,221 to 1,031 | No | ns | 0,9664 |
| 2010 vs. 2014 | -1,182 | -2,403 to 0,03969 | No | ns | 0,0666 |
| 2010 vs. 2015 | -1,083 | -2,439 to 0,2731 | No | ns | 0,2372 |
| 2010 vs. 2016 | -1,364 | -3,237 to 0,5087 | No | ns | 0,3584 |
| 2010 vs. 2017 | -1,337 | -3,088 to 0,4145 | No | ns | 0,2945 |
| 2010 vs. 2018 | -2,394 | -3,587 to -1,201 | Yes | * | <0,0001 |
| 2011 vs. 2012 | 1,585 | -0,08803 to 3,258 | No | ns | 0,079 |
| 2011 vs. 2013 | 0,09514 | -1,380 to 1,570 | No | ns | >0,9999 |
| 2011 vs. 2014 | -0,4912 | -1,504 to 0,5212 | No | ns | 0,8458 |
| 2011 vs. 2015 | -0,3926 | -1,564 to 0,7789 | No | ns | 0,9805 |
| 2011 vs. 2016 | -0,6736 | -2,417 to 1,070 | No | ns | 0,9539 |
| 2011 vs. 2017 | -0,6464 | -2,259 to 0,9662 | No | ns | 0,943 |
| 2011 vs. 2018 | -1,704 | -2,682 to -0,7256 | Yes | * | <0,0001 |
| 2012 vs. 2013 | -1,49 | -3,421 to 0,4420 | No | ns | 0,2811 |
| 2012 vs. 2014 | -2,076 | -3,682 to -0,4699 | Yes | * | 0,0023 |
| 2012 vs. 2015 | -1,977 | -3,688 to -0,2665 | Yes | * | 0,0107 |
| 2012 vs. 2016 | -2,258 | -4,402 to -0,1148 | Yes | * | 0,0304 |
| 2012 vs. 2017 | -2,231 | -4,270 to -0,1928 | Yes | * | 0,0203 |
| 2012 vs. 2018 | -3,289 | -4,873 to -1,704 | Yes | * | <0,0001 |
| 2013 vs. 2014 | -0,5863 | -1,986 to 0,8129 | No | ns | 0,9271 |
| 2013 vs. 2015 | -0,4877 | -2,006 to 1,031 | No | ns | 0,9851 |
| 2013 vs. 2016 | -0,7687 | -2,762 to 1,225 | No | ns | 0,9543 |
| 2013 vs. 2017 | -0,7416 | -2,621 to 1,138 | No | ns | 0,9479 |
| 2013 vs. 2018 | -1,799 | -3,174 to -0,4243 | Yes | * | 0,0019 |
| 2014 vs. 2015 | 0,09859 | -0,9755 to 1,173 | No | ns | >0,9999 |
| 2014 vs. 2016 | -0,1824 | -1,862 to 1,497 | No | ns | >0,9999 |
| 2014 vs. 2017 | -0,1552 | -1,699 to 1,388 | No | ns | >0,9999 |
| 2014 vs. 2018 | -1,213 | -2,072 to -0,3535 | Yes | * | 0,0005 |
| 2015 vs. 2016 | -0,281 | -2,061 to 1,499 | No | ns | >0,9999 |
| 2015 vs. 2017 | -0,2538 | -1,906 to 1,398 | No | ns | >0,9999 |
| 2015 vs. 2018 | -1,311 | -2,353 to -0,2693 | Yes | * | 0,0034 |
| 2016 vs. 2017 | 0,02717 | -2,070 to 2,124 | No | ns | >0,9999 |
| 2016 vs. 2018 | -1,03 | -2,689 to 0,6292 | No | ns | 0,5842 |
| 2017 vs. 2018 | -1,057 | -2,578 to 0,4637 | No | ns | 0,4247 |

