MICROBIAL AND PHYSICO-CHEMICAL QUALITY OF SOME SURFACE WATER RESOURCES IN DURBAN, SOUTH AFRICA

Presented by

K O V A S H N E E N A I C K E R

Submitted in fulfilment of the academic requirements for the degree of Master of Science (MSc) in the Discipline of Microbiology, School of Biochemistry, Genetics and Microbiology, Faculty of Science and Agriculture at the University of KwaZulu-Natal, Durban.

As the candidate's supervisor, I have approved this dissertation for submission.

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PREFACE

The experimental work described in this dissertation was carried out in the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa from January 2008 to December 2009, under the supervision of Professor B. Pillay and the co-supervision of Dr. A. O. Olaniran.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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Abstract

Microbial and chemical contamination of inland and coastal waters in Southern Africa is a major challenge facing the water industry and regulatory authorities. Increased stresses on these surface water resources through human and environmental influences have resulted in deteriorating water quality that has severely encumbered the country's capability to provide sufficient water to meet its needs and to ensure environmental sustainability. In addition, indiscriminate use of antibiotics has resulted in widespread contamination of surface waters, leading to accelerated development of antibiotic resistance and proliferation of resistant water-borne diarrhoeal-related pathogens, such as *Escherichia coli* and *Vibrio cholerae*. Despite the high level of contamination of South African surface waters, the microbiological quality of rivers and beaches in Durban, South Africa, have not been adequately investigated. Therefore, the current study assessed the seasonal fluctuations of the microbial and physico-chemical quality of two rivers (Umgeni River and Umdloti River) and six beaches (Virginia Aerodome, Beachwood, Umgeni South, Battery, Sunkist, Addington) in Durban, using several bacterial indicator organisms and physico-chemical parameters as indices. The antibiotic resistance profiles (ARPs) of *E. coli* and *V. cholerae* strains, recovered from the water samples, were determined and changes in the microbial community of the water samples were monitored over a seasonal cycle, using denaturing gradient gel electrophoresis (DGGE). Spatial and seasonal fluctuations of the physico-chemical parameters differed significantly ($p < 0.05$) among the water samples with high heavy metal concentrations detected across the seasonal cycle. Temperature profiles ranged from 13°C to 26.5°C for the Umgeni River, 13°C to 27°C for the beaches and 12°C to 26°C for the Umdloti River while pH ranged from 6.30 to 8.45 (Umgeni River), 6.37 to 8.30 (beaches) and 5.96 to 7.94 (Umdloti River). Turbidity ranged from 0.53 NTU to 15.6 NTU (Umgeni River); 0.57 NTU to 2.37 NTU (beaches) and 2.23 NTU to 18.8 NTU (Umdloti River). During spring and summer, all river and beach water samples had < 500 µg/L phosphate concentrations; however, these concentrations increased significantly ($p < 0.05$) during autumn and winter in both rivers. Majority of the samples had low concentrations of ammonia and nitrates. Sulphate concentrations for the beach samples ranged from 2355 mg/L (B5 – summer) to 2899 mg/L (B2 – winter) as compared to the Umgeni and Umdloti Rivers which ranged from 3.90 mg/L (A4 – autumn) to 2762 mg/L (A1 – summer) and 4.47 mg/L (C4 – winter) to 168 mg/L (C1 – winter), respectively. According to the South African Target Quality Range guidelines for the heavy metals (in surface waters), all river and beach water samples exceeded the set limits for lead (Pb^{2+}), mercury (Hg^{2+}) and cadmium (Cd^{2+}) across all seasons. During spring and summer all water samples complied with the aluminium guideline of 0 – 0.15 mg/L. Bacterial population profiles indicated that all sampling points failed to comply with the set guidelines (domestic use) for presumptive total coliform (TC), faecal coliform (FC) and total heterotrophic bacterial (THB) counts during all four seasons. Estimated TC, FC and THB populations as high as 8.6×10^1, 3.7×10^1 and 2.15×10^5 cfu/100ml,

respectively, were obtained for some of the samples with peak indicator levels and generally a higher microbial load observed during the summer season. High prevalence of resistance to ampicillin [67.82% (Umgeni River)] was encountered among the *E. coli* isolates from the water samples followed by amikacin [53.33% (Umdloti River)], augmentin [49.6% (Umdloti River)], tetracycline [42% (Umgeni River)], streptomycin [37.1% (beaches)] and cotrimoxazole [33% (Umgeni River)]. The most frequently encountered form of resistance among the *V. cholerae* isolates was against cotrimoxazole [93.34% (Umgeni River)], streptomycin [84% (beaches)], erythromycin [78.7% (Umgeni River)], trimethoprim [77.7% (Umdloti River)], rifampicin [70% (Umgeni River)] and cefoxitin [45% (Umdloti River)]. Multi-drug resistance among the *E. coli* isolates was indicated by twenty nine (Umgeni River), twenty six (beaches) and fourteen (Umdloti River) different resistance patterns, while the *V. cholerae* isolates produced eighteen (Umgeni River), thirty five (beaches) and twenty nine (Umdloti River) different resistance patterns. In addition, proportional resistances of the *E. coli* and *V. cholerae* strains to the different classes of antibiotics ranged from six to eleven and four to eleven different antibiotic classes, respectively. The present study suggests that the bacterial communities detected in the water samples collected from the rivers and beaches in Durban, followed seasonal dynamics and could possibly be the consequence of fluctuations in certain environmental factors. A total of 87 different DGGE bands were detected among the Umgeni River water samples, 127 different DGGE bands among the six beach water samples and 107 bands in the Umdloti River samples, over the four seasons. Twenty one dominant bands were found among all sampling sites, indicating widespread phylotypes, whereas 14 bands were exclusively detected at only one sampling site (C1) potentially indicating unique phylotypes. Some bands appeared all year-round, whereas some other bands were specific to a particular season. Overall, the present study successfully demonstrated the poor microbiological quality of the investigated river and beach water resources which raise concerns over the management of these water resources and the subsequent deleterious effects these waters could have on the end users. This emphasizes the need for implementation of improved management strategies of these river catchments and beaches for continued sustainability. Furthermore, the high level of multi-antibiotic resistance demonstrated by the *E. coli* and *V. cholerae* strains, recovered from the water samples, reiterates the need to continuously monitor the changing trends in antimicrobial resistance patterns of these diarrhoeal-related bacterial pathogens. Therefore, continued surveillance of these surface waters used for recreational or domestic purposes and development of adequate prevention strategies are needed for public health reasons. Lastly, combining the use of conventional faecal indicators with molecular-based techniques, such as DGGE, can provide more information on the microbial load and diversity of surface waters. In addition, information regarding the effects of seasonal variations on microbial diversity as observed in this study is important for the sustainable management of surface water resources.

1.1 Introduction

Water is the most important constituent of the life support system. Although water is generally considered a recyclable resource, meticulous management and protection is required as this resource is highly vulnerable to over-exploitation and pollution due to industrial growth, urbanization and anthropogenic problems mainly as a result of population growth (May *et al.,* 2006; Ouyang *et al.,* 2006). The chief sources of water pollution are sewage, industrial effluents, agricultural discharges and industrial wastes from chemical industries, fossil fuel plants and nuclear power plants (Taljaard and Kayombo, 2007). This has resulted in the degradation of a significant proportion of our usable water resources and aquifers, leading to deterioration of quality, reduced integrity of these resources and dramatic social repercussions. Reversing this threat and achieving sustainability will require an integrated approach in order to manage water, land and ecosystems, one that takes into account socio-economic and environmental needs (Durham *et al.,* 2002).

Recently, concerns over water quality have increased due to frequent contamination of coastal and inland water resources by water-borne bacterial, viral and protozoan pathogens. Pathogen contamination of water systems has become a frequent occurrence across the globe (Belkin and Colwell, 2006; Herwaldt *et al.,* 1992; Howe *et al.,* 2002; Hsu *et al.,* 2000; Lisle and Rose, 1995; MacKenzie *et al.,* 1994; Zuckerman *et al.,* 1997). Among the pathogens disseminated in water sources, enteric pathogens are most frequently encountered. Since these pathogens occur periodically in natural waters at low concentrations, the systematic search for all potential strains of entero-pathogens is impractical, making their detection and quantification difficult to perform (Savichtcheva and Okabe, 2006). Therefore, routine microbiological water analyses are based on the enumeration of various indicator organisms which are used to indicate the presence of pathogens in aquatic ecosystems and water resources, thereby evaluating the extent of faecal contamination of these waters and, the sanitary risk associated with utilization of the water (Ashbolt *et al.,* 2001). However, the ecological and survival characteristics of these pathogens vary under different environmental conditions, indicating that there is no universal indicator organism that can suggest the presence of all enteric pathogens for all types of waters (Ashbolt *et al.,* 2001). While the presence of coliforms could still indicate faecal contamination, their absence can no longer be assumed that the water is not polluted and safe for human use. Thus, existing bacterial indicators and indicator approaches do not in all circumstances identify all potential water-borne pathogens. The usefulness of an indicator organism is also influenced by its environmental resistance or even ability to multiply in the environment (Hurst *et al.,* 2002). Hence, viral, bacterial, parasitic protozoan and helminth pathogens are unlikely to all behave in the same way as a single indicator group. Furthermore, the occurrence of specific pathogens varies according to their seasonal occurrence (Ashbolt *et al.,* 2001). If the origin of faecal contamination and pathogens could be correctly identified, management and remediation efforts could be allocated in a more-cost effective and efficient manner.

In addition to the problem of the detection of pathogens in water samples above the recommended standards, is the wide antibiotic resistance commonly demonstrated by these pathogens. Several pathogens have been shown to demonstrate a significant increase in resistance to some specific antibiotics over a short period of time, either as a result of selective pressure, antibiotic abuse by humans or over use in animals. Previous studies indicate a direct correlation between antimicrobial use and the extent of antimicrobial resistance (Olaniran *et al.*, 2009). Also at risk are aquatic ecosystems, which are largely controlled by, and dependent upon, microbial organisms for crucial processes (e.g. denitrification), associations (e.g. nitrogen fixation) and services (e.g. organic breakdown), all of which can potentially be hindered by antibiotic substances (Costanzo *et al.*, 2005). Chemical pollution of surface waters can also create serious health risks and enter these water bodies through point and nonpoint sources (Table 1.1). Coastal pollution of seawater may give rise to health hazards because of local contamination of fish or shellfish like the mercury contamination of fish in the infamous Minamata disease outbreak in Japan in 1956 (WHO 1976). Pollution of seawater with persistent chemicals, such as polychlorinated biphenyls (PCBs) and dioxins, can also be a significant health hazard even at extremely low concentrations.

Table 1.1: Sources and resulting contaminants of chemical pollution of surface waters (adapted from McMichael *et al.*, 2001).

Source	Contaminant
Rain or irrigation water	Fertilizers (nitrogen and phosphates), herbicides, insecticides
Paper and pulp mills	Chlorinated organic compounds, sludge, lime sludge, ash
Sugar mills	Ammonium, pesticide residues
Leather tanneries	Chromium, acids, sulphides, chlorides
Textile industries	Toxic residues
Petrochemical manufacturing plants	Suspended solids, oils, grease, phenols, benzene, spent caustic
Mining	High levels of cadmium , lead, zinc, copper, nickel, chromium
Medical waste	Mercury
Water disinfection	Chlorine disinfection by-products

1.2 Current water situation in South Africa

South Africa is a semi-arid country making water availability one of our key limitations to development. The country's water resources have been severely jeopardized by pollution due to rapid demographic changes which have coincided with the establishment of informal settlements without access to basic necessities (Fatoki *et al.,* 2001). Approximately 30% of the population survives without adequate domestic water supplies while 17 million South Africans still lack access to appropriate sanitary infrastructure, forcing many of these communities, especially from human settlements, to rely on surface water resources to compensate for their water requirements (Momba *et al.,* 2006). A significant proportion of inhabitants of these rural communities are exposed to water-borne diseases (Olaniran *et al.,* 2009). A previous report on the microbial quality of river water sources located in certain rural communities of South Africa, indicated that these water bodies were unsafe for human consumption and *E. coli* was the predominant pathogen isolated (Obi *et al.,* 2002). The upper tributaries of river ecosystems are generally good to fair whilst the lower reaches of most river systems are affected by urbanization resulting in poor water quality (Meybeck *et al.,* 1996). This highly variable status of water quality within individual catchments makes it difficult to assess the overall state of South Africa's rivers.

The South African government has made progress in delivering basic water supplies by increasing the number of households with access to piped water from 2.4 million households since 1996 to 9.5 million households in 2001 (DEAT, 2006). To ensure further equitable access, the Free Basic Water policy was instituted in 2001, which mandates that households should receive up to 6000 liters of free water per month. Improving wastewater management has been identified as priority; however deteriorating water quality is having adverse effects on human health in some areas, compounded by the lack of sanitation and the non-functioning of a number of basic water supply schemes (Mackintosh and Colvin, 2003; Momba *et al.*, 2003; Swartz, 2000).

The South African marine and coastal resources provide important economic, social and commercial opportunities that have facilitated job creation. However, these ecosystems are increasingly been threatened by human and environmental pressure, including mismanagement which have declined marine productivity. This impacts the structure and functioning of such ecosystems leading to degradation and resource- and socio-economic loss. Pollution of coastal water sources can originate from land-based sources, shipping activity and atmospheric gases. Offshore discharges along the KwaZulu-Natal coast amount to 500000 m^3/day of which 61% is industrial and the rest domestic effluent (DEAT, 2006). As many as 67 discharge points releasing 1.3 million m^3 of wastewater daily into the marine environment, highlights the severity of wastewater pollution (DEAT, 2006).

Coastal bathing water quality standards and the associated human health risks have come under intense scrutiny in recent years where questions have been raised concerning the capacity of these standards to protect the public and the costs required to achieve compliance. Concerns over the often

unregulated discharge of sewage and storm water drains directly into bathing waters can result in gastrointestinal and upper respiratory tract infections (Bartram and Rees, 2000). South African water quality guidelines are based solely on *E. coli* as an indicator of pathogenic pollution. Recreational waters intended for full and intermediate contact are regulated by compliance to the following two limits enumerated for *E. coli*: (1) less than 20% of the samples can exceed 100 colony forming units per millilitre (cfu/ml), (2) less than 5% of the samples to exceed 2000 cfu/ml. These guidelines do not set limits for enterococcus concentrations nor is the sampling frequency specified (Mardon and Stretch, 2004). Regulation of recreational water quality in South Africa is fragmented due to several drawbacks that hinder proper management of its beaches (Georgiou and Langford, 2002): (a) conventional monitoring of recreational water quality is based on a system where action is only executed following exposure of a pathogen threat to humans, (b) traditional indicators used to monitor these waters can originate from other sources other than human faecal material which poses the main health risk to humans if sewage contaminated waters are ingested, (c) beaches are classified as either safe or unsafe however there is a gradient of increasing severity, variety and frequency of health effects with increasing sewage pollution therefore remediation strategies should differ among the unsafe beaches based on the severity of pollution, (d) focus on sewage treatment and outfall management as the only effective intervention methods. However, project initiatives such as Marine Protected Areas and improved management policies have been undertaken both nationally and abroad to protect these resources and curtail the current negative effects.

1.3 Water resource protection and management initiatives in South Africa

Increased stress on catchments and coastal zones renders protection of aquatic environments insufficient thus resulting in increased pathogen risks. This problem is further exacerbated in developing countries where surface water sources are subject to direct and unregulated effluent discharges (Hipsey, 2007). In order to fully assess the overall pathogen risk of polluted waters, it is imperative to understand crucial factors governing the transport and distribution of pathogens upon entering these receiving systems. This pathogen data together with a systematic approach to pathogen monitoring of fresh and coastal waters can help reduce the risk of exposure to the public (Allen *et al.*, 2000; Hipsey, 2007). This information would enable water custodians to quantify the risk posed by a pathogen threat on water quality. Revision of current monitoring strategies within a broader management framework and employing adaptive management strategies that are more effective can assist in reversing the current state of surface waters (Hipsey, 2007).

1.3.1 Inland surface water resource management

The Department of Water Affairs and Forestry (DWAF) is currently responsible for water resources management at the national level and has in place a national monitoring program equipped with established assessment procedures for assessing the microbiological and chemical water quality of surface waters (DWAF, 2007). To facilitate the management of water resources, the country has been divided into 19 catchment-based water management areas. Weekly to monthly sampling is conducted at 1600 monitoring stations at various river systems across southern Africa and the assessments form part of the River Health Programme through its State of Rivers initiative. The initiative collects; stores and interprets the data in a systematic and quality-controlled manner which then allows each section of a river to be allocated different river health categories (natural, good, fair or poor) based on the water quality situation (DEAT, 2006). However, monitoring points typically are located far upstream in the catchments and are generally not representative of the loads that ultimately enter the estuaries or marine environment and can be difficult in providing a national indication of the microbiological water quality (Taljaard and Kayombo, 2007). Very few rivers in South Africa qualify as truly natural systems, and many of them are located in protected areas such as national or provincial parks and wilderness areas. However, even the lower reaches of a river that flows through a protected area may be impacted by upstream developments that are located outside the protected area, as is the case for most of the rivers flowing through the Kruger National Park (DWAF, 2004a).

The National Water Resource Strategy (NWRS) was established to achieve integrated water resources management in South Africa by providing strategies that will protect, develop, conserve, utilize, control and manage the country's water resources in accordance with the requirements of the policy and law. The central objective of this initiative was to judiciously manage water resources in a way that ensures sufficient water of appropriate quality will be available to sustain a strong economy, high social standards and healthy aquatic ecosystems for many generations. A vital element of the NWRS is the delegation of responsibility and authority for water resources management to catchment management agencies and water user associations (local level) to allow effective management of water resources in their respective areas (DWAF, 2004a). A Trophic Status Monitoring Programme is conducted, on a much smaller scale, to investigate the nutrient status of selected South African impoundments that are managed by the DWAF. The design of a more extensive Eutrophication Monitoring Programme has been completed and the Trophic Status Programme was integrated with this program as soon as it became operational. In addition, the organic surface water resource quality monitoring occurs for a very small number of sampling sites and no national network is feasible at present because of the costs of sampling and analysis (DEAT, 2006).

There is a greater commitment to water conservation and demand-side management and more attention is being directed toward clean technology programs. The basic household water supply program

has led to improved and more equitable access to water resources. Stations that monitor water flow and quality are well established and additional emphasis being placed on monitoring microbes, toxic substances and radioactive materials in water. South Africa has also entered into several regional agreements on the management of transboundary watercourses. The National Toxicants Monitoring Programme includes monitoring of surface waters for organic and heavy metal pollutants and radioactivity monitoring is done at a regional level only where such problems exist.