*** = significant**

**ns= Not Significant**

**Green blocks = significantly different mean**

**Appendix B: post-hoc results of mean *E. coli* levels between years in the Olifants River from 2010 to 2018.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -165381 | -1459496 to 1128734 | No | ns | >0,9999 |
| 2010 vs. 2012 | 1229 | -1292887 to 1295344 | No | ns | >0,9999 |
| 2010 vs. 2013 | -1004096 | -2298211 to 290020 | No | ns | 0,2641 |
| 2010 vs. 2014 | -551482 | -1845597 to 742633 | No | ns | 0,9127 |
| 2010 vs. 2015 | -499306 | -1793421 to 794809 | No | ns | 0,9494 |
| 2010 vs. 2016 | -1323566 | -2617681 to -29451 | Yes | * | 0,0409 |
| 2010 vs. 2017 | -1181246 | -2475361 to 112870 | No | ns | 0,1025 |
| 2010 vs. 2018 | -734768 | -2028884 to 559347 | No | ns | 0,6816 |
| 2011 vs. 2012 | 166610 | -1127505 to 1460725 | No | ns | >0,9999 |
| 2011 vs. 2013 | -838715 | -2132830 to 455401 | No | ns | 0,5093 |
| 2011 vs. 2014 | -386101 | -1680216 to 908015 | No | ns | 0,9896 |
| 2011 vs. 2015 | -333925 | -1628040 to 960190 | No | ns | 0,9961 |
| 2011 vs. 2016 | -1158185 | -2452300 to 135930 | No | ns | 0,1175 |
| 2011 vs. 2017 | -1015865 | -2309980 to 278251 | No | ns | 0,2498 |
| 2011 vs. 2018 | -569387 | -1863502 to 724728 | No | ns | 0,897 |
| 2012 vs. 2013 | -1005324 | -2299440 to 288791 | No | ns | 0,2626 |
| 2012 vs. 2014 | -552711 | -1846826 to 741405 | No | ns | 0,9117 |
| 2012 vs. 2015 | -500535 | -1794650 to 793580 | No | ns | 0,9487 |
| 2012 vs. 2016 | -1324795 | -2618910 to -30679 | Yes | * | 0,0406 |
| 2012 vs. 2017 | -1182474 | -2476590 to 111641 | No | ns | 0,1018 |
| 2012 vs. 2018 | -735997 | -2030112 to 558118 | No | ns | 0,6796 |
| 2013 vs. 2014 | 452614 | -841501 to 1746729 | No | ns | 0,9716 |
| 2013 vs. 2015 | 504790 | -789326 to 1798905 | No | ns | 0,9461 |
| 2013 vs. 2016 | -319470 | -1613586 to 974645 | No | ns | 0,9971 |
| 2013 vs. 2017 | -177150 | -1471265 to 1116965 | No | ns | >0,9999 |
| 2013 vs. 2018 | 269327 | -1024788 to 1563443 | No | ns | 0,9991 |
| 2014 vs. 2015 | 52176 | -1241940 to 1346291 | No | ns | >0,9999 |
| 2014 vs. 2016 | -772084 | -2066200 to 522031 | No | ns | 0,6207 |
| 2014 vs. 2017 | -629764 | -1923879 to 664351 | No | ns | 0,8321 |
| 2014 vs. 2018 | -183286 | -1477402 to 1110829 | No | ns | >0,9999 |
| 2015 vs. 2016 | -824260 | -2118375 to 469855 | No | ns | 0,5334 |
| 2015 vs. 2017 | -681940 | -1976055 to 612176 | No | ns | 0,7623 |
| 2015 vs. 2018 | -235462 | -1529577 to 1058653 | No | ns | 0,9997 |
| 2016 vs. 2017 | 142320 | -1151795 to 1436436 | No | ns | >0,9999 |
| 2016 vs. 2018 | 588798 | -705317 to 1882913 | No | ns | 0,8781 |
| 2017 vs. 2018 | 446477 | -847638 to 1740593 | No | ns | 0,9738 |

*** = Significant**

**ns = Not Significant**

**Green blocks = significantly different means**

**Appendix C: post-hoc results of mean *E. coli* levels between years in the Inkomati River from 2010 to 2018.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Below threshold | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | 3082 | -4717 to 10880 | No | ns | 0,9569 |
| 2010 vs. 2012 | 2231 | -5567 to 10030 | No | ns | 0,9954 |
| 2010 vs. 2013 | 3573 | -4226 to 11371 | No | ns | 0,8974 |
| 2010 vs. 2014 | 3636 | -4163 to 11434 | No | ns | 0,8872 |
| 2010 vs. 2015 | -2907 | -10706 to 4891 | No | ns | 0,9703 |
| 2010 vs. 2016 | 2943 | -4856 to 10741 | No | ns | 0,9678 |
| 2010 vs. 2017 | 3605 | -4194 to 11403 | No | ns | 0,8923 |
| 2010 vs. 2018 | 4623 | -3176 to 12422 | No | ns | 0,6593 |
| 2010 vs. 2019 | 4725 | -3074 to 12524 | No | ns | 0,6306 |
| 2011 vs. 2012 | -850,3 | -8649 to 6948 | No | ns | >0,9999 |
| 2011 vs. 2013 | 491,1 | -7308 to 8290 | No | ns | >0,9999 |
| 2011 vs. 2014 | 553,9 | -7245 to 8353 | No | ns | >0,9999 |
| 2011 vs. 2015 | -5989 | -13788 to 1810 | No | ns | 0,2908 |
| 2011 vs. 2016 | -138,9 | -7938 to 7660 | No | ns | >0,9999 |
| 2011 vs. 2017 | 522,9 | -7276 to 8322 | No | ns | >0,9999 |
| 2011 vs. 2018 | 1541 | -6257 to 9340 | No | ns | 0,9997 |
| 2011 vs. 2019 | 1643 | -6155 to 9442 | No | ns | 0,9996 |
| 2012 vs. 2013 | 1341 | -6457 to 9140 | No | ns | >0,9999 |
| 2012 vs. 2014 | 1404 | -6394 to 9203 | No | ns | 0,9999 |
| 2012 vs. 2015 | -5139 | -12937 to 2660 | No | ns | 0,5121 |
| 2012 vs. 2016 | 711,3 | -7087 to 8510 | No | ns | >0,9999 |
| 2012 vs. 2017 | 1373 | -6425 to 9172 | No | ns | >0,9999 |
| 2012 vs. 2018 | 2392 | -5407 to 10190 | No | ns | 0,9923 |
| 2012 vs. 2019 | 2494 | -5305 to 10292 | No | ns | 0,9896 |
| 2013 vs. 2014 | 62,83 | -7736 to 7861 | No | ns | >0,9999 |
| 2013 vs. 2015 | -6480 | -14279 to 1319 | No | ns | 0,1935 |
| 2013 vs. 2016 | -630 | -8429 to 7169 | No | ns | >0,9999 |
| 2013 vs. 2017 | 31,83 | -7767 to 7830 | No | ns | >0,9999 |
| 2013 vs. 2018 | 1050 | -6748 to 8849 | No | ns | >0,9999 |
| 2013 vs. 2019 | 1152 | -6646 to 8951 | No | ns | >0,9999 |
| 2014 vs. 2015 | -6543 | -14342 to 1256 | No | ns | 0,1829 |
| 2014 vs. 2016 | -692,8 | -8491 to 7106 | No | ns | >0,9999 |
| 2014 vs. 2017 | -31 | -7830 to 7768 | No | ns | >0,9999 |
| 2014 vs. 2018 | 987,4 | -6811 to 8786 | No | ns | >0,9999 |
| 2014 vs. 2019 | 1089 | -6709 to 8888 | No | ns | >0,9999 |
| 2015 vs. 2016 | 5850 | -1949 to 13649 | No | ns | 0,3229 |
| 2015 vs. 2017 | 6512 | -1287 to 14311 | No | ns | 0,1881 |
| 2015 vs. 2018 | 7530 | -268,3 to 15329 | No | ns | 0,0677 |
| 2015 vs. 2019 | 7632 | -166,3 to 15431 | No | ns | 0,0604 |
| 2016 vs. 2017 | 661,8 | -7137 to 8460 | No | ns | >0,9999 |
| 2016 vs. 2018 | 1680 | -6118 to 9479 | No | ns | 0,9995 |
| 2016 vs. 2019 | 1782 | -6016 to 9581 | No | ns | 0,9992 |
| 2017 vs. 2018 | 1018 | -6780 to 8817 | No | ns | >0,9999 |
| 2017 vs. 2019 | 1120 | -6678 to 8919 | No | ns | >0,9999 |
| 2018 vs. 2019 | 102 | -7697 to 7901 | No | ns | >0,9999 |