1.3.2 Coastal surface water resource management

South African coastal environments are regulated and governed by a wide range of stringent enforcement efforts and joint coastal management initiatives that have in place management parameters to ensure sustainable utilization of these natural resources and prevent over-exploitation. Most programmes in operation form part of the National Coastal Management Initiative which is briefly described below. The South African Marine Protected Areas (MPAs) have come under scrutiny regarding the fragmented approach used to protect the coastal environments against fishing activities. Only 22% of South Africa's 3000 km coastline is under protection to a certain extent and a mere 9% is protected by the MPAs initiative (DEAT, 2006). The marine environments are also under surveillance and are monitored by one offshore and three inshore fisheries and environmental protection vessels. These vessels monitor all commercial fishing operations, are equipped to handle oil spillages and prevent further large-scale oil pollution incidents. The Department of Environmental Affairs and Tourism (DEAT) is responsible for ensuring compliance of South Africa's marine and coastal environments with regulatory water quality guidelines. DEAT also delegated marine and coastal law enforcement in KwaZulu-Natal to the Ezemvelo KZN Wildlife provincial conservation body. DEAT initiated the Mussel Watch Program to monitor heavy metal concentrations in the tissues of specific mussel species at 42 sites along the South African coastline (Western and Northern Cape, Durban, East London). Mussels serve as good indicators of water quality as they are sessile organisms and are able to bio-accumulate pollutants. Another marine and coastal ecosystem protection project established by DEAT was the Environmental Courts set up around the country where environmental offences and offenders could be formally reported and appropriate action taken. In 1999, the DEAT established a Red Tide Response Team who is tasked with monitoring stations between Doring Bay and Cape Agulhas for toxicity levels and informing the public of dangerous toxicity levels. Blue Flag is an international program that rewards beaches across 27 countries for excellence in coastal management. The program encourages continual improvement in managing beaches to comply with water quality guidelines thus promoting safety to its users. Public education initiatives in the form of Adopt-a-Beach program, South African Coastal Information Centre and the Coastal Indicator program provide and educate the public with information on how they are able to participate in improving the state of the coastal environments which will ultimately benefit them.

1.4 Surface water pollution: sources, indicator organisms and detection methods

Discharge of sullage and excreta from informal settlements into storm water drainage systems, flow directly into close proximity rivers causing faecal contamination (Paulse *et al.,* 2007). Low rainfall coupled with increasingly high temperatures also aids the proliferation of microorganisms which leads to a significant increase in water-borne diseases. This high microbial load in contaminated river water also affects farms, located downstream from the pollution source, that utilize the water as a means of irrigation (Paulse *et al.,* 2007). These major sources expose humans to disease transmission via three main routes: (a) direct consumption of pathogens contained in drinking water (fresh), (b) direct recreational contact (fresh; estuarine and marine) and (c) consuming microorganisms that have bio-accumulated in the tissue of consumable shellfish (estuarine and marine).

Poor sanitation and contaminated drinking water resulting from human activity and natural phenomena result in thousands of children dying each day from diarrhoea, cholera, dysentery and other water-; sanitation- and hygiene-related diseases when untreated water is consumed (Pandey, 2006; Zamxaka *et al.,* 2004). In South Africa, 2.6% of all deaths are attributable to unsafe water supplies, inadequate sanitation facilities and hygiene, with significantly higher figures applying to children under five years of age and an associated treatment cost of R 3.4 billion (Lewin *et al.,* 2007; Wenhold and Faber, 2009). The incidence of diarrhoea in South Africa is used as an indicator to identify potential environmental hazards and review the health status of the country's children. According to the South African Health Review (SAHR) released in 2007, the national figure for diarrhoea in 2006 was 214.9 children under the age of five were afflicted with this water-related illness per thousand children in the target population, with significant difference among provinces (Day and Gray, 2007).

1.4.1 Sources of water pollution

Water quality refers to the physical, chemical and biological characteristics of water. Quality of a water source indicates how suitable the water is for its intended purpose in nature or for use by different water users. Poor water quality is generally associated with human activities but can also originate from natural causes which reduce the residual natural assimilative capacity of the resource thus limiting its fitness for subsequent use (Vega *et al.,* 1998). Primary drivers of water stress include high population density, rapid industrial growth, extensive and inefficient irrigation, changes in rainfall patterns and salination due to over-exploitation (Boyacioglu, 2007). The main natural factor that influences water quality is the geology of the formations over which water flows or through which it percolates, which results in sediment load and mineralization of the water. Vegetation, the slope of the land and flow rate may also influence water quality (DWAF, 2004a). Water stress may be primarily a water quantity issue, but it can also occur as a consequence of deterioration of water quality or lack of appropriate water management. Water resource management interventions such as diversion, storage and inter-catchment

transfer of water can also impact water quality. Inland and coastal resources in South Africa are displaying signs of modification and degradation and in some instances, destruction. The section that follows highlights some of the key sources of surface water pollution and contributory damaging effects:

1.4.1.1 Microbiological contamination

Water-borne pathogens infect around 250 million people each year resulting in 10 to 20 million deaths around the globe (Wilkes *et al.,* 2009; Zamxaka *et al.,* 2004). Microbiological contamination of surface waters is typically associated with land-based sources of pollution, including inappropriate disposal of under-treated municipal wastewater, contaminated runoff or effluent from livestock rearing locations; solid waste dump sites; defecation on beaches; accidental spills and industrial effluents mainly from food processing industries (Taljaard and Kayombo, 2007). In the past, monitoring of offshore sewage outfalls in South Africa have indicated no negative impact on the marine environment, however, increased effluent discharges from malfunctioning or overloaded treatment facilities into the surf zone has adversely affected these environments (DWAF, 2004a).

1.4.1.2 Eutrophication

Eutrophication refers to artificially enhanced primary productivity and organic matter loading in water due to elevated concentrations of nutrients contributed by poorly regulated disposal of municipal wastewater with a high organic and inorganic content, nutrient-enriched irrigation return flows and seepage, over-application of fertilizers and pesticides, and certain industrial and mining processes (Chapman, 1996; Cloern, 2001). Nutrients such as ammonia and nitrates are fatally toxic to aquatic fauna and leads to excessive production of plants and nuisance algal blooms as was the case with the water hyacinth problem in Hartebeesport Dam, South Africa in 1970s and 1980s. Algae increase the cost of water purification and are a physical threat to treatment plants as they clog filters and pumps, while reducing the carrying capacity of pipelines and canals. It can take decades to reverse eutrophication in a dam, since phosphates settle into the sediment and are released back into the water when conditions are favourable, resulting in renewed algal bloom (Chapman and Kimstach, 1996; Smith *et al.,* 1999). The Middle Vaal River and the Hartebeesport, Inanda, Laing and Bridlesdrift dams in South Africa have all been affected by eutrophication (DWAF, 2004a). Impacts associated with eutrophication include (Campbell *et al.,* 1993; Codd, 2000; Vousta *et al.,* 2001): (1) promotion of the growth of potentially toxic cyanobacteria and cell rupture, thus releasing their toxic content into the water, (2) environmental impacts such as modification of species composition (3) depletion of excess nutrients causing decay of the algal blooms and exhaustion of the oxygen in the water by creating anoxic conditions and resulting in large-scale destruction of the aerobic biota and (4) socio-economic consequences where loss of aesthetic value of the affected water body results in loss of recreational value and tourism revenue of the area; artisanal

and/or commercial fisheries are impacted and risks to human health. Along the South African coast, estuarine systems typically act as purifying systems that absorb nutrients contained in catchments resulting in cleaner water entering the sea. Unfortunately, this nutrient removal function results in excessive growth of weeds and phytoplankton blooms in these estuaries instead of the neighbouring marine environment (Snow *et al.,* 2000). This is particularly evident during low flow periods (dry seasons) when the river runoff entering the estuaries can have high nutrients levels as well as water having longer residence times within the estuaries (Taljaard *et al.,* 2006).

1.4.1.3 Solid waste disposal

The introduction of solid waste into river and coastal water resources negatively affects these environments and can originate from land-based sources particularly ports, industrial and commercial areas, informal settlements, urban developments, mining, solid waste dumping sites (legal or illegal) located on the coast or along rivers and through river discharges transporting litter from adjacent catchments (Chapman and Kimstach, 1996). Older waste repositories (industry and mining) and landfill sites (domestic) that lack structured lining systems release contaminated leachate into adjacent water resources. Solid litter can degrade to release hazardous substances that can be potentially harmful to the biota, impede gas exchange thus depleting oxygen, flood rivers upstream due to blocked drains and drain downstream, reduce the amenity value of beaches due to public litter and damage vessels involved in commercial fisheries (Lane, 2007).

1.4.1.4 Suspended solids

Suspended solids are insoluble sediment material that can enter surface water bodies from excessive erosion due to inappropriate land use practices, destruction of riparian vegetation, land-based human activities such as construction activities; over-grazing and industrial or domestic discharges particularly during rainy seasons and river discharges carrying high suspended sediment loads (DEAT, 2006). Adverse effects of sediment pollution include, alteration of the habitat belonging to aquatic organisms leading to changes in the species community of that water body, feeding efficiency of fish are impaired as suspended solids can impair visibility and food becomes buried in silt, obstruction of gaseous exchange leading to impaired respiratory functions and light penetration is hindered which prevents photosynthesis (Kazunga *et al.*, 2002).

1.4.1.5 Chemical pollution

Chemical contaminants released into surface water resources are compounds that are toxic, recalcitrant and/or bioaccumulating and are categorically grouped as heavy metals, hydrocarbons and persistent organic compounds (Alonso *et al.,* 2004; Hem, 1989; Rovedatti *et al.,* 2001). Potential causes

of chemical pollution result from land-based human activities typically linked to inappropriate utilization; inadequate storage and discharges of agrochemicals (e.g. fertilizers and pesticides), atmospheric emissions (e.g. heavy metals), leachate from solid waste dump sites, dredging activities and bad operational practices in harbour areas (seven commercial ports and twelve proclaimed fishing harbours along the South African coastline) and accidental oil or chemical spills along transport routes near the coast (e.g. heavy metals and hydrocarbons) (Järup, 2003). Since 1987, approximately 82000 tonnes of oil was accidently or deliberately discharged into South African coastal waters (DEAT, 2006; Taljaard and Kayombo, 2007). Pollutants from agricultural activities usually enter surface water resources through river discharges that act as a transport mechanism by draining intensive inland agricultural areas (Xiao-long *et al.,* 2007). In addition, agricultural activities taking place adjacent to coastal areas can result in direct contamination of coastal waters through surface or groundwater seepage. Unless appropriate and environmentally sustainable agricultural practices are promoted, chemical pollution of surface water resources associated with these activities will increase (Kunwar *et al.,* 2005).

Atmospheric emissions result from fossil fuel fires that are used for domestic energy needs, incineration of solid waste at dumping sites as well as traffic and industrial emissions. The energy sector can also influence marine water quality through thermal discharges (cooling water) from power generation installations. These activities generate smoke which contains pollutants such as nitrogen, trace metals and hydrocarbons which contribute to higher atmospheric emission loads and can deposit into the marine environment. Rapid increase in urbanization and tourism development is expected to result in increased emissions and consequently higher pollutant loads (Mohammed *et al.,* 2006).

Hydrocarbons include petrochemicals (e.g. lubricating oil, petrol, diesel, paraffin, greases and tar), synthetic organic solvents and oils and fats of biological origin from food processes. These pollutants can have serious toxic effects upon entering water environments where oil films block or smother animal respiratory organs. Other consequences arising from hydrocarbons entering water environments involve environmental impacts where growth and reproduction of aquatic organisms are severely affected, socio-economic losses where the quality of seafood products are reduced and exposure of toxic substances to humans through contact recreation (Chapman and Kimstach, 1996).

South Africa's marine and coastal environment is mined in the northeast for heavy metals (titanium and zirconium), in the south for fossil fuel (oil) and in the northwest for diamonds. Mining activities can result in elevated salinity, increased metal content, unavoidable disruption of sediment, and complete destruction of biological community as well as a change in the pH of the water (Younger, 2001). High salinity may also be the natural result of catchment geology, such as in the Fish River in the Eastern Cape, which flows through the saliferous Karoo region, or the Breede River in the Western Cape. The increase in salinity of the water can lead to salinization of irrigated soils, reduced crop yields, increased scale formation and corrosion of domestic and industrial pipes (Thomas and Meybeck, 1996).

Acidification (pH of the water decreases below neutral) can result from anthropogenic activities such as mining, industry, waste disposal and certain biological processes such as acid rain. However, acidification of surface waters may only take place if the buffering capacity of the river basin soil is very low. The decrease in pH of water resources can mobilize metals (cadmium and lead) which can adversely affect the aquatic ecosystem and the water users (Friedrich *et al.*, 1996; Meybeck *et al.*, 1996).

Studies investigating heavy metal accumulation in Cape Town (South Africa) revealed that the coastal environment is generally in good condition, except in localized areas such as the Port of Cape Town (Brown, 2005; CSIR, 2006a; 2006b). For example, the Mussel Watch Programme conducted along South Africa's west coast revealed that the concentrations of cadmium (Cd), lead (Pb), zinc (Zn) and mercury (Hg) did reflect inter-annual fluctuations, however no clear long-term (increasing) trends were deduced. Pesticides were detected in the fatty tissue of seals and dolphins habituating along the South African (and Namibian) coast, however, these levels did not indicate serious pollution (Vetter *et al.*, 1999). Furthermore, in South Africa municipal and industrial wastewater discharges (point sources) are regulated and licensed under the National Water Act (36 of 1998). Therefore proper environmental impact assessments and legislative controls have a positive influence on sustaining acceptable environmental quality.

Furthermore, major industries such as oil refineries dispose effluent into the South African marine environment and are located in Cape Town, Mossel Bay and Durban whereas the chemical, textile, paper and pulp industries and aluminium smelters (classified as chemical industries) are concentrated along the east coast (Durban and Richards Bay) of the country. Fish processing industries disposing wastewater into coastal waters are mainly located on the west and south west coasts. Tanneries, breweries, cement factories, sugar factories and fertilizer factories also contribute significantly to chemical pollution. It is estimated that a daily discharge volume of 0.49×10^{6} m^{3} of industrial wastewater is being released directly into the South African marine environment (DWAF, 2004b).

1.4.1.6 Urbanization and land utilization

Urbanization results in the alteration of land surfaces which augments the volume of runoff entering surface waters. Industrialization, urbanization and changing patterns of land use will continue to affect water flow and quality which is further aggravated by overstretched management and regulatory capacity. For example, hydrological patterns are significantly altered by human activities including encroachments into floodplains, the building of dams; weirs; bridges and canalization and the diversion of watercourses (Tong and Chen, 2002). Ongoing impacts on water in urban areas also include larger impervious surfaces that increase runoff volumes and reduce groundwater recharge, land disturbance and over-grazing that result in increased flooding and sedimentation of water courses; dams and estuaries, the continued intrusion of alien vegetation into catchments, increased nutrient loads and microbial

contamination from fertilized fields; animal feedlots and the discharge of sewage (Igbinosa and Okoh, 2009).

Increased and often uncontrolled urbanization is also linked to a rapid increase in municipal wastewater and solid waste loads which results in deteriorating standards in wastewater management (Schuster-Wallace *et al.,* 2008). As mentioned earlier, the staggering proportion of the South African population that lacks sanitation and regular refuse services results in little or no treatment taking place in these circumstances, such as at informal settlements (Mackintosh and Colvin, 2003). Where treatment is available, sewer reticulation systems can be inadequate or poorly maintained, resulting in uncontrolled releases such as seepage and overflow to the natural environment (DEAT, 2006). Urban runoff containing high concentrations of organic substances and nutrients can lead to eutrophication and microbial contamination in urban streams and impoundments. Therefore an urgent need exists for improving the current level of treatment of domestic wastewater to decrease the current negative impact which includes the cost of damage to our critical inland water resources.

1.4.2 Microbial indicators of water quality and their detection methods

It is well established that the risks associated with the consumption of microbiologically contaminated water are of great concern from a health perspective (OECD, 2003). Microbial water quality indicators are used in a variety of ways within public health risk assessment frameworks, including assessment of potential hazard, exposure assessment, contaminant source identification, and evaluating effectiveness of risk reduction actions. Presence or absence of these indicator organisms are key elements of most drinking water quality guidelines, water supply operating licenses' and agreements between bulk water suppliers and retail water companies (Colford *et al.,* 2004). Whether continued reliance on microbial indicators is sufficient to ensure microbial water quality is a big question to the international water industry therefore, a more holistic approach is being adopted to delivering safe water, through the development and adoption of risk management plans for drinking water quality. The current bacterial indicator approaches have become standardized, are relatively easy and inexpensive to use, and constitute a keystone of local and global monitoring and regulatory programs. Currently, the bacterial indicators used in water quality and health risk assessments include total coliforms, faecal coliforms, *E. coli*, and enterococci and the use of *E. coli* as an indicator of faecal contamination is subject to strict governmental regulations (Meays *et al.,* 2004). The indicator bacteria themselves are usually not pathogenic, however, indicator bacteria such as faecal coliforms, faecal streptococci and *E. coli* are used because they are much easier and less costly to detect and enumerate than the pathogens themselves. None of the bacterial indicators currently used for monitoring, meet all ideal criteria established for water quality (Ashbolt *et al.*, 2001; Bitton, 2005; Stevens *et al.,* 2003). An appropriate faecal indicator should not grow outside the host; however indicators like *E. coli* and enterococci are able to survive and proliferate in natural

environments such as fresh water lakes and streams (Byappanahalli *et al.*, 2003; Power *et al.*, 2005), algal wrack (Olapade *et al.*, 2006), beach sand (Byappanahalli *et al.*, 2006b), soils and sediments (Anderson *et al.*, 2005; Byappanahalli and Fujioka, 2004; Byappanahalli *et al.*, 2006a; Fujioka *et al.*, 1999; Ishii *et al.*, 2006; Solo-Gabriele *et al.*, 2000; Whitman *et al.*, 2004). In addition, these indicators do not correlate well with the presence of pathogens due to differing survival profiles between the pathogens and indicators (Bonadonna *et al.*, 2002; Harwood *et al.*, 2005; Hörman *et al.*, 2004; Lemarchand and Lebaron, 2003; Lund, 1996). Bacterial indicators also correlate poorly with viruses (Fong and Lipp, 2005; Harwood *et al.*, 2005; Hörman *et al.*, 2004; Jiang *et al.*, 2001; Lemarchand and Lebaron, 2003; Noble and Fuhrman, 2001; Pusch *et al.*, 2005).