**ns = Not Significant**

**Appendix D: post-hoc results of mean *E. coli* between years in the Mzimvubu River from 2010 to 2017.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -217513 | -346171 to -88855 | Yes | * | <0,0001 |
| 2010 vs. 2012 | -80230 | -208888 to 48428 | No | ns | 0,529 |
| 2010 vs. 2013 | 19664 | -108994 to 148322 | No | ns | 0,9997 |
| 2010 vs. 2014 | 15181 | -116108 to 146470 | No | ns | >0,9999 |
| 2010 vs. 2015 | 19584 | -109074 to 148242 | No | ns | 0,9997 |
| 2010 vs. 2016 | 19305 | -111984 to 150595 | No | ns | 0,9998 |
| 2010 vs. 2017 | 19921 | -108737 to 148579 | No | ns | 0,9997 |
| 2011 vs. 2012 | 137284 | 14613 to 259954 | Yes | * | 0,0174 |
| 2011 vs. 2013 | 237177 | 114507 to 359848 | Yes | * | <0,0001 |
| 2011 vs. 2014 | 232694 | 107267 to 358122 | Yes | * | <0,0001 |
| 2011 vs. 2015 | 237097 | 114426 to 359767 | Yes | * | <0,0001 |
| 2011 vs. 2016 | 236819 | 111391 to 362246 | Yes | * | <0,0001 |
| 2011 vs. 2017 | 237434 | 114764 to 360105 | Yes | * | <0,0001 |
| 2012 vs. 2013 | 99894 | -22777 to 222564 | No | ns | 0,1969 |
| 2012 vs. 2014 | 95410 | -30017 to 220838 | No | ns | 0,2724 |
| 2012 vs. 2015 | 99813 | -22857 to 222484 | No | ns | 0,1977 |
| 2012 vs. 2016 | 99535 | -25892 to 224963 | No | ns | 0,2242 |
| 2012 vs. 2017 | 100151 | -22520 to 222821 | No | ns | 0,1943 |
| 2013 vs. 2014 | -4483 | -129911 to 120944 | No | ns | >0,9999 |
| 2013 vs. 2015 | -80,42 | -122751 to 122590 | No | ns | >0,9999 |
| 2013 vs. 2016 | -358,6 | -125786 to 125069 | No | ns | >0,9999 |
| 2013 vs. 2017 | 257,1 | -122413 to 122928 | No | ns | >0,9999 |
| 2014 vs. 2015 | 4403 | -121025 to 129830 | No | ns | >0,9999 |
| 2014 vs. 2016 | 4125 | -124000 to 132250 | No | ns | >0,9999 |
| 2014 vs. 2017 | 4740 | -120687 to 130168 | No | ns | >0,9999 |
| 2015 vs. 2016 | -278,2 | -125706 to 125149 | No | ns | >0,9999 |
| 2015 vs. 2017 | 337,5 | -122333 to 123008 | No | ns | >0,9999 |
| 2016 vs. 2017 | 615,7 | -124812 to 126043 | No | ns | >0,9999 |

*** = Significant**

**ns = Not Significant**

**Green blocks = significantly different means**

**Appendix E*:* post-hoc results of mean *E. coli* levels between years in the Breede River from 2010 to 2019.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -2100 | -38701 to 34500 | No | ns | >0,9999 |
| 2010 vs. 2012 | -770,8 | -38194 to 36653 | No | ns | >0,9999 |
| 2010 vs. 2013 | -21300 | -57900 to 15301 | No | ns | 0,577 |
| 2010 vs. 2014 | 7385 | -29216 to 43986 | Yes | * | 0,0327 |
| 2010 vs. 2018 | 7040 | -29561 to 43641 | Yes | * | 0,0014 |
| 2010 vs. 2019 | 7006 | -29594 to 43607 | No | ns | 0,9972 |
| 2011 vs. 2012 | 1330 | -36094 to 38753 | No | ns | >0,9999 |
| 2011 vs. 2013 | -19199 | -55800 to 17402 | No | ns | 0,6898 |
| 2011 vs. 2014 | 9485 | -27115 to 46086 | Yes | * | 0,0169 |
| 2011 vs. 2018 | 9141 | -27460 to 45741 | Yes | * | 0,0004 |
| 2011 vs. 2019 | 9107 | -27494 to 45708 | No | ns | 0,9885 |
| 2012 vs. 2013 | -20529 | -57952 to 16895 | No | ns | 0,6433 |
| 2012 vs. 2014 | 8156 | -29268 to 45579 | No | ns | 0,9943 |
| 2012 vs. 2018 | 7811 | -29612 to 45234 | No | ns | 0,9955 |
| 2012 vs. 2019 | 7777 | -29646 to 45201 | No | ns | 0,9956 |
| 2013 vs. 2014 | 28684 | -7916 to 65285 | Yes | * | 0,0156 |
| 2013 vs. 2018 | 28340 | -8261 to 64941 | Yes | * | 0,0003 |
| 2013 vs. 2019 | 28306 | -8295 to 64907 | No | ns | 0,238 |
| 2014 vs. 2018 | -344,7 | -36945 to 36256 | No | ns | >0,9999 |
| 2014 vs. 2019 | -378,6 | -36979 to 36222 | No | ns | >0,9999 |
| 2018 vs. 2019 | -33,92 | -36635 to 36567 | No | ns | >0,9999 |