The greater survival ability of pathogenic viruses and protozoa in environments, questions the reliance on relatively short-lived coliforms as indicators of the microbiological quality of water. With increased attention being placed on non-bacterial pathogens, effective testing of microbial water quality clearly requires more than simply testing for total coliforms and *E. coli* (Stevens *et al.*, 2003). After so many years, the great question is: is it time for a complete change or do the traditional microbial indicators still have some merit in water quality monitoring?

1.4.2.1 Total coliforms and faecal coliforms

In developing countries, these indicator organisms are presently used for monitoring drinking water, surface waters resources and the efficiency of wastewater treatment facilities, although the reliance on these indicators as the main source of information about the safety of reclaimed water for public health is under review in many jurisdictions (Ashbolt *et al.*, 2001; Fewtrell and Bartram, 2001). These indicators are useful for determining the quality of potable water, shellfish harvesting waters, and recreational waters (Field and Samadpour, 2007). Coliforms are disseminated in relatively high numbers in human and animal faeces, but not all of them are of faecal origin (Tallon *et al.*, 2005). As a consequence, coliforms, detected in higher concentrations than pathogenic bacteria, are used as an index of the potential presence of entero-pathogens in aquatic environments. Even though the coliform group is used in many countries as a monitoring tool for microbiological impairment of water; many authors have reported water-borne disease outbreaks in water meeting the coliform regulations (Gofti *et al.*, 1999; MacKenzie *et al.*, 1994; Moore *et al.*, 1994; Ootsubo *et al.*, 2002, 2003; Ottson and Stenstrom, 2003; Payment *et al.*, 1991). In addition, several limitations associated with their application including: short survival in water body, non-faecal source, ability to multiply after release into water column, great weakness to the disinfection process, inability to identify the source of faecal contamination (point and non-point), low levels of correlation with the presence of pathogens and low sensitivity of detection methods have been reported (Savichtcheva and Okabe, 2006).

Coliforms that produce acid and gas from lactose at 44.5 ±0.2°C within 24 ±2 hrs are known as faecal coliforms due to their role as faecal indicators and this group comprises bacteria such as *E. coli* and *Klebsiella pneumonae.* Some investigators have suggested the sole use of *E. coli* as an indicator of faecal pollution as it can be easily distinguished from the other members of the faecal coliform group. In general, international drinking water guidelines and national legislation adopted worldwide require that acceptable drinking water may not have *E. coli* detected in 100 ml sample (Hörman and Hänninen, 2006). Faecal coliforms have been widely used as an indicator of the microbiological quality of surface and ground waters (Colford *et al.,* 2007; Hagedorn *et al.,* 1999; Hartel *et al.,* 2002; Harwood *et al.,* 2000; Scott *et al.,* 2002). In addition, several studies have identified the sources of faecal contamination using various molecular techniques (Ahmed *et al.,* 2005, 2007; Anderson *et al.,* 2006; Colford *et al.,* 2007; Dombek *et al.,* 2000; Ishii *et al.,* 2007; Shanks *et al.,* 2006a, 2007; Yan *et al.,* 2007). Faecal coliforms display a survival pattern similar to that of bacterial pathogens but their usefulness as indicators of protozoan or viral contamination is limited. Hence, coliform standards are unreliable with regard to contamination of aquatic environments with viruses and protozoan cysts. Studies have shown that *E. coli* is the only coliform almost exclusively associated with a faecal source (Tallon *et al.,* 2005) and although there is much support for *E. coli* as the definitive indictor of faecal pollution, some studies have shown that high levels of *E. coli* can be found in tropical natural water systems and effluents from pulp and paper mills with no known sources of faecal contaminations (Tallon *et al.,* 2005). Therefore, further investigation is required to correlate the presence of *E. coli* with that of relevant pathogens in order to assess the need for the use of additional indicators.

Classical methods for detection of total and faecal coliforms in natural waters include the Most Probable Numbers (MPN) and the Membrane Filtration (MF) techniques (APHA, 1998). Although the tests are simple to perform, they are time-consuming, requiring 48 hrs for the presumptive results and do not allow to detect all the target bacteria in natural environments. Several factors can influence the detection and recovery of coliform bacteria in environmental samples, resulting in the low recovery of injured coliforms and underestimation of their numbers as these stressed bacteria do not grow well on selective detection media used under temperatures much higher than those encountered in the environment (Domek *et al.,* 1984; McFeters *et al.,* 1982). Chemical and physical factors involved in drinking water treatment processes can also cause sublethal injury and increased sensitivity to bile salts or to the replacement surface-active agents (sodium desoxycholate or Tergitol 7) contained in some selective media, resulting in a damaged cell unable to proliferate on these media. A growth medium, m-T7 agar was proposed for the recovery of injured microorganisms (LeChevallier *et al.,* 1983; Reasoner *et al.,* 1979), as it is known that injured pathogens may retain their pathogenicity following injury (Singh and McFeters, 1987) and the medium has shown a higher coliform recovery, from routine drinking and surface water samples compared with that on the m-Endo medium. Also, since MacConkey's

development of selective media for *E. coli* and coliforms, various workers have shown that these selective agents inhibit environmentally or oxidatively stressed coliforms. As a consequence, alternative methods have been developed since 1990 to detect and enumerate faecal coliforms (including *E. coli*) in waters (Rompre´ *et al.,* 2002). These are direct enzymatic methods (George *et al.,* 2000; Van Poucke and Nelis, 2000), immunological methods (Pyle *et al.,* 1999), quantitative polymerase chain reaction (Q-PCR) (Juck *et al.,* 1996) and Fluorescent *in situ* Hybridization (FISH) (Garcia-Armisen and Servais, 2004; Lepeuple *et al.,* 2003; Regnault *et al.,* 2000). Media without harsh selective agents but specific enzyme substrates provide an alternative approach for rapid and sensitive detection of total coliforms and *E. coli* in environmental samples, allowing significant improvements in recoveries and identification of target bacteria (Farnleitner *et al.,* 2001, 2002; Wutor *et al.,* 2007).

1.4.2.2 Faecal streptococci and enterococci

The most commonly considered alternative indicator or adjunct to coliform bacteria is Faecal Streptococci (FS). They are regarded as suitable indicators of faecal pollution as they occur in relatively high numbers in human and other warm-blooded animals' excreta and are generally absent from natural environments having no contact with human and animal life. In addition, they persist without proliferation outside the animal host (Sinton *et al.,* 1998). Early studies have indicated that faecal streptococci are more persistent than faecal coliforms in receiving waters and groundwater (Bitton *et al.,* 1983; Evison, 1988; Evison and Tosti, 1980; Keswick *et al.,* 1982; Sinton and Donnison, 1994). The enterococcus group is a subgroup of faecal streptococci and has been used successfully as faecal pollution indicators as well as reliable as indicators of health risk in marine environments and recreational waters (Scott *et al.,* 2002). This group of bacteria generally does not grow in the environment and have been shown to survive longer than faecal coliforms (Sinton *et al.,* 1998). They also require greater amounts of cumulative solar radiation to be inactivated in seawater compared to faecal coliforms (Davies-Colley *et al.,* 1994; Sinton *et al.,* 1994). However, it is possible that regrowth of environmental reservoirs of enterococci may occur once deposited into the environment (Desmarais *et al.,* 2002).

Faecal streptococci/enterococci can be detected, using selective growth media in MPN or MF techniques. Media for the recovery and enumeration of faecal streptococci are usually based on their ability to grow in the presence of azide, and their fermentation of carbohydrates to produce lactic acid. Over 70 enumeration media have been proposed for faecal streptococci determination by the MF technique. Media for the recovery and enumeration of enterococci are usually based on the ability of the genus to hydrolyze the complex carbohydrate, esculin in the presence of high concentrations of bile salts. Rapid and simple methods, based on defined enzyme substrate technology, are available for the detection and enumeration of faecal streptococci and enterococci. These tests are based on the detection of the activity of two specific enzymes, pyroglutamyl aminopeptidase and β-D-glucosidase (Manafi and

Sommer, 1993), using fluorogenic or chromogenic substrates incorporated into the selective media. Enterolert is a semi-automated 24 hr MPN test that is commercially available for the detection of enterococci, and is based, on the use of a methylumbelliferyl substrate (Budnick *et al.,* 1996). The test has been used successfully to test fresh and marine waters for fecal indicator organisms (Budnick *et al.,* 1996).

Three different approaches have been proposed for the use of faecal streptococci as indicators of the source(s) of faecal contamination, including, the faecal coliforms vs. faecal streptococci ratio (FC/FS); species identification and the FC/FS ratio shift (Cimenti *et al.,* 2007). According to Geldreich and Kenner, (1969) a ratio of > 4.0 indicates human pollution and a ratio of ≤ 0.7 indicates animal pollution, therefore making it theoretically possible to ascribe a human or animal source to the faecal pollution based on the determined ratio. The assay is rapid, provides satisfactory results and requires minimal training to perform. However, the method is unable to accurately differentiate between human and animal sources due to differences in die-off rates between faecal coliforms and faecal streptococci as well as variability of the survival rates among faecal streptococcal species. Therefore, this ratio is no longer recommended as a viable approach to faecal source tracking unless very recent faecal pollution has occurred (Howell *et al.,* 1995). Species identification is based on the ratio of enterococci to other streptococci in faeces among vertebrate species. Human faeces and sewage is predominantly composed of enterococci, whereas animal sources contain significant numbers of non-enterococci (Sinton *et al.,* 1998). However, enterococci are also present in animals, and are more resistant to environmental stress than other faecal streptococci (Sinton *et al.,* 1998) therefore; this approach to identifying sources of faecal pollution is generally regarded as unreliable (APHA, 1995). The ratio shift approach is used to determine the differential die-off coefficients of faecal coliforms and faecal streptococci in stored samples. Faecal streptococci and faecal coliform counts in receiving waters are generally correlated, although there is a shift in the ratio with time/distance from the faecal source due to unquantifiable factors (Sinton *et al.,* 1994; Sinton and Donnison, 1994). This method requires further investigation as its application has produced variable results.

1.4.3 Alternative microbial indicators of water quality

While the aforementioned microbial indicators of water quality are useful in predicting possible faecal contamination in water, their shortcomings as putative tools for risk assessment are becoming increasingly apparent (Savichtcheva and Okabe, 2006). This has led to the development and application of alternative faecal indicators such as faecal anaerobes, viruses and faecal sterol biomarkers. The increasing use of these alternative indicators combined with traditional indicators holds promise in predicting potential sources of faecal pollution and associated pathogens; however adequate

epidemiological studies to support their use are needed (Savichtcheva and Okabe, 2006). Among the alternative microbial indicators commonly used, are the following:

1.4.3.1 Faecal anaerobes

A significant portion of faecal flora is composed of faecal anaerobes (Matsuki *et al.*, 2002). The use of these organisms as indicators of faecal pollution is attractive; however their survival span outside the host (i.e. aerobic conditions) is reduced due to their low oxygen tolerance (Savichtcheva and Okabe, 2006). Therefore detection of large numbers of these microorganisms in water environments is an evidence of recent or extensive faecal contamination. The advantage of using anaerobes is their inability to reproduce once deposited into the environment, therefore their presence can provide evidence of recent faecal contamination (USEPA, 2005). However, the relatively short and variable survival time is presently a problem in terms of recovery of these microorganisms from the water body. Commonly used faecal anaerobes as indicators of water quality include: *Clostridium perfringens; Bifidobacterium spp.*, *Bacteroides spp.*, and *Rhodococcus coprophilus.*

1.4.3.2 *Clostridium perfringens*

Although there is considerable controversy surrounding the use of *C. perfringens* as a water quality indicator due to its extended viability in the environment, which is considered to be significantly longer than enteric pathogens (Cabelli, 1978), a number of scientists continue to recommend its use. Therefore, these indicators may only be detected long after a pollution event has occurred and a distance from contamination sites, indicating remote or old faecal pollution (Desmarais *et al.*, 2002). However, *C. perfringens* has been successfully used as a faecal indicator for sewage-contaminated streams, ocean environments (Hurst *et al.*, 2002) and sea water (Roll and Fujioka, 1997). A protocol developed by Bisson and Cabelli (1979) is today generally used for the recovery and enumeration of *C. perfringens*. The method involves processing the environmental sample using the MF technique and inoculating the selective medium, m-CP, with the membrane filters. The plates are then incubated for 24 to 36 hrs at 45°C in an anaerobic atmosphere. All uninoculated m-CP medium plates are reduced prior to use by storing them in an anaerobic chamber for at least 24 hrs. Isolated colonies exhibiting a characteristic salmon-pink colour after exposure to sodium hydroxide fumes are counted as *C. perfringens* (Lisle *et al.*, 2004).

Spores of *C. perfringens* are largely of faecal origin (Sorensen *et al*., 1989) and are always present in sewage which may be useful in determining the fate of sewage or storm water released into a water body. The WHO (1996) suggests that their presence in filtered supplies may not be an indication of treatment inefficiencies. The spores produced by this organism are extremely resistant to environmental and disinfection stress and therefore persist for longer periods of time than most indicator bacteria

(Hörman *et al.*, 2004). Therefore, spores of *C. perfringens* represent one of the most conservative indicators of faecal pollution. There is evidence to show that *C. perfringens* may be a suitable indicator for viruses and parasitic protozoa when sewage is the suspected cause of contamination (Payment and Franco, 1993). *C. perfringens* standards have not yet been evaluated based on epidemiological studies on the acceptable risk associated with faecal pollution, therefore their preferred role is to aid identification of faecal contamination in sanitary surveys. In addition, *C. perfringens* has been shown to be a useful indicator for *Cryptosporidium* oocysts and *Giardia* cysts; therefore, it can be used as a model organism for the presence of human pathogenic protozoan cysts (Payment and Franco, 1993).

1.4.3.3 *Bifidobacterium spp.*

Bifidobacteria are obligate anaerobic, non-spore forming bacteria and are one of the most numerous groups of bacteria in the faeces of warm-blooded animals, in particular, *B. adolescentis* and *B. longum*. This group of bacteria is primarily associated with humans and rarely found in animals and if present is isolated at different frequencies from different animals. Therefore they have been suggested as possible tools to differentiate human from animal faecal contamination sources (Bitton, 2005). The human isolates of *Bifidobacteria* have the ability to ferment sorbitol, and presently only one growth medium has been formulated called the human *Bifidobacterium* sorbitol-fermenting agar (HBSA) (Mara and Oragui, 1983; Rhodes and Kator, 1999), which can be used in conjunction with established enumeration techniques (membrane filtration) for processing large volumes of water (Bonjoch *et al.*, 2005; Long *et al.*, 2005).

PCR, multiplex PCR and real-time PCR approaches with strain-specific primers have been developed (Bonjoch *et al.*, 2004; Matsuki *et al.*, 2004; Nebra *et al.*, 2003) and applied for detection and quantification of host-associated *Bifidobacterium* spp. used as source-specific indicators. General PCR primers have been developed to detect all *Bifidobacterium* (Kaufmann *et al.*, 1997), and several PCR platforms have been designed to detect individual species. Lynch *et al.* (2002) used the Bifidobacterium medium (BFM), developed by Nebra and Blanch (1999), combined with colony hybridization (digoxigenin (DIG-)-labelled oligonucleotide probe) to identify *B. adolescentis.* On the other hand, Nebra *et al.* (2003) proposed the use of *B. dentium* as an indicator organism, while the distribution of *Bifidobacteria* in different environments has been described by Gavini (2003) and Ventura *et al.* (2001). Multiplex PCR for the detection of *B. adolescentis* and *B. dentium* was demonstrated by Bonjoch *et al.* (2004). They can also be detected with rRNA probes (Bonjoch *et al.*, 2004; Langendijk *et al.*, 1995). Quantitative PCR (QPCR) assays have been designed to quantify *B. longum* (Malinen *et al.*, 2003; Matsuki *et al.*, 2004), *B. adolescentis* and *B. dentium* (Matsuki *et al.*, 2004). *B. dentium* and *B. adolescentis* have been found in human sewage but not animal wastewaters using multiplex PCR approaches (Bonjoch *et al.*, 2004). In addition, the detection of human associated *Bifidobacterium* in

water samples may indicate recent contamination events. Molecular detection of *Bifidobacteria* for source tracking has undergone comparative testing in Europe, and was not considered to be a strong method (Blanch *et al.,* 2006). High background bacterial levels could prevent growth and/or detection of *Bifidobacterium* spp. in the aquatic environment (Rhodes and Kator, 1999). Also, the transit time before filtration of the environmental sample must be kept to a minimum (3 hrs), as only 60 – 70% of the population would be recovered (Resnick and Levin, 1981). Since these survival issues tend to reduce or alter the numbers of *Bifidobacteria* present in the environmental sample, new techniques must be developed that can improve both specificity and sensitivity of the detection methods used. Comprehensive studies on survival ability and correlation with presence of pathogens and water-borne diseases outbreaks should be further performed before this organism can be used as a reliable indicator of faecal pollution.

1.4.3.4 *Bacteroides spp.*

The genus *Bacteroides* are non-spore forming obligate anaerobic bacteria which occur at very high densities in human faeces (Savage, 2001). Since they are almost absent in animal faeces, these species have the potential role as an indicator of anthropogenic sources. Even though strict anaerobic bacteria are desirable because they are restricted to warm-blooded animals, and do not survive long once deposited in waters (Meays *et al.,* 2004), the need to maintain anoxic conditions for cultivation, isolation and biochemical identification limits the use of *Bacteroides* species as faecal indicators. The *Bacteriodes* bile esculin agar (BBE) is currently the most common medium for isolating *B. fragilis* while the use of WCPG medium following membrane filtration is also widely used (Cimenti *et al.,* 2007).

The increasing use of molecular methods allows for the identification of the source of faecal contamination by tracking *Bacteroides* species that are highly host-specific (Simpson *et al.,* 2004). Terminal restriction fragment length polymorphism (T-RFLP) and length heterogeneity PCR (LH-PCR) have been used for differentiating *Bacteroides–Prevotella* species from human and cow faecal material (Bernhard and Field, 2000a, b). The generated host-specific genetic markers were then successfully applied to real water samples (Bernhard and Field, 2000a; Boehm *et al.,* 2003). Other developments include the detection and quantification of the human-specific *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessing human faecal pollution in fresh waters (Seurinck *et al.,* 2005b) and the rapid estimation of total faecal *Bacteroides* using a quantitative Taq nuclease assay for 16S rRNA gene (Dick and Field, 2004). *Bacteroidales* host-specific PCR primers based on uncultivated microbes can identify faeces from ruminants, humans, dogs, pigs, horses, and elk (Bernhard and Field, 2000b; Dick *et al.,* 2005; Fogarty and Voytek, 2005; Layton *et al.,* 2006; Okabe *et al.,* 2007; Shanks *et al.,* 2006b). *Bacteroidales* markers persist long enough to be detectable in natural water samples and also correlate with sewage and faecal indicator bacteria (Layton *et al.,* 2006), while the occurrence of zoonotic pathogens has also been predicted (Walters *et al.,* 2007). These assays are geographically stable, and have

been used around the world (Betancourt and Fujioka, 2006; Gilpin *et al.*, 2003; Okabe *et al.*, 2007; Seurinck *et al.*, 2005a, b; Vogel *et al.*, 2007; Walters *et al.*, 2007).

These bacteria have been shown to release bacteriophages in great quantities in the environment. Testing for the presence of these phages in environmental samples by demonstrating their attack on a strain of *Bacteroides* that demonstrates host specificity is another promising and fairly straightforward procedure (Payan *et al.*, 2005). A QPCR assay for the detection of *B. fragilis* from human faecal samples has been developed (Malinen *et al.*, 2003). Degradative processes observed during summer months are more active at high temperatures resulting in a rapid decrease in PCR-detectable *Bacteroides*, while human-specific *Bacteroides* marker could persist in freshwaters for up to 24 days at 4 and 12°C, and up to 8 days at 23°C, indicating high possibilities of being detected after the discharge event (Seurinck *et al.*, 2005b). Other factors like sunlight and organic matter also play an important role in survival of faecal *Bacteroides* (Rozen and Belkin, 2001). Therefore, seasonal changes in persistence should also be considered when *Bacteroides* genetic markers are applied as indicators of faecal contamination. Kreader (1995) developed PCR primers and specific hybridization probes to distinguish three *Bacteroides* species and demonstrated that the *B. fragilis* group (*B. distasonis* and *B. thetaiotaomicron*) and *B. vulgatus* were at higher concentrations in human faeces than in farm animal species. QPCR assays have been developed for the detection of all *Bacteroides* species (Dick and Field, 2004) and human-specific *Bacteroides* (Seurinck *et al.*, 2005b). Limitations of this approach include lack of host-specific markers and horizontal transfer of faecal bacteria among species in close contact resulting in potential movement of molecular markers from one species to another. Therefore specificity must be tested whenever *Bacteroidales* PCR markers are used (Dick and Field, 2004; Seurinck *et al.*, 2005b).

1.4.3.5 *Rhodococcus coprophilus*

Rhodococcus coprophilus is an actinomycete that inhabits the digestive system of almost all grazing animals and is usually transmitted to other animals via the faecal-oral route. This organism has therefore been proposed as an indicator of faecal contamination from farm animals (Mara and Oragui, 1981). Its persistence in the environment is considerably longer than that of faecal streptococci, *Bacteroides* or *Bifidobacterium* as it is aerobic and is passed between grazing animals. This target has not been used in any MST studies and is still being tested for its distribution among hosts. The design of a TaqMan-based QPCR assay by Savill *et al.* (2001) allows continued testing of this bacterium as an indicator, however; additional information is needed on the prevalence of this bacterium contained in faeces.

1.4.3.6 Bacteriophages

Bacteriophages are viruses that infect bacteria and those that infect coliforms are known as coliphages. Phages have proved to be most valuable tools in research on viruses and have been proposed as microbial indicators as they share many fundamental properties with human enteric viruses which pose a health risk, if present in water contaminated with human faeces (Grabow, 2001). The DNA containing tailed coliphages (T type) and RNA-containing phages that infect via the F pili [sex factor] (F-RNA coliphages) have been the most used. The use of phages as water quality indicators has been extensively investigated and the limitations of their use as reliable water quality indicators, widely debated. For example, the use of phages as indicators are limited owing to the fact that differences in the electrostatic charges on phages differ from those on enteric viruses, which affects host-phage interaction and recovery efficiency of techniques based on adsorption-elution principles (Grabow, 2001). Other limitations reported include the fact that: (i) phages are excreted by a certain percentage of humans and animals all the time while viruses are excreted only by infected individuals for a short period of time - no direct correlation in numbers of phages and viruses in human faeces (Grabow *et al.,* 1999); (ii) enteric viruses have been detected in water environments in the absence of coliphages (Morinigo *et al.,* 1992); (iii) human enteric viruses associated with water-borne diseases are excreted almost exclusively by humans, whereas phages used as surrogates in water quality assessment are excreted by humans and animals (Grabow *et al.,* 1998) and (iv) some coliphages may replicate in water environments (Borrego *et al.,* 1990).

Phage detection in environmental samples consists of concentrating the sample, decontaminating the concentrate, and carrying out the phage assay by the double or single-layer methods (Bitton, 2005). A wide range of bacterial host cells have been used as some are more efficient than others in hosting phages. Stanek and Falkinham (2001) developed a rapid coliphage detection assay based on the phage-induced release of β-galactosidase from *Escherichia coli.* The assay has a low detection limit and does not require an overnight incubation period. The incidence of phages in water environments has been extensively reported, however, the data obtained are not consistent as several variables affect the incidence, survival and behaviour of phages in different water environments, including the densities of both host; bacteria and phages, temperature, pH etc. (Ashbolt *et al.,* 2001). The host bacteria used for the detection of various groups of phages, varied techniques used for recovery, detection and enumeration of phages from water environments, all contribute to discrepancies in results (Green *et al.,* 2000). Nonetheless, international collaboration is now leading to meaningful, universally accepted guidelines for the recovery and detection of phages in water environments. Three groups of bacteriophages have been considered as indicators: somatic coliphages, male-specific RNA coliphages (F-RNA phages) and phages infecting *Bacteroides fragilis.*

1.4.3.7 Somatic coliphages

These phages belong to a wide variety of lytic members that infect *E. coli* and closely related members of the bacterial family, *Enterobacteriacea.* Somatic coliphages generally outnumber F-RNA phages in wastewater and raw water sources by a factor of about 5, and cytopathogenic human viruses by about 500 (Cimenti *et al.,* 2007), making them valuable indicators of the potential presence of enteric viruses in water environments like estuaries, seawater, freshwater, potable water, wastewater and biosolids (Mocé-Llivina *et al.,* 2003). Phages can also serve as biotracers to identify pollution sources in surface waters and aquifers (Harvey, 1997). Genetically modified phages have been proposed to avoid interference with indigenous phages present in environmental samples (Bitton, 2005). A unique DNA sequence was inserted into the phage genome which can then be detected, using PCR or plaque hybridization (Daniell *et al.,* 2000). The United States Environmental Protection Agency (USEPA) has proposed two methods (methods # 1601 and 1602) to detect somatic coliphages (host is *E. coli* CN-13) in aquatic environments. Method 1601 includes an overnight enrichment step followed by "spotting" onto a host bacterial lawn. In method 1602, the water sample is supplemented with $MgCl_2$, host bacteria, and double-strength molten agar and the plaques are counted after overnight incubation (USEPA, 2001a; 2001b). Wild-type strains of *E. coli* are poor hosts for the detection of the wild variety of coliphages in wastewaters, as these strains have a complete O-antigen that masks the majority of phage receptor sites and their defense mechanisms which include nuclease enzymes that destroy phage nucleic acid recognized as foreign, thus preventing phage replication (Grabow, 2001).

1.4.3.8 Male-specific F-RNA coliphages

F-RNA coliphages (ss-RNA phages) include the families *Inoviridae* (F-DNA) and *Leviviridae* (F-RNA). They infect *E. coli* cells as the receptor sites for male-specific coliphages are located on the fertility fimbriae of this bacterium. These fimbriae are produced only by *E. coli* (strain K12), that carry the F plasmid which codes for the F or sex pilus to which the phage attach. Since F-encoded pili are only synthesized at temperatures above 30°C, F-RNA phages are typically found in low concentrations in the environment (Grabow, 2001). To date, there are four serogroups of F-RNA phages that are selectively excreted by humans or animals, which offers an attractive tool to distinguish between faecal pollution of human and animal origin (Brion *et al.,* 2002; Cole *et al.,* 2003), however, they cannot distinguish between animals sources (Sobsey, 2002). F-RNA coliphages closely resemble many human enteric viruses making them attractive models of human enteric viruses. Grabow *et al.* (1998) found that FRNA phages outnumber cytopathogenic enteric viruses, in wastewaters and raw water sources, by a factor of about 100 implying that their absence from raw and treated water supplies offers a meaningful indication of the absence of human enteric viruses. Several studies confirmed that the resistance of F-RNA coliphages to unfavourable conditions and disinfection processes resembles or exceeds that of most human enteric

viruses (Bitton, 1987; Burge *et al.,* 1981; Grabow *et al.,* 1983; Havelaar *et al.,* 1990; Kapuscinski and Mitchell, 1983; Olivieri *et al.,* 1999).

Detection of F-RNA coliphages by plaque assays is not simple as the F fimbriae are produced only by host bacteria in the logarithmic growth phase making preparation of the host cultures especially difficult. The USEPA has proposed the use of specific host cells such as *Salmonella typhimurium* strain WG49 or *E. coli* strain HS[pFamp]R to detect male-specific phages in aquatic environments. This strain of *S. typhimurium* is not susceptible to a large number of somatic coliphages in water environments which tend to interfere with the detection of F-RNA coliphages using *E. coli* hosts. Once detected, the phage can be further characterized as being human or animal derived by immunological or genetic methods (Griffin *et al.,* 1999; Hsu *et al.,* 1995). In serotyping, group-specific antisera are used whereas in genotyping, hybridization with group specific oligonucleotides is used (Beekwilder *et al.,* 1996; Sundram *et al.,* 2006). The latter involves plating the phage on a particular host, transferring the plaques to a nylon membrane, denaturing the phage to expose the nucleic acid, cross-linking the nucleic acid to the membrane, and then detecting group-specific nucleic acid sequences with ^{32}P- or digoxigenin-labelled oligonucleotide probes. This technique is useful for identifying the four groups of F^+RNA bacteriophages and therefore can be used in tracking sources of faecal pollution (Scott *et al.,* 2002). Molecular methods have also been developed that allow for more rapid characterization of coliphages and identification of more refined and host-specific subgroups. For example, Vinjé *et al.* (2004) have developed an RT-PCR and Reverse Line Blot (RLB) hybridization technique capable of rapid detection and genotyping of coliphages. According to the authors, the RLB method is rapid, reproducible, low-cost, and easy to perform with a high throughput of samples, and could be used in microbial source tracking, however, quantitative source tracking using F-RNA coliphage typing may be problematic owing to differential survival characteristics of the subgroups (Brion *et al.,* 2002; Schaper *et al.,* 2002). A sensitive, culture-independent method was developed by Kirs and Smith (2007) for the simultaneous quantification and identification of all four subgroups of F^+-specific RNA coliphages using a novel molecular beacon-based multiplex real-time reverse transcriptase-PCR (RT-PCR) assay.

1.4.3.9 Phages of *Bacteroides fragilis*

Phages infecting obligate anaerobic bacteria have been reported; among these are phages which infect *Bacteroides* species, notably *B. fragilis. Bacteroides* bacteria themselves have limited value as indicators as they are non-sporing and quickly inactivated by environmental oxygen levels. However, the phages of *Bacteroides* bacteria are specific and significantly more resistant to unfavourable conditions compared to coliphages and enteric viruses and have proven to be valuable indicators for the potential presence of enteric viruses in water environments (Duràn *et al.,* 2003). The failure of *B. fragilis* phages to multiply in the environment makes them favourable models to assess the survival of human enteric

viruses in water treatment and disinfection processes. Bacteriophages infecting *Bacteroides* are potential tools for MST, however, it is well documented that *Bacteroides* host strains vary in their ability to discriminate between phages of different sources but also that phage detection by a given host strain varies geographically (Payan *et al.,* 2005).

Plaque assays for *B. fragilis* phages are more complicated, expensive, labour-intensive and time-consuming than those for somatic and F-RNA coliphages. Other difficulties arising from performing the *B. fragilis* phage assay include the use of anaerobic *Bacteroides* as a host and the low concentration of these phages in environmental samples. Complex growth media amended with antibiotics for selection of resistant host strains and strictly anaerobic incubation conditions are also required (Cimenti *et al.,* 2007; Grabow, 2001). A less complicated method of enumerating *B. fragilis* bacteriophages is the double-layer technique (with plaque detection), using *Bacteroides* phage recovery medium (BPRM) however, there are still concerns about the reliability of this method (Maier *et al.,* 2000). Puig *et al.* (2000) used PCR for the detection of *B. fragilis* HSP40 phages, which proved to be more sensitive than plaque assays and yielded higher counts of the phages in sewage as well as river and seawater. Attempts to identify additional host strains of *Bacteroides* in order to detect additional phage, lead to the discovery of *B. fragilis* RYC4023, which was almost phenotypically identical to strain HSP40 and showed similar sensitivity to infection by bacteriophages. In addition, strain RYC2056 was identified and detected higher numbers of phage than strain HSP40 in waters with a known human impact, making it a potential candidate as an indicator of human faecal pollution in environmental waters (Scott *et al.,* 2002). These phages do not occur commonly in certain parts of the world, including the US and Canada (Payan *et al.,* 2005). Therefore a promising method of isolating new *B. thetaiotaomicron* and *B. ovatus* host strains, useful for source discrimination in different geographic areas, was developed (Payan *et al.,* 2005). *Bacteroides* phage source discrimination has been tested in Europe and South Africa and was considered strong in a comparative study (Blanch *et al.,* 2006; Payan *et al.,* 2005), but has not been tested with blind samples.

1.5 Toxigenic *Escherichia coli* and *Vibrio cholerae*: classification, pathogenesis and virulence determinants

Enteric infections causing diarrhoeal diseases remain a leading global health problem. Two to four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting in 3 million to 5 million deaths (Sánchez and Holmgren, 2005). Almost half of all cases of diarrhoea are due to bacteria that cause disease by producing one or more enterotoxins (Sánchez and Holmgren, 2005). The virulence of a pathogen is dependent on a distinct set of genetic elements and their regulated expression (Chakraborty *et al.,* 2000). During evolution, bacterial species have become capable of transferring virulence genes not only between members of a particular species but also between different bacterial species, creating new pathotypes with new combinations of different virulence genes (Kaper *et al.,* 2004; Schubert *et al.,* 1998). The detection of specific virulence attributes of a given strain allows for the determination of potential reservoirs of virulence genes which are expected to play a key role as the origin of emerging diseases caused by pathogens and is a useful tool in the analysis and detection of new strains (Chassagne *et al.,* 2009; Feng and Monday, 2000; Hidaka *et al.,* 2009; Kuhnert *et al.,* 2000; Ram *et al.,* 2009; Sharma, 2002; Sharma and Dean-Nystrom, 2003).

Virulent strains of *E. coli* causes a series of human diseases, such as gastroenteritis, urinary tract infections and neonatal meningitis as well as haemolytic uremic syndrome (HUS), peritonsis, mastitis, and septicemia in rare cases (Todar, 2007). Also, *V. cholerae*, a motile Gram-negative curved-rod shaped bacterium with a polar flagellum causes cholera in humans (Faraque and Nair, 2002), which is a common pandemic disease, especially in Africa. This section therefore provides an overview of the enterohaemorrhagic and enterotoxigenic *E. coli* as well as toxigenic *V. cholerae*, their virulence determinants and their associated malaises.

1.5.1 *Escherichia coli*

Certain strains of *E. coli*, the widely used 'indicator' of the microbiological quality of surface waters, have virulence properties that may account for life-threatening infections. The pathogenicity of a particular *E. coli* strain is primarily determined by specific virulence factors which include adhesins, invasins, haemolysins, toxins, effacement factors, cytotoxic necrotic factors and capsules (Galane and Le Roux, 2001; Kuhnert *et al.,* 2000). Additional genes that were detected in pathogenic *E. coli* encode various virulence factors which directly indicate their virulence and pathotype (Kuhnert *et al.,* 2000). Three general clinical syndromes resulting from infection with natural pathogenic *E. coli* strains include: urinary tract infections, sepsis/meningitis and enteric/diarrhoeal diseases (Nataro and Kaper, 1998).

Currently, six *E. coli* pathotypes recognized (Turner *et al.,* 2006) to cause diarrhoea in humans are entero-pathogenic *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli* (EHEC) or shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli*, enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli*. However, certain pathotypes of *E. coli* including STEC and ETEC are potent pathogens

associated with water-borne disease outbreaks and mortality in humans (Ram *et al.,* 2007). Although ETEC and STEC are known to be associated with food and water-borne diseases, relatively few studies have been performed to determine their distribution in environmental surface waters (Begum *et al.,* 2005; Hamelin *et al.,* 2006; Higgins *et al.,* 2005; Obi *et al.,* 2004; Ram *et al.,* 2008c; Shelton *et al.,* 2006).

1.5.1.1 Enterohaemorrhagic *E. coli* (EHEC)

Enterohaemorrhagic *E. coli* or shiga toxin-producing *E. coli* (STEC) has been shown to be the most important group among the six characterized groups of diarrheagenic *E. coli* in developed countries since numerous outbreaks have been reported where patients have developed life-threatening complications such as HUS and hemorrhagic colitis (HC) (Leelaporn *et al.,* 2003; Matsell and White, 2009). *E. coli* O157:H7 is a dominant STEC serotype in many parts of the world and historically has been the type most commonly associated with large outbreaks (Paton and Paton, 1998). The STEC have been classified into pathogroups A to E based on the severity of diseases they cause and their association with outbreaks (Karmali *et al.,* 2003). The ingestion of as few as 1 – 10 cells may cause illness in humans (Chart, 2000; Kuhnert *et al.,* 2000). Although STEC/EHEC is asymptomatic in animals, cattle, sheep and goats represent the main reservoir of STEC as the organism is shed in their faeces (Shelton *et al.,* 2006; Williams *et al.,* 2006). This in turn may serve as a means of maintenance and spread of these pathogens among cattle sheep and goat herds (Franz *et al.,* 2007). The organism has also sporadically been detected in chickens, pigs, horses and dogs (Beutin *et al.,* 1993; Fratamico *et al.,* 2008; Karch *et al.,* 1999).

The ability of STEC to cause diseases in humans is related to the production of one or more *Stx* (shiga toxins) and additional virulence factors such as EHEC hemolysin encoded by *hlyA* gene which acts as pore forming cytolysin on eukaryotic cells, intimin, an outer membrane protein responsible for the attaching and effacing phenotype, encoded by a chromosomal *eaeA* gene, the *chuA* gene is part of heme transport locus that encodes for a 69-kDa outer membrane protein responsible for heme transport, the bifunctional catalase peroxidase *KatP* enzyme and the secreted serine protease which can cleave human coagulation factor V (Leelaporn *et al.,* 2003; Ram *et al.,* 2009; Torres and Payne, 1997). STEC may also possess genes that encode fimbrial and non-fimbrial adhesins, proteases, and other toxins, including *astA* (enteroaggregative *E. coli* heat-stable enterotoxin, *EAST*1) and *cdt* (cytolethal distending toxin) (Gyles, 2007).