*** = Significant**

**ns = Not Significant**

**Green blocks = significantly different means**

**Appendix F*:* post-hoc results of mean *E. coli* between years in the Berg Olifants River from 2010 to 2019.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -420838 | -2426738 to 1585061 | No | ns | 0,9979 |
| 2010 vs. 2012 | -360012 | -2365911 to 1645888 | No | ns | 0,9992 |
| 2010 vs. 2013 | 38398 | -1967502 to 2044298 | No | ns | >0,9999 |
| 2010 vs. 2014 | 733013 | -1272887 to 2738913 | Yes | * | <0,0001 |
| 2010 vs. 2016 | 733039 | -1317943 to 2784021 | Yes | * | <0,0001 |
| 2010 vs. 2018 | -6970 | -2110775 to 2096836 | Yes | * | <0,0001 |
| 2010 vs. 2019 | -1097052 | -3102952 to 908848 | No | ns | 0,6874 |
| 2011 vs. 2012 | 60827 | -1945073 to 2066727 | No | ns | >0,9999 |
| 2011 vs. 2013 | 459237 | -1546663 to 2465137 | No | ns | 0,9964 |
| 2011 vs. 2014 | 1153852 | -852048 to 3159752 | Yes | * | <0,0001 |
| 2011 vs. 2016 | 1153878 | -897104 to 3204859 | Yes | * | <0,0001 |
| 2011 vs. 2018 | 413869 | -1689937 to 2517674 | Yes | * | <0,0001 |
| 2011 vs. 2019 | -676214 | -2682114 to 1329686 | No | ns | 0,9655 |
| 2012 vs. 2013 | 398410 | -1607490 to 2404310 | Yes | * | 0,0424 |
| 2012 vs. 2014 | 1093025 | -912875 to 3098925 | Yes | * | <0,0001 |
| 2012 vs. 2016 | 1093051 | -957931 to 3144033 | Yes | * | <0,0001 |
| 2012 vs. 2018 | 353042 | -1750764 to 2456847 | Yes | * | <0,0001 |
| 2012 vs. 2019 | -737041 | -2742941 to 1268859 | No | ns | 0,9455 |
| 2013 vs. 2014 | 694615 | -1311285 to 2700515 | Yes | * | <0,0001 |
| 2013 vs. 2016 | 694641 | -1356341 to 2745623 | Yes | * | <0,0001 |
| 2013 vs. 2018 | -45368 | -2149174 to 2058437 | Yes | * | 0,0007 |
| 2013 vs. 2019 | -1135451 | -3141350 to 870449 | No | ns | 0,6488 |
| 2014 vs. 2016 | 25,8 | -2050956 to 2051008 | No | ns | >0,9999 |
| 2014 vs. 2018 | -739983 | -2843789 to 1363822 | No | ns | 0,9566 |
| 2014 vs. 2019 | -1830066 | -3835965 to 175834 | Yes | * | <0,0001 |
| 2016 vs. 2018 | -740009 | -2886842 to 1406824 | No | ns | 0,961 |
| 2016 vs. 2019 | -1830091 | -3881073 to 220890 | Yes | * | <0,0001 |
| 2018 vs. 2019 | -1090082 | -3193888 to 1013723 | Yes | * | <0,0001 |