The *Stx* family contains two major groups called *Stx*1 and *Stx*2. A single EHEC strain may express *Stx*1 only, *Stx*2 only, or both toxins or even multiple forms of *Stx*2. *Stx*1 from EHEC is identical to the Shiga toxin from *S. dysenteriae* I (O' Brien *et al.,* 1992; Takeda, 1995). Members of the *Stx* family are compound toxins (the holotoxin is approximately 70 kDa), comprising a single 32-kDa A subunit and a multimeric B subunit (7.7-kDa monomers) which binds the toxin to a specific glycolipid receptor, globotriaosylceramide or Gb_3, which is present on the surface of eukaryotic cells (Sandvig and van Deurs,

1994). Following internalization by receptor-mediated endocytosis, the holotoxin is transported to the Golgi apparatus and then to the endoplasmic reticulum (Paton and Paton, 1998; Sandvig and van Deurs, 1994). Briefly, internalization involves the formation of a clathrin-coated pit within the cell membrane, which subsequently pinches off to form a sealed coated vesicle with toxin bound to the internal surface (Paton and Paton, 1998). The A subunit is translocated to the cytoplasm and is proteolytically nicked by a membrane-bound protease furin to yield A_1 (27-kDa N-terminal) and A_2 (4-kDa C-terminal) peptides; which remain linked by a disulfide bond. The A_1 peptide contains the enzymatic activity, and the A_2 peptide serves to bind the A subunit to a pentamer of five identical 7.7-kDa B subunits (O' Brien *et al.,* 1992; Paton and Paton, 1998). The disulfide bond is subsequently reduced, thereby releasing the active A1 component which enzymatically inactivates the 60S ribosomal subunit by depurination of specific residues of the host cell ribosomes, thus inhibiting the peptide chain elongation step of protein synthesis. This leads to the death of renal endothelial cells, intestinal epithelial cells or any cells, which possess the Gb_3 receptor (Sandvig and van Deurs, 1994).

The STEC isolates also produce a hemolysin, encoded by the *hlyA* gene which is located on a large virulence 60-MDa plasmid (O' Brien *et al.,* 1992). The hemolysin antigen has been detected in the serum of 95% of patients with HUS (Schmidt *et al.,* 1995). The role of the hemolysin in the pathogenesis of infection is unclear although it has been shown to increase the secretion of interleukin (IL) 1-β which enhances the toxicity of the shiga toxin toward human vascular epithelial cells through up-regulation of the cell surface receptor for the toxin. *E. coli* O157 contains a specialized iron transport system which allows this organism to use heme or haemoglobin as an iron source and the lysis of erythrocytes by one or more of the hemolysins could release these sources of iron, thereby aiding infection (Law and Kelly, 1995; Mills and Payne, 1995). The final virulence factor is the ability of *E. coli* O157 to adhere to and colonize intestinal surfaces. Attachment to mucosal surfaces prevents the loss of the bacteria via peristalsis and promotes delivery of the toxin to the cell surface in a concentrated manner. The *eaeA* gene of O157 encodes the intimin protein which facilitates actin polymerization and cytoskeletal rearrangement of the intestinal cells, causing the development of characteristic attaching and effacing (A/E) lesions (Caprioli *et al.,* 2005; Donnenberg *et al.,* 1993). The complex mechanism of A/E adhesion is genetically governed by a large pathogenicity island defined as the Locus of Enterocyte Effacement (LEE), consisting of three functionally different modules; the first encodes a type III secretion system (TTSS) that exports effector molecules, the second encodes the secreted proteins *EspA, B,* and *D,* which function as part of the type III secretion apparatus, while the third encodes the intimin and the translocated intimin receptor (*Tir*), which is translocated into the host cell plasma membrane by the TTSS (Caprioli *et al.,* 2005).

The mechanisms by which *E. coli* O157 causes HC and HUS are not fully understood. The organism is believed to adhere closely to mucosal cells of the large bowel, disrupting the brush border

(Mead and Griffin, 1998). This process alone may be sufficient to produce non-bloody diarrhoea. Shiga toxins have effects on the intestine and are probably critical to the development of bloody diarrhoea. Damage of the endothelial cells, mediated by Shiga toxins, may trigger platelet and fibrin deposition, leading to injury of passing erythrocytes (haemolysis) and occlusion of renal microvasculature (renal failure) (Mead and Griffin, 1998). Thrombocytopenia is believed to reflect trapping of platelets in involved organs and removal by the liver and spleen. Although the kidneys are preferentially involved, other organs including the brain may be affected, resulting in a wide range of complications (Mead and Griffin, 1998).

1.5.1.2 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* causes secretary diarrhoea in humans throughout the world (Albert *et al.,* 1995; Sack, 1975), especially in children below 5 years due to the consumption of contaminated water and food (Qadri *et al.,* 2005). ETEC is also an important pathogen in the farming industry and causes diseases in cattle, neo-natal and post-weaning pigs (Turner *et al.,* 2006). The main virulence determinants of ETEC are *LT1* and *ST1* genes. ETEC causes travelers diarrhoea by producing different combinations of heat labile (*LT*) and heat stable (*ST*) enterotoxins along with one or more of the 22 colonizing factors (Turner *et al.,* 2006). The *LT*s of *E. coli* are oligomeric toxins that are closely related to the cholera enterotoxin (*CT*) expressed by *V. cholerae* (Sixma *et al.,* 1993). There are two major serogroups of *LT*, *LT-I* and *LT-II*, which do not cross-react immunologically. *LT-I* is expressed by *E. coli* strains that are pathogenic for both humans and animals. *LT-II* is found primarily in animal *E. coli* isolates and rarely in human isolates, but in neither animals nor humans has it been associated with disease (Prescott *et al.,* 2005). In contrast to the large, oligomeric *LT*s, the *ST*s are small, monomeric toxins that contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins (Prescott *et al.,* 2005). The genes for the *LT* and *ST* enterotoxins could be encoded together or separately on large variable plasmids called *Ent* plasmids (Prescott *et al.,* 2005; Turner *et al.,* 2006).

ETEC first colonize and multiply in the small intestine and produce the abovementioned toxins. Colonization is usually associated with the presence of surface adhesins known as the colonization factors (CFs) that allow attachment to the small intestine (Ansaruzzaman *et al.,* 2007). After binding to the host cell membranes, the toxin is endocytosed and translocated through the cell in a process involving trans-Golgi vesicular transport (Lencer *et al.,* 1995). *ST* binds to a glycoprotein receptor that is coupled to guanylate cyclase on the surface of intestinal epithelial cells. Activation of guanylate cyclase stimulates the production of cyclic guanosine monophosphate (cGMP), which leads to the secretion of electrolytes and water into the lumen of the small intestine, manifested as watery diarrhoea characteristic of an ETEC infection (Crane *et al.,* 1992; Mezoff *et al.,* 1992). *LT* binds to specific gangliosides on the epithelial cells and activates membrane-bound adenylate cyclase, which leads to an increased production of cyclic

adenosine monophosphate (cAMP), resulting in the hypersecretion of electrolytes and water into the intestinal lumen (Prescott *et al.,* 2005). The illness is typically abrupt in onset with a short incubation period (14 to 50 hrs) (DuPont *et al.*, 1971; Nalin *et al.,* 1975). The diarrhoea is watery, usually without blood, mucus, or pus; fever and vomiting are present in a minority of patients (Levine *et al.,* 1987). Administration of antibiotics to which ETEC strains are susceptible has been shown to decrease both the duration of diarrhoea and the intensity of ETEC excretion (Black *et al.*, 1982).

1.5.2 *Vibrio cholerae*

Cholera is an epidemic disease of major global and public health significance and is caused by the organism, *V. cholerae* which is an autochthonous inhabitant of riverine, brackish and estuarine ecosystems. The seventh cholera pandemic started in Indonesia in 1961, reached Africa in 1970 and arrived in Latin America in 1991 with Peru being the first country to be hit with cholera. In 1999, a small outbreak occurred in Paranaguá Bay, Paraná State (south of Brazil) where cholera incidents had never been reported (Rivera *et al.,* 2003). In Argentina, there were seven epidemics since 1992, with these outbreaks occurring mainly during the summer months and were consistent with reports from other geographic regions of the world (Binsztein *et al.,* 2004). In August 2000, South Africa was experiencing one of the worst cholera epidemics in the country's history. Initial reports of the cholera outbreak came from the largely rural and impoverished communities on the outskirts of the Ngwelezane Township, near the Empangeni town. The source of the epidemic was traced to the Mhlathuze River, also in the northern part of the KwaZulu-Natal province (Jenkins, 2000). However, by the end of the year 2000, the northern KwaZulu-Natal cholera outbreak had replicated itself in eight of South Africa's nine provinces and registered over 114,000 cases and 260 reported deaths by the end of January 2002, nearly all from KwaZulu-Natal (KZN Department of Health media release, 7 February 2002). The disease also claimed at least 5000 lives in Angola in 2006 and neighbouring countries (Thompson *et al.,* 2008).

V. cholerae is a heterogeneous species with 206 serotypes identified to date based on the heat-stable somatic O antigen. Among these only two serotypes, O1 and O139, have been characterized as toxigenic and have caused epidemics of cholera (Rivera *et al.,* 2003). *V. cholerae* non O1 or non O139 is isolated in abundance from aquatic environments whereas *V. cholerae* O1 is seldom recovered from these ecosystems in the inter-epidemic periods of the disease or it may be found in the non-toxigenic form, "viable but non-culturable" form or in the form of biofilms (Leal *et al.,* 2008). Also, interaction with plankton appears to play an important role in the ecology of the microorganism and to facilitate persistence, mainly in response to low temperatures and reduced nutrient concentrations (Binsztein *et al.,* 2004). *V. cholerae* O1 is divided into two biotypes, classical and El Tor, which are distinguished by a variety of phenotypic markers (Kaper *et al.,* 1995). However, three variants of the El Tor biotype were recently described in Bangladesh, Mozambique and other regions of Asia and Africa (Taneja *et al.,* 2009).

The major virulence factors for *V. cholerae* are the cholera toxin (*CT*) and the toxin-co-regulated pilus (*TCP*). *CT* is a heterodimeric protein exotoxin which consists of two parts: (1) an enzymatically active A subunit which acts as an ADP-ribosyl transferase and elevates intracellular cyclic AMP levels, known as CTA and (2) a pentamer of B subunits (CTB) which bind the holotoxin to its receptor which is the ganglioside GM_1 located on eukaryotic cells (Murley *et al.,* 2000). *CT* initiates its toxic action on cells by binding with high affinity and exquisite specificity to cell membrane receptors (GM_1), which appears to be localized mainly in lipid rafts on the cell surface, and is endocytosed by the cell. In order for cell intoxication to occur, the A subunit needs to be transported to the cytosol to induce the activity of adenylate cyclase (*AC*). After endocytosis, *CT* travels to the endoplasmic reticulum (ER) via a retrograde transport pathway after which *CT*A dissociates from *CT*B (Feng *et al.,* 2004). The arrival of *CT*A in the cytosol is the crucial step for intoxication because this peptide catalyzes the ADP ribosylation of a specific component of *AC*. This causes *AC* to remain in its GTP-bound state, resulting in enhanced *AC* activity and an increased intracellular cAMP concentration (Sànchez and Holmgren, 2005). Higher levels of cAMP produce an imbalance in electrolyte movement in the epithelial cell. Decreased sodium uptake reduces water intake by the enterocyte, and, at the same time, increased anion extrusion causes sodium outflow, and thus water secretion; the overall result is abundant net fluid loss from the intestine (Sànchez and Holmgren, 2005).

More than 95% of *V. cholerae* strains belonging to the O1 and O139 serogroups produce *CT* which is central to the disease process (Chakraborty *et al.,* 2000). *TCP* is an adhesin that is coordinately regulated with *CT* production (Taylor *et al.,* 1987) and is the only *V. cholerae* pilus that plays a role in colonization of the gut mucosa of humans (Chakraborty *et al.,* 2000). Both *CT* and *TCP* are presumed to be exclusively associated with clinical strains of *V. cholerae* as reports on the incidence of *CT* and *TCP* among environmental strains are rare, suggesting these virulence factors are associated only with virulent *V. cholerae* O1 or O139 (Chakraborty *et al.,* 2000).

Both the O1 and O139 pathogenic serogroups contain two main regions related to pathogenicity: CTX genetic element and VC pathogenicity island (VPI) (Karaolis and Kaper, 1999; Schmidt and Hensel, 2004). Under certain environmental conditions, non-toxigenic *V. cholerae* can transform into the toxigenic form with epidemic potential by acquisition of the CTXΦ (lysogenic filamentous bacteriophage) and VPIΦ phages which carry the genes for the *CT* as an operon (*ctxAB*) and the gene for the major subunit of the *TCP* (*tcpA*) (Faraque *et al.,* 2006; Faraque and Nair, 2002). Transcription of the *tcpA* gene together with an operon of 12 genes is involved in processing and assembly of *TCP* on the surface of *V. cholerae* (Murley *et al.,* 2000). Regulation of expression of these virulence genes are dependent on *ToxR* (transmembrane DNA-binding protein) and *ToxS* (second regulatory protein) and these are both encoded in an operon (Miller and Mekalanos, 1984). These proteins are essential for the transcription of a two genes encoding the outer membrane protein of *V. cholerae* known as *ompU*

(Crawford *et al.*, 1998) and a second regulatory protein known as *ToxT* (Higgins and DiRita, 1994). In turn, *ToxT* activates the transcription of the *ctxAB* and *tcpA* operons (Higgins and DiRita, 1994). Although *CT* and *TCP* are undoubtedly the main virulence factors of *V. cholerae*, several accessory factors have also been described which range from additional pilus structures, such as the fructose-binding and mannose-binding hemagglutinins which might or might not contribute to colonization of classical and El Tor biotypes, respectively, to various soluble factors. In addition to mucinase and neuraminidase, the latter group includes a variety of 'minor toxins' that might contribute to cholera diarrheoa (Sànchez and Holmgren, 2005).

1.6 Antibiotics in surface water environments: sources, classification and microbial resistance mechanisms

Surface waters have become the main receptacle for antibiotic-resistant bacteria and antibiotics being discharged in various amounts into the environment (Begum *et al.,* 2007; Ram *et al.,* 2009). Indiscriminate and inappropriate use of antibiotics, and related compounds, for various purposes (medical, veterinary, and agricultural practices) can cause significant antibiotic contamination of the natural environment (Mellon *et al.,* 2001; McEwen and Fedorka-Cray 2002) and the development and proliferation of antimicrobial resistant pathogens (Begum *et al.,* 2005; Furuya and Lowy, 2006; Hamelin *et al.,* 2006; Hu *et al.,* 2008; Ram and Shanker, 2005; Ram *et al.,* 2008a, b). Most of the antibiotic compounds employed in medicine are only partially metabolized by patients and are then discharged into the hospital sewage system or directly into municipal waste water if used at home which eventually ends up in the environment, mainly in the water compartment (Kümmerer, 2004). Antibacterial substances used for promoting rapid growth of livestock enter the environment when manure is applied to fields. These antibiotics may either end up in soil or sediment, surface waters or in groundwater (Kümmerer, 2004). Antibacterial treatments used in aquaculture to treat infections are most often administered to fish through medicated feed or added directly to the water resulting in high local antibiotic concentrations in the water compartment and adjoining sediments and the contamination of the surrounding environment occurs principally from the leaching of uneaten food and faeces (Gordon *et al.,* 2006). Industrial and human activities might contribute to the multi-drug resistance patterns and since rivers are at the receiving end of industrial effluents, exposure to environmental pollutants and changes in nutrient composition can lead to selective pressure that favours antibiotic resistance in certain organisms (Furuya and Lowy 2006; Ram *et al.,* 2008b). This has resulted in surface waters becoming major reservoirs of multi-antimicrobial resistant pathogenic microbes and this pollution may contribute to the maintenance and even the development and spread of bacterial antibiotic resistance (Goni-Urriza *et al.,* 2000). Antibiotics released into the aquatic environment can have the following potential impacts: (i) contamination of raw, treated and recycled water used for drinking, irrigation and recreation; (ii) acceleration of widespread bacterial resistance to antibiotics; and (iii) negative effect (through death or inhibition) on important bacteria in the ecosystem (Costanzo *et al.,* 2005). Antimicrobial resistant bacteria with virulence genes can spread to all regions of the world through global travel and generate significant health and economic impacts (Turner *et al.,* 2006). Antibiotic use selects for existing resistance mechanisms and for novel resistance mutations. Resistance can also be acquired through horizontal gene transfer via uptake of resistance determinants via conjugation, transduction and transformation (Tenover, 2001). In this section, the various classes of antibiotics commonly found in water environments, based on the target cell component, their mode of action and the various resistance mechanisms employed by the microorganisms was discussed.

1.6.1 Antibacterial agents: their targets, classification and mechanism(s) of antibacterial resistance

Targets for antimicrobial agents are a diverse group involved in various cellular functions. The association between antimicrobial agents and targets may be difficult to reverse due to covalent or firm non-covalent binding or it can be readily reversible. Antimicrobial agents may be categorized according to their principal mechanism of action, chemical structure, or spectrum of activity (Calderón and Sabundayo, 2007). The primary mode of action of antibiotics usually involves inhibiting the growth or function of the target microorganism by inhibiting bacterial or fungal cell wall synthesis; protein synthesis; metabolic pathway and nucleic acid synthesis and disrupting cell membrane function (Table 1.2). Antibiotics can be bacteriostatic (temporarily inhibits the growth of the organism and once removed, the organism will resume growth) or bactericidal (causes cell death) (Walsh, 2000). Antibacterial resistance can be attained through intrinsic and acquired mechanisms. Intrinsic resistance mechanisms are a result of naturally occurring genes located on the host's chromosome whereas acquired resistance mechanisms involve mutations in genes which are targeted by antibacterial drugs and transfer of the resulting resistance traits to other organisms on mobile genetic elements via processes of transduction (bacteriophages), conjugation (plasmids and conjugative transposons) and transformation (incorporation of chromosomal DNA, plasmids and other DNAs into the chromosome) (Levy and Marshall, 2004).

Table 1.2: Antibiotic mechanisms of action (adapted from Calderón and Sabundayo, 2007).

Interference with cell wall synthesis	Inhibition of nucleic acid synthesis
• **β-Lactams** ✓ Penicillins (bactericidal) ✓ Cephalosporins (bactericidal) ✓ Carbapenems (bactericidal) ✓ Monobactams (bactericidal) • **Glycopeptides** (bactericidal) ✓ Vancomycin ✓ Teicoplanin	• Fluoroquinolones (bactericidal) • Rifamycins (bactericidal) • Sulfonamides (bacteriostatic) • Trimethoprim (bacteriostatic) • Cyclic lipopeptides (bactericidal)
Inhibition of protein synthesis	**Inhibition of cell membrane function**
• Aminoglycosides (bactericidal) • Tetracyclines (bacteriostatic) • Glycylcyclines • Clindamycin (bacteriostatic) • Chloramphenicol (bacteriostatic) • Macrolides (bacteriostatic or bactericidal) • Ketolides • Oxazolidinones • Streptogramins (bactericidal)	• Amphotericin B • Imidazoles (fungistatic) • Triazoles (fungistatic) • Echinocandins

Plasmids carry resistance genes among other traits and multiple plasmids can exist in a single bacterial cell becoming part of the total genetics of that organism. Transposons are pieces of DNA that code for proteins that facilitate their incorporation into specific genomic regions of plasmids or host's chromosome through recombination (Mazel, 2006). Conjugative transposons assist the transfer of endogenous plasmids from one organism to another. Integrons contain gene cassettes (collection of genes) that are classified according to the sequence of the protein that facilitated the recombination function (Mazel, 2006). These mobile genetic elements can stably integrate into regions of DNA from other organisms and deliver several new genes, particularly resistance genes, in a single exchange.

Efflux pumps (intrinsic mechanism) expel various antibiotics and are now recognized as major contributors to multidrug resistance (Fig. 1.1). Energy-dependent removal of tetracyclines from the bacterial cell is commonly mediated by membrane-associated proteins (*Tet*) which exchange a proton for a tetracycline-cation complex. Twenty different tetracycline efflux proteins belonging to six groups contain 12 or 14 putative transmembrane-spanning segments. Some of these efflux proteins achieve resistance to tetracyclines by engaging in electroneutral reactions and others in electrogenic reactions (Chopra and Roberts, 2001). Efflux proteins specific for the phenicols are classified into 8 different groups (E1 – E8). Expression of some chloramphenicol efflux proteins (*CmlA*) is mediated by an inducible mechanism of translational attenuation (Butaye *et al.,* 2003). *MsrA* is an ABC efflux protein that provides resistance to 14- and 15-membered macrolides and streptogramin B drugs in streptococci and staphylococci, with the exception of clindamycin.

Mef efflux transporters are the predominant macrolide resistance proteins in streptococci. Multi-drug resistance efflux proteins are specified on plasmids which facilitates their movement e.g. *B. subtilis Bmr* and *S. aureus Qac* are two multi-drug resistance efflux proteins where *Bmr* is constitutively expressed and provides intrinsic resistance to chloramphenicol and fluoroquinolones and the *Qac* system provides resistance to antiseptics and disinfectants (Butaye *et al.,* 2003). Another mechanism of drug resistance called gene amplification can result in the over-expression of multi-drug transporters and drug targets (Albertson, 2006); however the resulting phenotype will be an unstable form of resistance which can revert to the wild-type phenotype in the absence of the drug (Nicoloff *et al.,* 2007). With regard to poor permeability of the Gram-negative cell envelope, porin proteins embedded in these envelopes act as nonspecific entrance and exit points for antibiotic and other small molecule organic chemicals. Mutations of the genes encoding these porin proteins results in decreased expression of the porin thus providing resistance to antibiotics. Imipenem passes through *OprD* (*P. aeruginosa* porin protein) as mutations decreased expression of this porin, contributing to clinical imipenem resistance (Alekshun and Levy, 2007; Nikaido, 2001).

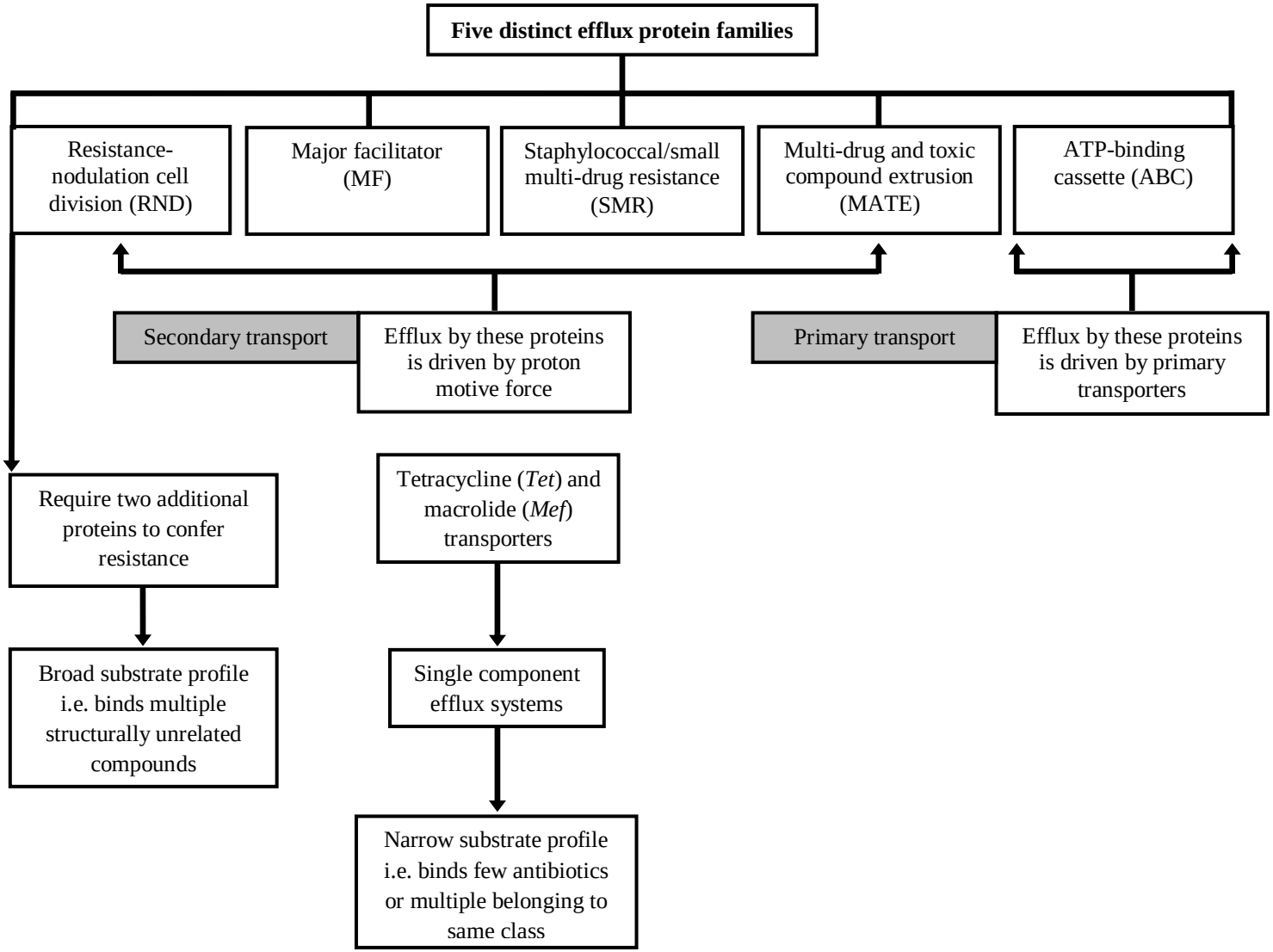

Fig. 1.1: Efflux as a mechanism of antibiotic resistance with most drug efflux proteins belonging to five protein families (adapted from Alekshun and Levy, 2007).

1.6.1.1 Cell wall synthesis as a target

All β-lactam antibiotics are inhibitors of the bacterial cell wall synthesis. The antibiotic exerts their antibacterial effect by first binding to the penicillin-binding protein (PBP) located within the cytoplasmic membrane of the cell wall. Once bound, the antibiotic can cause various effects that eventually result in the failure to make peptidoglycan cross links, leading to a weaker wall that predisposes the treated bacteria to a killing lysis of the cell wall layer (Walsh, 2000). Bacteria have three main survival strategies that they use to combat β-lactam antibiotics which involve destruction of the chemical warhead in the antibiotic by hydrolytic deactivation of the β-lactam ring in the penicillins and cephalosporins by elaboration of the hydrolytic enzyme, β-lactamase. β-lactamases are divided into four groups: class A, C and D have serine active sites and class B requires zinc as a metal ion cofactor (Walsh, 2000). To overcome this resistance mechanism, some antibiotics are combined with β-lactamase inhibitors that prevent the destruction of the β-lactam ring and some penicillin-like nafcillin are resistant to β-lactamase destruction due to the positioning of their side chain (McEvoy, 2006). The second strategy results in decreased penetration of the antibiotic to reach the PBP and the third is mutation of PBPs to lower-affinity forms as well as by expression of new PBPs with lower affinity for the antibiotic (Chambers and Neu, 1995; Mayer *et al.,* 1995). Plasmid-borne *ampC* genes that encode the inducible and usually chromosomal enzyme, *AmpC* can be transferred among *E. coli*, *Klebsiella spp.*, and *Salmonella spp*. (Jacoby and Munoz-Price, 2005). Resistance in staphylococci and streptococci frequently occurs following the acquisition of genes for PBPs that are not sensitive to β-lactam inhibition. The altered PBP of methicillin- (penicillin derivative) resistant *S. aureus*, PBP2a, is specified by *mecA* and is transported on a mobile genetic element called the "staphylococcal cassette chromosome" (Fuda *et al.,* 2005). These new strains include the nearly complete β-lactam resistance phenotype and have the ability to cause serious life-threatening infections.

1.6.1.1.1 Penicillins

Penicillins are categorized based on their spectrum of activity: the natural penicillins were, until recently, considered the drug of choice for many infections caused by Gram-positive bacteria, the aminopenicillins having activity against Gram-positive and Gram-negative bacteria, the carboxypenicillins, with carbenicillin and ticarcillin making up this group, having the same antibacterial spectrum of activity as ampicillin and lastly the ureidopenicillins having the broadest spectrum of activity in the penicillin class (McEvoy, 2006; Neu, 1982).

1.6.1.1.2 Glycopeptides

The glycopeptide agents (vancomycin and teicoplanin) also target cell wall synthesis but do so by sequestering the peptide substrate (terminal D-alanine residues) of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis (Walsh, 2000). The antibiotic binds to the peptide substrate preventing it from reacting with either the transpeptidases or the transglycosylases, which accounts for the high affinity of the antibiotic for its target (Lee *et al.,* 2004). The clinical application of vancomycin is limited to infections caused by Gram-positive bacteria with methicillin-resistant strains of *S. aureus* (MRSA) and most strains of coagulase-negative staphylococci being highly susceptible to the antibiotic (Cui *et al.,* 2006). Bacterial resistance to glycopeptides is dependent upon the minimum inhibitory concentration (MIC) on the target organism and ability of the organism to induce and transfer resistance to vancomycin and teicoplanin. In enterococci, glycopeptide resistance is acquired and attributable to *VanA, B, D, E* and *G* phenotypes whereas *VanC* affords intrinsic resistance (Alekshun and Levy, 2007; Zirakzadeh and Patel, 2005). Bacterial strains that possess the *VanA* and *VanD* phenotypes can enjoy resistance to both vancomycin and teicoplanin whilst the others confer lower resistance level to vancomycin alone (Courvalin, 2006). The resistance phenotype is accomplished using multiple proteins specified in gene clusters and each enzymes activity contributes to the production of a modified peptidoglycan and thus removes the antibiotic's normal target. The movement of the enterococcal *vanA* gene cluster (plasmid-borne) into the methicillin-resistant *S. aureus* has imparted full-blown vancomycin resistance to this pathogen (Weigel *et al.,* 2003).

1.6.1.1.3 Cephalosporins

Cephalosporins are divided into four groupings based on their activity range: first-generation cephalosporins attack Gram-positive cocci, staphylococci, and streptococci, but not enterococcus species and are rarely employed to treat any infection; second-generation cephalosporins display less activity against the Gram-positive cocci but are more active against selected Gram-negative bacilli; third-generation cephalosporins are administered to combat nosocomial, multi-resistant, Gram-negative bacterial infections; and the fourth-generation cephalosporins (composed of only one antimicrobial agent, cefepime) was developed to sustain activity against Gram-positive and Gram-negative bacteria (Calderón and Sabundayo, 2007).

1.6.1.1.4 Carbapenems

Carbapenems (ertapenem, imipenem and meropenem) possess the broadest antimicrobial spectrum of activity, among the β-lactam antibiotics that are presently available, having activity against both aerobic and anaerobic Gram-positive and Gram-negative bacteria. Therefore, many clinicians prefer to reserve these antibiotics for treatment of mixed bacterial infections and aerobic Gram-negative bacteria

that are resistant to other β-lactam antibiotics (Calderón and Sabundayo, 2007). They are effective in treating severe infections at diverse sites, with relatively low resistance rates and a favourable safety profile (Kattan *et al.,* 2008). Carbapenems occupy a unique position in the β-lactam family of antibacterials (Kattan *et al.,* 2008). Their broad spectrum of activity and their stability in the presence of a wide range of β-lactamases, make them important therapeutic options for treating serious infections including resistant *Enterobacteriaceae,* anaerobes, *P. aeruginosa* and *Acinetobacter sp*. (Shahid *et al.,* 2009). Carbapenems bind to unifocal penicillin-binding proteins, disrupting the growth and structural integrity of bacterial cell walls (Nicolau, 2008).

1.6.1.1.5 Monobactams

The monobactam group consists of one antibiotic agent called aztreonam due to its monocyclic chemical structure composed solely of the four-membered β-lactam ring and a side chain as opposed to the five- or six-membered side ring possessed by the penicillins and cephalosporins, respectively. The antibiotic has a narrow spectrum of activity as it binds primarily to the PBP-3 located on *Enterobacteriaceae, Pseudomonas* spp., and other Gram-negative aerobic organisms, but not to the PBPs found on Gram-positive bacteria (Bush *et al.,* 1995).

1.6.1.2 Protein synthesis as a target

Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins, and oxazolidinones produce their antibacterial effects by inhibiting protein synthesis (Tenover, 2006). Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells; therefore antibacterial agents take advantage of these differences to selectively inhibit bacterial growth. Given the large number of steps involved in initiation, elongation and termination of protein assembly by the ribosome, it is not surprising that there would be many steps of binding or catalysis that could be interdicted by these and many other classes of protein-synthesis inhibitors (Walsh, 2000). Tetracyclines and aminoglycosides interact with 16S rRNA (*rrs*) and the macrolides-lincosamides-streptogramins (MLS) family binds to 23S rRNA (*rrl*) (Alekshun and Levy, 2007). Resistance to these drugs via chromosomal mutations is uncommon as multiple *rrs* and *rrl* operons are present in most bacteria. Therefore, resistance is difficult to attain without mutations in all or majority of these operons (Alekshun and Levy, 2007). Also, genes encoding inactivating enzymes act on the MLS antibiotics e.g. esterases act on erythromycin and azithromycin (macrolides), nucleotidyltransferases confer resistance to lincosamides like clindamycin, phosphotransferases modify 14-; 15- and 16-membered macrolides and the hydrolases and acteyltransferases affect/inactivate streptogramin B and A antibiotics, respectively (Matsuoka and Sasaki, 2004).

1.6.1.2.1 Chloramphenicol

Chloramphenicol binds to the 50S ribosomal subunit resulting in bacteriostasis; however, ribosomal binding is reversible as the drug can be removed from the ribosome (Hermann, 2005). The drug also inhibits binding of aminoacyl-tRNA and peptide bond formation (Hermann, 2005). Acetyltransferases that inactivate this antibiotic are the most common resistance mechanisms for this drug and are separated into type A and B proteins. These enzymes are regulated through constitutive expression and translational attenuation (Schwarz *et al.,* 2004).

1.6.1.2.2 Streptogramins

Quinupristin-dalfopristin is the first antibiotic in a class known as streptogramins, a combination shown to be bacteriostatic against most strains of *E. faecium*, including vancomycin resistant *E. faecium* (VREF), but has no activity against *E. faecalis* (Kvirikadze *et al.,* 2006). It is also active against *S. pyogenes* and therefore also indicated for the treatment of complicated skin and skin-structure infections caused by these microorganisms (Calderón and Sabundayo, 2007). Mutations in the large ribosome protein L22 (*rplV*) affects streptogramins susceptibility by interfering with the synergistic relationship that is imperative to this combination's bactericidal mechanism (Hershberger *et al.,* 2004). *E. faecalis* is naturally resistant to quinupristin-dalfopristin as the organism expresses *lsa* which specifies a streptogramin efflux protein belonging to the ABC family (Hershberger *et al.,* 2004).

1.6.1.2.3 Oxazolidinones

Linezolid is the first clinically available oxazolidinone and inhibits bacterial protein synthesis by binding to the 50S subunit on the bacterial ribosome and eventually through a variety of steps, prevents protein synthesis (Bozdogan and Appelbaum, 2004). Linezolid is considered to be bacteriostatic against enterococci and staphylococci and bactericidal against the majority of streptococci strains (Meka and Gold, 2004). Resistance to linezolid has been linked to point mutations in *rrl* in *S. aureus* and *E. faecalis* with the level of resistance increasing with increases in mutations in multiple *rrl* (23S rRNA) alleles in *S. aureus* which can lead to clinically relevant resistance (Wilson *et al.*, 2003).

1.6.1.2.4 Macrolides

Macrolides and lincosamides inhibit RNA-dependent protein synthesis by reversibly binding to the 50S ribosomal subunit, however, the latter binds to ribosomes less strongly than does the former. As a result erythromycin can antagonize the action of lincosamides by displacing it from its binding site even though the binding sites are not identical (Silver, 2007). The primary effect of erythromycin is probably to interfere with the positioning of peptidyl-tRNA and to inhibit translocation (Steigbigel, 1995). These antibiotic classes are primarily bacteriostatic in nature but can be bactericidal, depending on the

microorganism and drug concentration. Macrolides are highly active against many Gram-positive organisms and have good activity against some Gram-negative organisms, especially those commonly isolated from the respiratory tract (Zhanel *et al.*, 2001).

1.6.1.2.5 Ketolides

Telithromycin is the first member in this class of antibiotics known as the ketolides and was derived from erythromycin A. It is very similar in structure to the macrolides but has a ketone bond in the 3-position of the erythronolide ring instead of the sugar, cladinose (Hisanaga *et al.*, 2005). They inhibit protein synthesis via the same route as the macrolides [binds to 23S rRNA (*rrl*)] but provide a broader coverage against macrolide-resistant streptococci. They are being developed for respiratory indications, including community-acquired pneumonia, acute bacterial exacerbations of chronic bronchitis, sinusitis, and pharyngitis, especially when resistant *S. pneumoniae* strains may be a concern (Flamm and Valdes, 2000). Resistance to telithromycin has been found in *S. pneumoniae* with mutations in *rrl*, *rplD* and large ribosome protein L22 (*rplV*) (Hisanaga *et al.*, 2005).