*** = Significant**

**ns = Not Significant**

**Green blocks = significantly different means**

**Appendix G: post-hoc results of mean *E. coli* levels between years in the Pongolo River from 2010 to 2017.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | 214,6 | -317915 to 318344 | No | ns | >0,9999 |
| 2010 vs. 2012 | -716 | -318845 to 317413 | No | ns | >0,9999 |
| 2010 vs. 2013 | -18078 | -336207 to 300051 | No | ns | >0,9999 |
| 2010 vs. 2014 | -43703 | -361832 to 274426 | No | ns | 0,9999 |
| 2010 vs. 2015 | -208101 | -526230 to 110028 | No | ns | 0,4674 |
| 2010 vs. 2016 | 1290 | -316839 to 319419 | No | ns | >0,9999 |
| 2010 vs. 2017 | 4578 | -313552 to 322707 | No | ns | >0,9999 |
| 2011 vs. 2012 | -930,6 | -319060 to 317199 | No | ns | >0,9999 |
| 2011 vs. 2013 | -18292 | -336421 to 299837 | No | ns | >0,9999 |
| 2011 vs. 2014 | -43918 | -362047 to 274211 | No | ns | 0,9999 |
| 2011 vs. 2015 | -208315 | -526444 to 109814 | No | ns | 0,466 |
| 2011 vs. 2016 | 1076 | -317053 to 319205 | No | ns | >0,9999 |
| 2011 vs. 2017 | 4363 | -313766 to 322492 | No | ns | >0,9999 |
| 2012 vs. 2013 | -17362 | -335491 to 300767 | No | ns | >0,9999 |
| 2012 vs. 2014 | -42987 | -361116 to 275142 | No | ns | 0,9999 |
| 2012 vs. 2015 | -207385 | -525514 to 110744 | No | ns | 0,4719 |
| 2012 vs. 2016 | 2006 | -316123 to 320135 | No | ns | >0,9999 |
| 2012 vs. 2017 | 5294 | -312836 to 323423 | No | ns | >0,9999 |
| 2013 vs. 2014 | -25626 | -343755 to 292504 | No | ns | >0,9999 |
| 2013 vs. 2015 | -190023 | -508152 to 128106 | No | ns | 0,5851 |
| 2013 vs. 2016 | 19368 | -298761 to 337497 | No | ns | >0,9999 |
| 2013 vs. 2017 | 22655 | -295474 to 340784 | No | ns | >0,9999 |
| 2014 vs. 2015 | -164397 | -482527 to 153732 | No | ns | 0,7463 |
| 2014 vs. 2016 | 44994 | -273136 to 363123 | No | ns | 0,9998 |
| 2014 vs. 2017 | 48281 | -269848 to 366410 | No | ns | 0,9998 |
| 2015 vs. 2016 | 209391 | -108738 to 527520 | No | ns | 0,4592 |
| 2015 vs. 2017 | 212678 | -105451 to 530807 | No | ns | 0,4385 |
| 2016 vs. 2017 | 3287 | -314842 to 321416 | No | ns | >0,9999 |