1.6.1.2.6 Aminoglycosides

Aminoglycosides exert their bactericidal effect by penetrating the cell wall by active transport and passive diffusion and irreversibly binds to the 30S subunit of the bacterial ribosome, disrupting protein synthesis, and eventually causes cell death through leakage of essential bacterial constituents (Chen and Kaye, 2009; Vicens and Westhof, 2003). They also impair the permeability barrier allowing loss of molecular weight material and preferentially prevent synthesis of membrane proteins allowing membrane defects. Streptomycin interacts with polysomes (ribosomes engaged in chain elongation), slows down protein synthesis and causes misreading on such ribosomes (Shoji *et al.*, 2009). Passive diffusion of an aminoglycoside can be enhanced by the addition of a cell wall synthesis inhibitor, such as a β-lactam antibiotic, to the antibiotic regimen (Vicens and Westhof, 2003). Mechanisms of resistance to aminoglycosides include: (1) alteration of active transport or passive diffusion preventing the drug from entering the bacterial cell, (2) chromosomal mutations resulting in the inability of the drug to bind to the receptor on the 30S subunit of the bacterial ribosome and (3) production of aminoglycoside-modifying enzymes by microorganisms to inactivate the drug (Kotra *et al.*, 2000). These enzymes are specified by genes on transferable elements and resistance is achieved by (Davies and Wright, 1997): acetyltransferases that N-acetylate tobramycin; gentamicin, netilmicin and amikacin; phosphotransferases that phosphorylate amikacin and nucleotidyltransferases that adenylate tobramycin.

Gentamicin is usually considered the aminoglycoside of choice in most institutions because of its low cost, although in institutions with higher rates of resistance, tobramycin or amikacin may be prescribed more frequently. To decrease the likelihood of resistance formation, aminoglycosides are

commonly used in combination for the treatment of serious Gram-negative infections, especially those caused by *P. aeruginosa* (Chen and Kaye, 2009; Shoji *et al.,* 2009). Even though aminoglycosides have some limited activity against Gram-positive bacteria, they are never used alone for this purpose (Calderón and Sabundayo, 2007).

1.6.1.2.7 Tetracyclines

Tetracyclines prevent enzymatic and non-enzymatic binding of aminoacyl-tRNA to the A-site of the ribosome and inhibit polypeptide chain termination by inhibiting the interaction of the termination factors RF_1 or RF_2 with the termination codons (Brodersen *et al.,* 2000; Pioletti *et al.,* 2001). At low concentrations tetracyclines stimulate RNA synthesis by inhibiting the production of guanosine tetra- and penta-phosphates which are responsible for the stringent control of RNA synthesis by amino acids (Brodersen *et al.,* 2000; Chopra and Roberts, 2001). Tetracyclines are classified based on their duration of action. Once a microorganism develops resistance to one tetracycline, it generally confers resistance to the entire class of drugs. Resistance to tetracyclines commonly occurs via (Chopra and Roberts, 2001): (a) chromosomal mutations in the outer membrane of the organism resulting in decreased penetration of the drug into the cell, (b) energy-dependent pumping of the drug out of the cell, (c) biological or chemical inactivation of tetracyclines by resistant bacteria and (d) production of proteins that allow protein synthesis to continue in the presence of tetracycline within the cell.

Tetracycline resistance due to a point mutation in 3 rRNA operons in *Propionibacterium acnes* and 2 copies of *rrn* in *Helicobacter pylori* has been documented (Wu *et al.,* 2005). Prevalent mechanisms of tetracycline resistance are drug efflux and ribosome protection, where determinants for the latter have sequence similarity to bacterial elongation factors (EF-G and EF-Tu), possess GTPase activity and help release tetracycline from the ribosome target in an energy-dependent process (Connell *et al.,* 2003). Moore *et al.* (2005) characterized a flavin-dependent monooxygenase, encoded by the *tet*(X) gene identified in *B. fragilis*, which acts on older tetracyclines (such as oxytetracycline and chlortetracycline) as well as the newer tetracyclines (like doxycycline and minocycline).

1.6.1.2.8 Glycylcyclines

Tigecycline, a new broad-spectrum antimicrobial agent makes up the class of semi-synthetic glycylcyclines, was designed to overcome the two mechanisms of resistance to tetracyclines: efflux pumps and ribosomal protection proteins (Dean *et al.,* 2003). A bulky side chain added at position 9 of the minocycline molecule renders the drug less affected by resistance mutation while retaining the same mechanism of action as tetracyclines (Dean *et al.,* 2003; Hirata *et al.,* 2004). Improved activity and tolerability allow these glycylcycline derivatives to bind approximately five times more effectively than tetracycline. Tigecycline has activity *in vitro* against many Gram-positive organisms including MRSA,

glycopeptide-intermediately resistant *S. aureus* (GISA), penicillin-resistant *S. pneumoniae*, and vancomycin-resistant *enterococci* (VRE) (Olson *et al.*, 2006; Stein and Craig, 2006).

1.6.1.3 Nucleic acid synthesis as a target

1.6.1.3.1 Fluoroquinolones

The fluoroquinolones are classified into four generations based on their antimicrobial activity. Nalidixic acid, a first-generation fluoroquinolone has a narrow Gram-negative coverage and limited tissue distribution. Second-generation fluoroquinolones such as ciprofloxacin, enoxacin, levofloxacin, lomefloxacin, norfloxacin, ofloxacin, and temafloxacin, were developed from changing the basic structure of nalidixic acid and have a broader spectrum of antibacterial activity. The third-generation fluoroquinolones include gatifloxacin, sparfloxacin, moxifloxacin, grepafloxacin and posses improved Gram-negative and -positive pathogen coverage as compared to the second-generation. Fourth-generation group have improved Gram-positive coverage, maintain Gram-negative coverage, and gain anaerobic coverage (Fass, 1985; Wolfson and Hooper, 1985). These drugs inhibit bacterial DNA synthesis by inhibiting the enzyme DNA gyrase, which is responsible for breaking the super helical twist of DNA to allow the incorporation of new DNA and resealing the break. Currently, 12 fluoroquinolones are commercially available that are bactericidal and have a wide range of activity against Gram-negative and some Gram-positive organisms (Pankey and Sabath, 2004). Compared with the other quinolones, ciprofloxacin is more active against pseudomonads and most Gram-negative organisms (Calderón and Sabundayo, 2007; Hooper, 1995). Fluoroquinolone resistance is attributed to mutations within the drug's targets (DNA gyrase and topoisomerase IV) and these mutations are predominantly located in the fluoroquinolone-resistance-determining-region (QRDR) of the subunits that make up the targets (Drlica and Malik, 2003; Silver, 2007). High fluoroquinolone resistant isolates can bear multiple QRDR mutations which afford other resistance mechanisms to this class of antibiotics such as drug efflux. Also, the plasmid-mediated *qnr* determinants (pentapeptide repeat family of proteins) mediate resistance by protecting the targets from the inhibitory action of fluoroquinolones (Silver, 2007; Tran and Jacoby, 2002). Another fluoroquinolone resistance mechanism called *MfpA* found in *M. tuberculosis* inhibits DNA gyrase by mimicking the structure of B-form DNA (Alekshun and Levy, 2007; Hegde *et al.*, 2005).

1.6.1.3.2 Sulfonamides and Trimethoprim

The sulfonamides and trimethoprim exert their antibacterial activity by reducing the rate of synthesis of functional dihydrofolate (Huovinen *et al.*, 1995; Projan, 2002). Either drug when used alone is bacteriostatic, but when combined they are bactericidal to susceptible organisms. Sulfonamides act as inhibitors of dihydropteroate synthetase (DHPS) by competing with *p*-aminobenzoic acid to form adducts; however these adducts diffuse out of cells and do not reach high enough concentrations to inhibit

enzymes of folate synthesis or to inhibit cell growth (Projan, 2002). Trimethoprim is a potent inhibitor of the enzyme dihydrofolate reductase and interferes with the conversion of folic acid to folinic acid (tetrahydrofolic acid) (Projan, 2002). Folinic acid is necessary in the production of purines, the backbone of both bacterial and mammalian DNA, and therefore inhibition of such results in cell death. A combination of these drugs results in a sequential blockade in the folate synthesis pathway in bacteria (Smith and Powell, 2000). Trimethoprim-sulfamethoxazole has a wide range of activity against both Gram-positive and Gram-negative organisms, including *H. influenzae*, *S. pneumoniae*, *S. aureus*, *Salmonella* spp., *Shigella* spp., *Nocardia* spp., *Listeria* spp., *E. coli*, most *Enterobacteriaceae*, and *Pseudomonas* species other than *P. aeruginosa* (Skold, 2001). Mutations in the gene encoding DHPS decreases the enzyme's affinity for the sulfonamides whereas mutations in the chromosomal gene specifying dihydrofolate reductase can result in over-expression of the enzyme with a reduced affinity for trimethoprim, thus offering resistance to these drugs (Skold, 2001). Acquired resistance to these antibiotics results when genes specifying enzymes that are insensitive to drug inhibition are attained. The main determinants of sulfonamide resistance are the genes (*sul1* and *sul2*) that specify sulfonamide-insensitive DHPS which are present on class 1 integrons (*sul1*) and plasmids (*sul2*) (Skold, 2001). Trimethoprim resistance determinants (chronologically numbered from *dfr1*) are present on the Tn*7* transposons and move between organisms on class 1 and 2 integrons (Adrian *et al.,* 2000; Levings *et al.,* 2006).

1.6.1.3.3 Rifamycins

The rifamycins are a class of antibiotics used primarily for the treatment of mycobacterial infections but are also active against some Gram-positive and Gram-negative organisms (Chen and Kaye, 2009). They arrest transcription by interacting with *RpoB* (β subunit of DNA dependent RNA polymerase). Point mutations in the rifampin-binding region of *rpoB* results in resistance to this class of drugs, which remain the primary choices for front-line therapy of *M. tuberculosis* infections (Heep *et al.,* 2000).

1.6.1.3.4 Cyclic lipopeptides

Daptomycin represents a new class of antibiotics known as cyclic lipopeptides. The antibiotic introduces its lipid tail into the cytoplasmic membrane of Gram-positive bacteria causing membrane depolarization resulting in a release of intracellular ions (Camargo *et al.,* 2008). Cell death results when extensive dysfunction occurs, such as disruption of DNA, RNA, and protein synthesis (Silverman *et al.,* 2003). Daptomycin is only active against Gram-positive bacteria because it is unable to penetrate the outer membrane of Gram-negative bacteria (Alder, 2005). Laboratory experiments have established that

mutations in multiple chromosomal loci (*mprF*, *yycG*, *rpoB* and *rpoC*) affect daptomycin susceptibility (Friedman *et al.*, 2006; Rose *et al.*, 2008).

1.6.1.4 Cell membrane function as a target

A less well characterized mechanism of action involves the disruption of bacterial membrane structure which include, agents producing membrane disorganization, agents acting as ionophores; true ionophores and channel forming ionophores and agents that inhibit the action of bacterial membrane ATPase (Bryan, 1982). A variety of antibacterial agents act to modify the function of some component of the cytoplasmic membrane, however, many of these agents have poor selective toxicity and cannot be used for systemic infections (Cooke and Rodgers, 2005). Other agents can be used for such infections but only with a significant risk of toxic effects on specific host tissues. The major agents available for systemic use as antibacterial agents are polymyxins. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, through disruption of the membrane lipid packing (Bryan, 1982). Polyene antibiotics (amphotericin) are only active on membranes which contain sterols. Some polyenes (filipin) may act to cause disorganization of the membrane rather than to cause channel formation (Ghannoum and Rice, 1999).

1.7 Scope of the present study

Many rivers and beaches across Durban, South Africa are continuously subjected to pollution from land-based human activities and improper management of these water resource catchments. This has serious consequences for health care management and disease prevention within the local communities and the potential outbreaks of water-borne diseases continue to grow. These water resources might also serve as natural habitats of pathogenic *E. coli* and *V. cholerae* strains which harbour virulence factors, which could play a role in the disease process, as well as various multi-drug resistant water-borne pathogens. The quality of surface waters in Durban has not been adequately investigated, despite the fact that the microbiological qualities of water sources in other provinces in the country have been widely reported. Another gap in the corpus of research on water resources in the Durban region is the paucity of data on physico-chemical profiles, which are indices of water quality. Also, water resource management is hugely dependent on monitoring programs that rely on cultivation-dependent methods for evaluating the microbiological quality of surface waters. In light of the above discussion, this study was therefore undertaken to determine the level of contamination of two rivers and six beaches in and around Durban, over a seasonal cycle, using several bacterial indicator organisms and physico-chemical profiles as indices. The antibiotic resistance profiles (ARPs) of *E. coli* and *V. cholerae* strains, recovered from the water samples, were determined. The changes in the microbial community of the selected catchments were monitored during the four seasons, using denaturing gradient gel electrophoresis (DGGE) to provide information on the possible presence of water-borne pathogens that were not easily detected via culture-dependent methods.

The present study is presented in five chapters namely chapter one which provides a comprehensive review of the relevant literature pertaining to the entire dissertation and touches on the current water situation in South Africa, as well as, water resource protection and management initiatives undertaken in the country. Thereafter, surface water pollution is discussed including the sources of water pollution, factors affecting water quality, the conventional, as well as, the alternative microbial indicators of water quality and their detection methods. The next subsection entails information on the classification, pathogenesis and virulence determinants of toxigenic *Escherichia coli and Vibrio cholerae.* Finally, the contamination of surface waters with antibacterial agents is discussed, including the potential sources of the antibioitcs, classifcation of these substances and microbial resistance mechanisms. Chapter two provides baseline information on the microbial and physico-chemical quality of selected surface water resources in Durban. Furthermore, the effects of seasonal variability on the microbial and physico-chemical qualities of the surface waters were established. Chapter three focused on establishing the antibiotic resistance profiles and multi-drug resistant patterns of *E. coli* and *V. cholerae* strains recovered from the above-mentioned samples. Chapter four analyzed the seasonal changes in the bacterial community profiles of the water samples using DGGE. Finally, chapter five provides an overview of the

significant findings in the various chapters of the dissertation, places the entire research in perspective, identifies limitations and/or shortcomings of the study and finally future perspectives are discussed.

1.7.1 Hypotheses

The microbiological quality of most of the surface waters in the Durban region are generally poor and are not suitable for domestic and/or recreational uses. It was further hypothesized that monitoring and understanding the microbiological quality of these waters, using culture-dependent and culture-independent techniques, will provide relevant information for improved and sustainable water resource management and for implementing appropriate measures to ensure public health safety.

1.7.2 Objectives of the study

a) To evaluate the effects of seasonal variability on the microbial and physico-chemical quality of two rivers and six beaches in Durban, South Africa.

b) To establish the antibiotic resistance and multi-drug resistance profiles of *E. coli* and *V. cholerae* isolates recovered from the river and beach water sources.

c) To analyze the bacterial community profiles of the different water samples using denaturing gradient gel electrophoresis.

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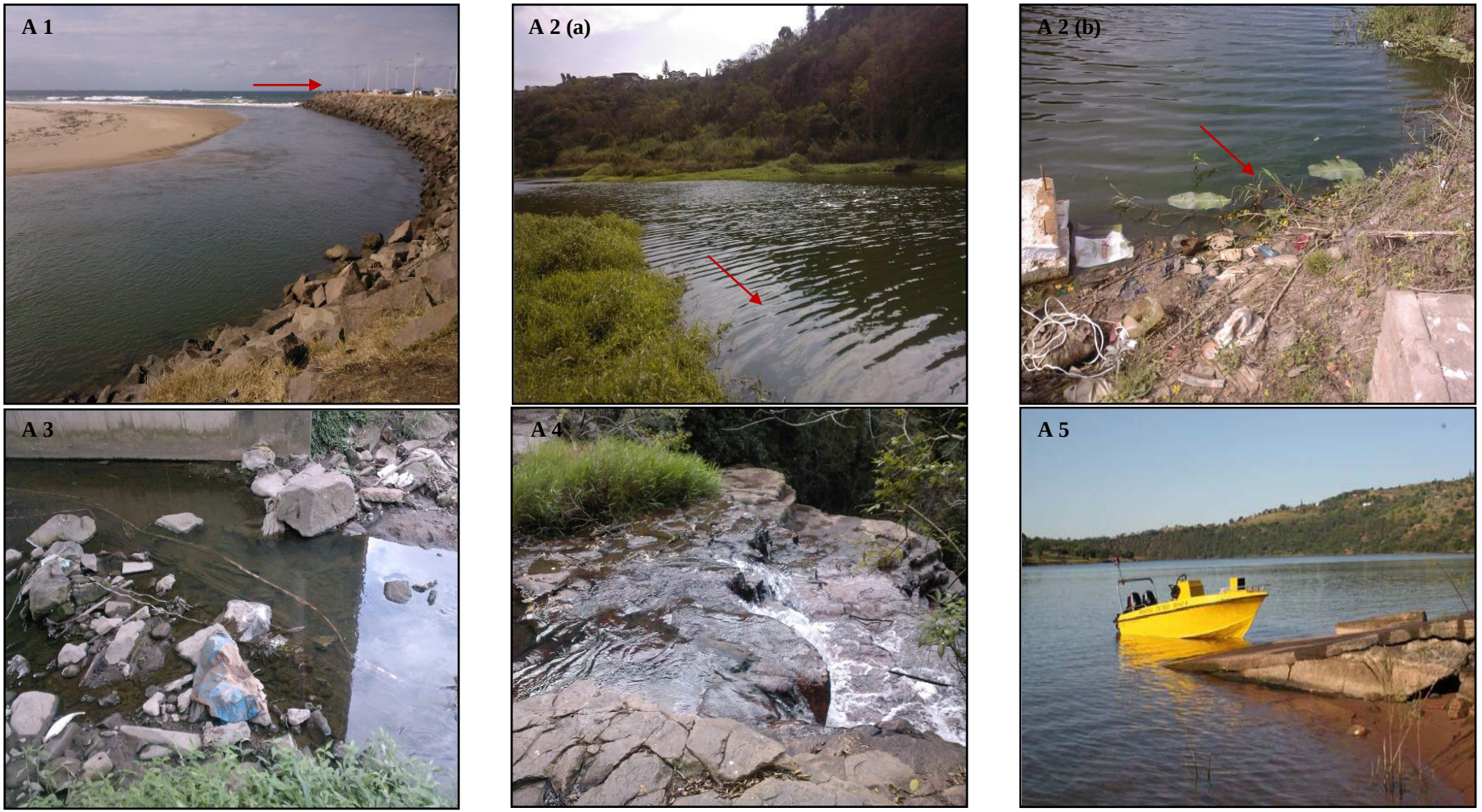


Fig. 2.2: Images illustrating various activities taking place during the collection of water samples from the Umgeni River: A1: Umgeni River mouth (fishing pier indicated by arrow), A2 (a): Further upstream where access to this part of the river is restricted by the Clare Estate crematorium (Reservoir Hills) and the presence of green algae in the water around this region (arrow) could indicate high nutrient loads, A2 (b): By products from funeral rituals, performed by the crematorium lined the bank of the river at this sampling station (arrow), A3: North-west up the river is the next sampling point located in New Germany where informal settlements lined the banks of the river and domestic waste impeded water flow, A4: This sampling point is located within the KrantzKloof Nature Reserve, therefore access is limited, A5: Inanda dam is the final sampling point along the river and here a rudimentary water craft launching pad is seen.