**ns = Not Significant**

**Appendix H: post-hoc results of mean *E. coli* levels between years in the Vaal River from 2010 to 2017.**

| Tukey's multiple comparisons test | Mean Diff, | 95,00% CI of diff, | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 2010 vs. 2011 | -166848 | -1243500 to 909804 | No | ns | 0,9997 |
| 2010 vs. 2012 | 27449 | -1049203 to 1104101 | No | ns | >0,9999 |
| 2010 vs. 2013 | -169012 | -1245664 to 907640 | No | ns | 0,9997 |
| 2010 vs. 2014 | -187024 | -1263676 to 889628 | No | ns | 0,9994 |
| 2010 vs. 2015 | 102100 | -974552 to 1178752 | No | ns | >0,9999 |
| 2010 vs. 2016 | -375 | -1077027 to 1076277 | No | ns | >0,9999 |
| 2010 vs. 2017 | -911225 | -1987877 to 165427 | No | ns | 0,16 |
| 2011 vs. 2012 | 194297 | -882355 to 1270949 | No | ns | 0,9992 |
| 2011 vs. 2013 | -2163 | -1078815 to 1074489 | No | ns | >0,9999 |
| 2011 vs. 2014 | -20176 | -1096828 to 1056477 | No | ns | >0,9999 |
| 2011 vs. 2015 | 268948 | -807704 to 1345600 | No | ns | 0,994 |
| 2011 vs. 2016 | 166473 | -910179 to 1243125 | No | ns | 0,9997 |
| 2011 vs. 2017 | -744376 | -1821028 to 332276 | No | ns | 0,394 |
| 2012 vs. 2013 | -196460 | -1273112 to 880192 | No | ns | 0,9992 |
| 2012 vs. 2014 | -214473 | -1291125 to 862179 | No | ns | 0,9985 |
| 2012 vs. 2015 | 74651 | -1002001 to 1151303 | No | ns | >0,9999 |
| 2012 vs. 2016 | -27824 | -1104476 to 1048828 | No | ns | >0,9999 |
| 2012 vs. 2017 | -938673 | -2015325 to 137979 | No | ns | 0,1342 |
| 2013 vs. 2014 | -18012 | -1094664 to 1058640 | No | ns | >0,9999 |
| 2013 vs. 2015 | 271112 | -805540 to 1347764 | No | ns | 0,9937 |
| 2013 vs. 2016 | 168637 | -908015 to 1245289 | No | ns | 0,9997 |
| 2013 vs. 2017 | -742213 | -1818865 to 334439 | No | ns | 0,3978 |
| 2014 vs. 2015 | 289124 | -787528 to 1365776 | No | ns | 0,9906 |
| 2014 vs. 2016 | 186649 | -890003 to 1263301 | No | ns | 0,9994 |
| 2014 vs. 2017 | -724201 | -1800853 to 352451 | No | ns | 0,4303 |
| 2015 vs. 2016 | -102475 | -1179127 to 974177 | No | ns | >0,9999 |
| 2015 vs. 2017 | -1013325 | -2089977 to 63327 | No | ns | 0,0803 |
| 2016 vs. 2017 | -910850 | -1987502 to 165802 | No | ns | 0,1604 |

**ns = Not Significant**

**Appendix I: post-hoc results of mean *E. coli* concentrations between rivers (2010 to 2019).**

**Tukey HSD**

| (I) River | River (J) | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval Lower Bound | 95% Confidence Interval Upper Bound |
|---|---|---|---|---|---|---|
| Limpopo | Olifants | 19652,45688 | 19988,25789 | >0,977 | -40940,6516 | 80245,5653 |
| | Inkomati | 151854.43609* | 20276,24473 | <0,000 | 90388,3142 | 213320,5580 |
| | Mzimvubu | 107597.01786* | 28206,92265 | <0,003 | 22089,5598 | 193104,4759 |
| | Breede | 151356.53841* | 24669,88470 | <0,000 | 76571,3817 | 226141,6951 |
| | Berg Olifants | 74182.67852* | 18704,05471 | <0,002 | 17482,5488 | 130882,8082 |
| | Pongolo | 139976.28652* | 16654,05140 | <0,000 | 89490,6090 | 190461,9641 |
| | Vaal | 134803.47759* | 19773,65093 | <0,000 | 74860,9363 | 194746,0189 |
| Olifants | Limpopo | -19652,45688 | 19988,25789 | >0,977 | -80245,5653 | 40940,6516 |
| | Inkomati | 132201.97921* | 18240,22661 | <0,000 | 76907,9143 | 187496,0441 |
| | Mzimvubu | 87944.56098* | 26780,78138 | <0,023 | 6760,3578 | 169128,7642 |
| | Breede | 131704.08153* | 23025,70254 | <0,000 | 61903,1565 | 201505,0066 |
| | Berg Olifants | 54530.22164* | 16474,87266 | <0,021 | 4587,7129 | 104472,7304 |
| | Pongolo | 120323.82964* | 14104,50972 | <0,000 | 77566,9225 | 163080,7368 |
| | Vaal | 115151.02071* | 17679,84834 | <0,000 | 61555,7062 | 168746,3352 |
| Inkomati | Limpopo | - 151854.43609* | 20276,24473 | <0,000 | - 213320,5580 | -90388,3142 |
| | Olifants | - 132201.97921* | 18240,22661 | <0,000 | - 187496,0441 | -76907,9143 |
| | Mzimvubu | -44257,41824 | 26996,40528 | >0,726 | - 126095,2713 | 37580,4349 |
| | Breede | -497,89768 | 23276,13851 | >1,000 | -71058,0031 | 70062,2078 |
| | Berg Olifants | -77671.75757* | 16823,11136 | <0,000 | - 128669,9294 | -26673,5857 |
| | Pongolo | -11878,14957 | 14509,74987 | >0,992 | -55863,5160 | 32107,2168 |
| | Vaal | -17050,95850 | 18004,79614 | >0,981 | -71631,3312 | 37529,4142 |
| Mzimvubu | Limpopo | - 107597.01786* | 28206,92265 | <0,003 | - 193104,4759 | -22089,5598 |
| | Olifants | -87944.56098* | 26780,78138 | <0,023 | - 169128,7642 | -6760,3578 |
| | Inkomati | 44257,41824 | 26996,40528 | >0,726 | -37580,4349 | 126095,2713 |
| | Breede | 43759,52055 | 30434,89788 | >0,840 | -48501,9000 | 136020,9411 |
| | Berg Olifants | -33414,33934 | 25836,43669 | >0,902 | - 111735,8228 | 44907,1441 |
| | Pongolo | 32379,26867 | 24393,38488 | >0,889 | -41567,6968 | 106326,2341 |
| | Vaal | 27206,45973 | 26620,98926 | >0,971 | -53493,3440 | 107906,2635 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Breede | Limpopo | -151356.53841* | 24669,88470 | <0,000 | -226141,6951 | -76571,3817 |
| | Olifants | -131704.08153* | 23025,70254 | <0,000 | -201505,0066 | -61903,1565 |
| | Inkomati | 497,89768 | 23276,13851 | >1,000 | -70062,2078 | 71058,0031 |
| | Mzimvubu | -43759,52055 | 30434,89788 | >0,840 | -136020,9411 | 48501,9000 |
| | Berg Olifants | -77173.85989* | 21920,17761 | <0,010 | -143623,4578 | -10724,2620 |
| | Pongolo | -11380,25189 | 20199,25622 | >0,999 | -72612,9881 | 49852,4843 |
| | Vaal | -16553,06082 | 22839,65400 | >0,996 | -85789,9918 | 52683,8701 |
| Berg Olifants | Limpopo | -74182.67852* | 18704,05471 | <0,002 | -130882,8082 | -17482,5488 |
| | Olifants | -54530.22164* | 16474,87266 | <0,021 | -104472,7304 | -4587,7129 |
| | Inkomati | 77671.75757* | 16823,11136 | <0,000 | 26673,5857 | 128669,9294 |
| | Mzimvubu | 33414,33934 | 25836,43669 | >0,902 | -44907,1441 | 111735,8228 |
| | Breede | 77173.85989* | 21920,17761 | <0,010 | 10724,2620 | 143623,4578 |
| | Pongolo | 65793.60800* | 12216,72639 | <0,000 | 28759,3937 | 102827,8223 |
| | Vaal | 60620.79907* | 16213,82887 | <0,005 | 11469,6276 | 109771,9705 |
| Pongolo | Limpopo | -139976.28652* | 16654,05140 | <0,000 | -190461,9641 | -89490,6090 |
| | Olifants | -120323.82964* | 14104,50972 | <0,000 | -163080,7368 | -77566,9225 |
| | Inkomati | 11878,14957 | 14509,74987 | >0,992 | -32107,2168 | 55863,5160 |
| | Mzimvubu | -32379,26867 | 24393,38488 | >0,889 | -106326,2341 | 41567,6968 |
| | Breede | 11380,25189 | 20199,25622 | >0,999 | -49852,4843 | 72612,9881 |
| | Berg Olifants | -65793.60800* | 12216,72639 | <0,000 | -102827,8223 | -28759,3937 |
| | Vaal | -5172,80893 | 13798,69602 | >1,000 | -47002,6617 | 36657,0438 |
| Vaal | Limpopo | -134803.47759* | 19773,65093 | <0,000 | -194746,0189 | -74860,9363 |
| | Olifants | -115151.02071* | 17679,84834 | >0,000 | -168746,3352 | -61555,7062 |
| | Inkomati | 17050,95850 | 18004,79614 | >0,981 | -37529,4142 | 71631,3312 |
| | Mzimvubu | -27206,45973 | 26620,98926 | >0,971 | -107906,2635 | 53493,3440 |
| | Breede | 16553,06082 | 22839,65400 | >0,996 | -52683,8701 | 85789,9918 |
| | Berg Olifants | -60620.79907* | 16213,82887 | <0,005 | -109771,9705 | -11469,6276 |
| | Pongolo | 5172,80893 | 13798,69602 | >1,000 | -36657,0438 | 47002,6617 |

***. The mean difference is significant at the 0.05 level.**
**Shaded blocks = significantly different mean.**