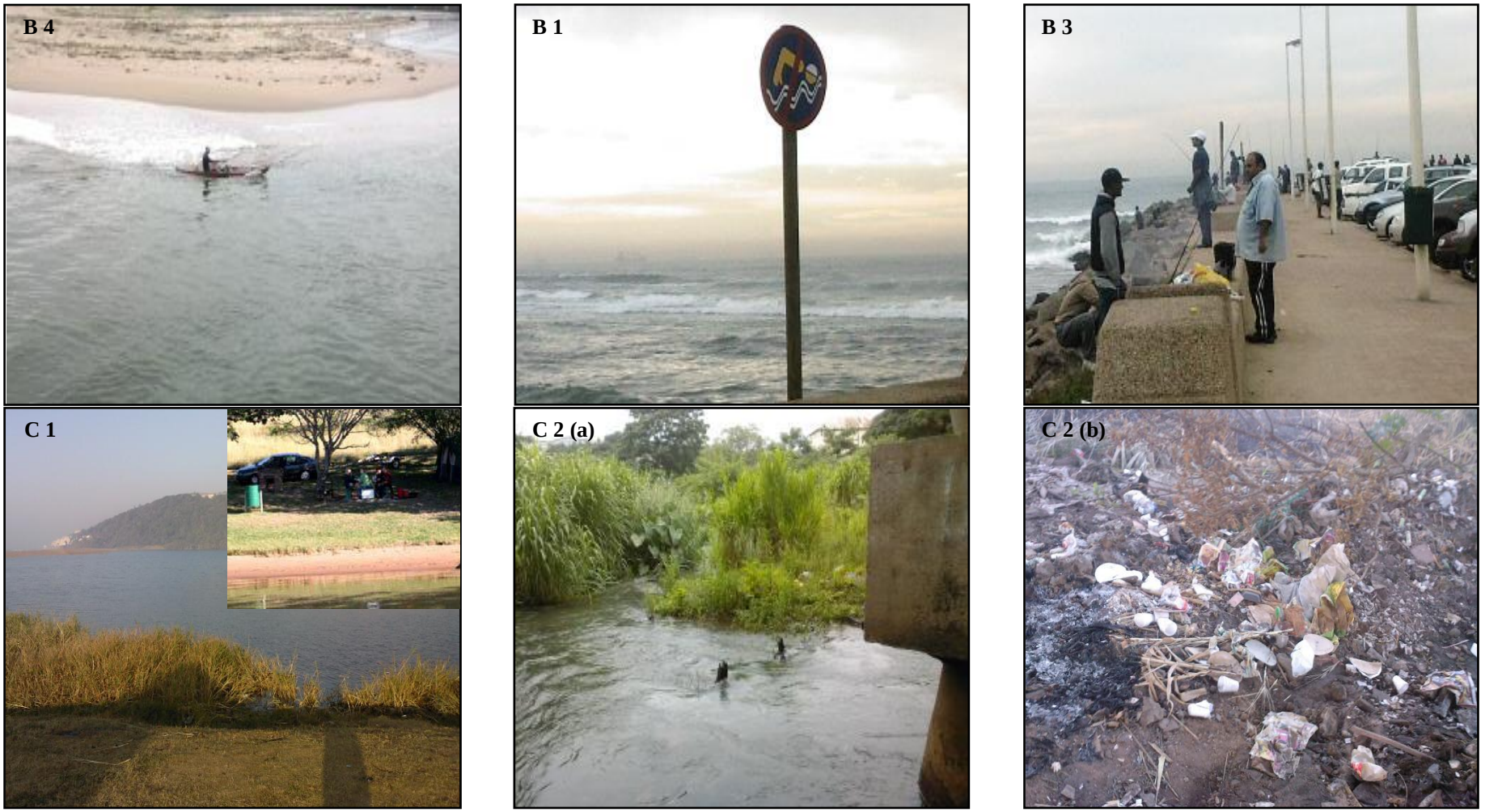


Fig. 2.3: Images illustrating activities taking place during the collection of water samples from the beaches and Umdloti River: B4: Battery Beach where regular canoeing, surfing, swimming, fishing and other activities were seen, B1: Virginia Beach where no swimming is allowed; however fishing is allowed, B3: Umgeni South Beach is a popular fishing and recreational spot among Durbanites which was abuzz on most weekends, C1: Umdloti River mouth was generally not subjected to heavy human traffic with few families enjoying the braai facilities (insert), C2 (a): Umdloti River flowing under Conco bridge in Verulam which is surrounded by thick vegetation and disturbance is caused by near-by road works and construction in preparation for the soccer World Cup in 2010, C2 (b): Unauthorized dumping (domestic waste, construction material etc.) at this sampling point washed into the river below during heavy rains which impeded flow.

Fig. 2.4: Images illustrating various activities taking place during collection of water samples from the Umdloti River: C3 (a): Canelands or Palmer's Estate in the Verulam region where truck tyres and large concrete slabs were in the river which caused some parts of the river to become stagnant and highly turbid, C3 (b): Locals use this spot for subsistence fishing, C3 (c): This sampling spot is characterized by thick vegetation which caused the river to become less visible, C4 (a): Hazelmere dam (access point to the dam which was used by visitors for fishing activities, jet skiing, swimming etc. (insert), C4 (b): Springbok and wildebeest, grazing in the early hours of the day and use the dam as a source of drinking water, C4 (c): Zebra grazing along banks of the dam.

2.1 Introduction

The increasing stress on fresh and marine water resources due to ever rising demand and profligate use, as well as the growing pollution worldwide, is of serious concern. This problem is exacerbated with rapidly growing urban areas that place heavy pressure on neighbouring water resources (Rijsberman, 2006). Currently, almost 900 million people, worldwide, are deprived of safe water supplies while 2.5 billion people live without proper sanitary facilities, majority (at least 80%) of whom live in underdeveloped areas (JMP, 2008). Consequently, an estimated 10% of the total global burden of illness results from water-related diseases. In addition, 1.4 million children die as a result of diarrhoea with an estimated 4 billion cases of diarrhoea occurring each year (Prüss-Üstün *et al.*, 2008).

According to the Food and Agriculture Organization (FAO) of the United Nations, by 2025, 1.8 billion people will be living in countries or regions with absolute water scarcity, and two-thirds of the world population could be under stress conditions. The poor spatial distribution of rainfall in South Africa makes the availability of water across the country highly uneven, thus resulting in the country's water resources being extremely limited. South Africa is among the driest on earth with an average rainfall of about 18 inches per annum, which is just above half the world average of 34 inches per annum (Otieno and Ochieng, 2004). This water shortage situation is further compounded by the strong seasonality of rainfall, as well as high within-season variability over almost the entire country which results in highly variable surface runoff (Otieno and Ochieng, 2004). To meet the country's growing water requirements; water resources are highly developed and utilized in large parts of the country which has significantly altered their flow regime (Otieno and Ochieng, 2004). In some instances this has resulted in severe degradation of the water quality of numerous surface water resources.

Major sources of pollution of surface waters are agricultural drainage and wash-off (irrigation return flows, fertilizers, pesticides and runoff from feedlots), urban wash-off and effluent return flows (bacteriological contamination, salts and nutrients), industries (chemical substances), mining (acids and salts), insufficient sanitation services (microbial contamination), leachate from landfills, human settlements and intrusion of sea water (Pandey, 2006; Wang *et al.*, 2007). These polluting sources have had a significant effect on the quality of surface water bodies in Durban, South Africa, such as the Umgeni and Umdloti Rivers, as well as several beaches along this subtropical coastal city. The water quality of the lower reaches of these river catchments are being affected by urbanization (human activities and influences) which poses a high risk to users due to microbial contamination. Furthermore, some of the beaches have lost their Blue Flag status following failure to meet recommended recreational water quality standards which places it's users at risk and can negatively affect tourism. Therefore, deteriorating water quality is one of the major threats to South Africa's capability to provide sufficient water of appropriate quality to meet its needs and to ensure environmental sustainability.

Pollutants normally enter surface waters through transport pathways such as surface runoff and since surface runoff is a seasonal phenomenon, it is largely affected by climate (Pejman *et al.*, 2009; Singh *et al.*, 2004). Seasonal variations in rainfall patterns, surface runoff, groundwater flow and water interception and abstraction can also have a strong effect on the microbial load and pollutant concentration of surface water resources (Khadka and Khanal, 2008; Mtethiwa *et al.*, 2008). Effects of seasonal changes on the microbiological quality of surface waters could include more frequent and intense rainfall events during certain seasons, leading to increased overland and shallow sub-surface flow which can mobilize pathogens and other contaminants (Schuster-Wallace *et al.*, 2008). Changes in temperature regimes could result in changes in reproduction, survival and infectivity rates of various pathogens. Also, changes in ecology within seasonal cycles could provide either more or less favourable habitat conditions which may result in areas being able to support pathogens (or vectors and their hosts) during certain seasons (Schuster-Wallace *et al.*, 2008).

Climate change can affect water quality in at least five ways (Anderson *et al.*, 2008): (1) a rise in water temperature can affect the rate of operation of biogeochemical processes which determine water quality; (2) changes in flow volumes can alter residence times and dilution; (3) increased atmospheric CO_2 could affect the rate at which this gas is dissolved in water and hence the rate of operation of many biogeochemical processes; (4) a change in soil properties and flow pathways may alter the transport of chemical load from river catchments and (5) changes in inputs of chemicals to the catchment perhaps due to the effects of climate change on agriculture, may alter water chemistry. Predictions of the direct effects of climate change on aquatic ecosystems are very complex, and projecting these in combination with other human impacts poses an even greater challenge. The consequences of human activities on freshwaters are considerable, including: acidification by sulphur and nitrogen compounds; mobilization of organic substances from soils; accelerated erosion and sedimentation in river channels; damming and diversion of river flows; eutrophication by nitrogen and phosphorus compounds; structural alteration of rivers for flood prevention in the interests of agriculture; fragmentation of habitats and introduction of alien species and selective removal of others (Anderson *et al.*, 2008).

According to the 2007 IPCC report, water temperatures, increased precipitation intensity and longer periods of low flows will exacerbate many forms of water pollution and jeopardize water quality. In addition, seasonal variations can affect the function of water infrastructure, operation and management practices (Kundzewicz *et al.*, 2008). Therefore, characterization of seasonal changes in surface water quality is important for evaluating temporal pollution due to natural or anthropogenic sources. Thus, this chapter focused on the effects of seasonal fluctuations on the microbiological quality and physico-chemical aspects of two rivers and six beaches in Durban, South Africa.

2.2 Materials and methods

2.2.1 Description of study area

In this study, two rivers and six beaches located in and around the metropolitan municipality of Durban, KwaZulu-Natal, were chosen for investigation based on the direct and indirect activities involving these surface water resources. The two rivers, Umgeni River (5 sampling points along river) and Umdloti River (4 sampling points along river), were sampled from the river mouths leading up to the dams (Inanda and Hazelmere Dam, respectively) which are situated within these catchments (Fig. 2.1).

The Umgeni River is 230 km long with a catchment area of approximately 5000 km²; making it the largest catchment in the KwaZulu-Natal region and the general location of the river mouth lies approximately 5 km north of the centre of Durban (WRC, 2002). The river provides water to over 3.5 million people and supports an area that is responsible for approximately 65% of the total economic production in the province (WRC, 2002). The Albert Falls, Midmar and Nagle Dams are also situated within this catchment. The Inanda Dam is located in the valley of a Thousand Hills, 42 km north of Durban. The dam is 23 km long from the bridge to the dam wall and is 1.5 km at the widest point and 50 m deep at its deepest point (IDPRMP, 2007). The water surface of the dam covers 1440 hectares and has a peripheral distance of approximately 100 km around the dam, when full (IDPRMP, 2007). From Inanda Dam, the river flows from the Thousand Hills with a gentle gradient for 24 km before it flows out to sea just north of Durban (IDPRMP, 2007). This part of the river is extensively modified, with riparian vegetation and the direction of flow is significantly altered to accommodate human settlements and activities. Historically the river entered Durban bay over a large swampy plain where Durban is now situated.

The Umdloti River flows perennially with a mean annual average flow of 2 m^3/sec (WRC, 2002). The river estuary mouth is approximately 25 km north east of Durban Central and the length of the river is < 90 km (WRC, 2002). Approximately 20 km upstream of the estuary, lies the Hazelmere dam in a gorge on the river, about 5 km upstream (north-west) of the town of Verulam (HDPRMP, 2007). The dam was constructed to supply domestic, industrial and agricultural requirements within the area and in expectation of Durban's new international airport. Water is released from the dam for downstream irrigators and Tongaat Hullett. Access to the dam (northern bank only) is along a secondary (tarred) road which runs north from Verulam to Hazelmere for 8 km, followed by 2 km of minor (gravel) road (HDPRMP, 2007). Hazelmere Dam has a low retention time and a high flushing rate. The average length of time that water is held in the reservoir (when full, and before sedimentation), is estimated to be 120 days (HDPRMP, 2007). This gives an average flushing rate of three times per year when the reservoir is full. Access to the Msinsi Holdings Recreational Facility, on the southern bank of the reservoir and 8km from north of Verulam, is along a minor road that passes the Barnes Sewage Disposal Works.

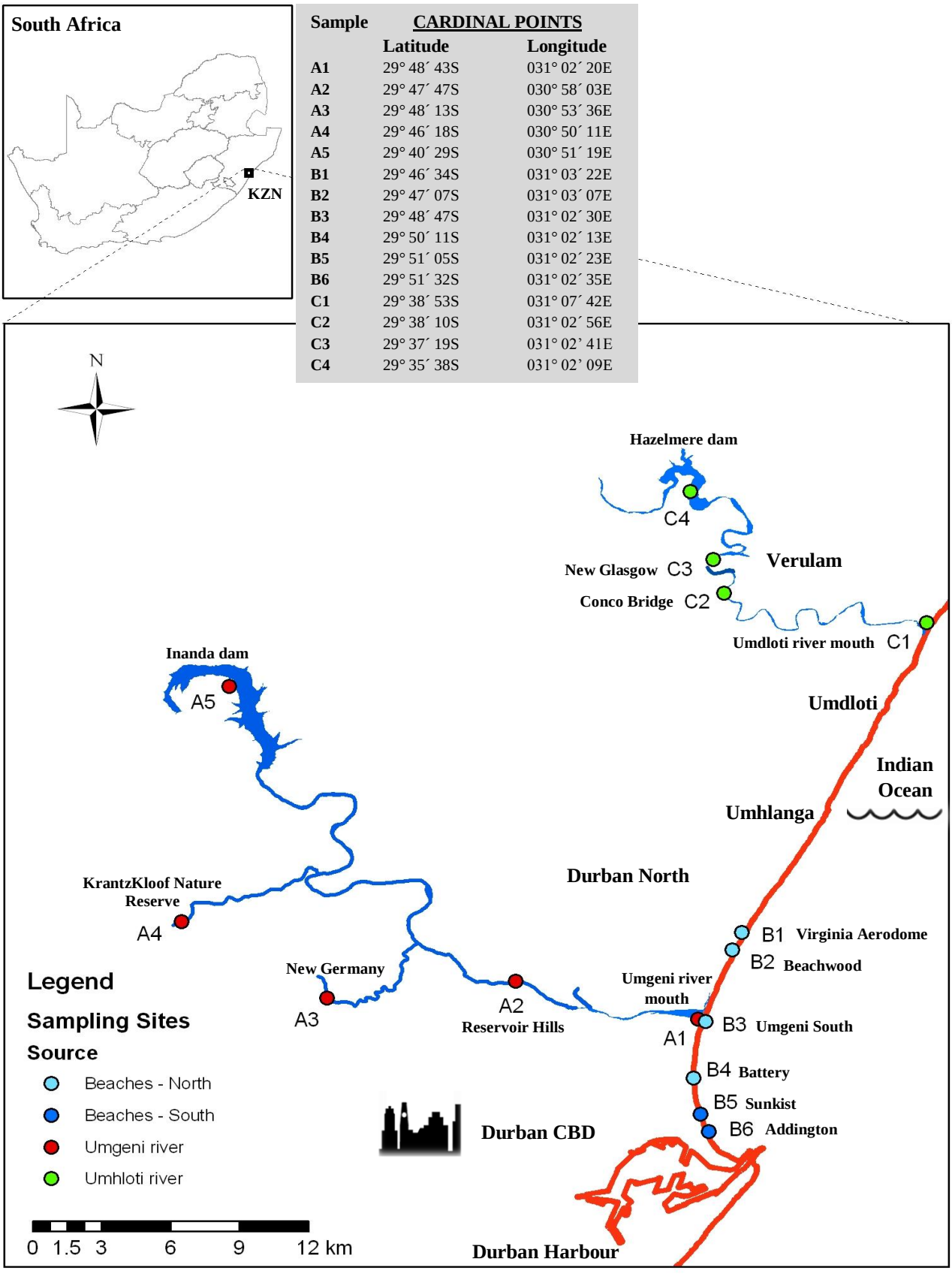


Fig. 2.1: Map of the study region within Durban and surrounding areas. Shown are the locations of the water resources and 15 sampling sites investigated in this study.

The six beaches sampled are located from north to south of the Durban coastline, each being designated as either a bathing beach (B3 – B6), utilized for full contact recreational activities (e.g. diving and boating) (B1 – B6), primarily used for non-contact activities (e.g. fishing) (B1 – B3) or in combination. Beaches B3 to B6 are utilized more frequently for various activities than the remaining two beaches which are located further north. The water quality situation at these beaches raises concern as they are used extensively for public bathing and they support the hotels that are situated within the area and tourist activities (Mardon and Stretch, 2004).

Generally, the physical characteristics of these surface water resources varied at each sampling point, with different activities affecting the water at each point. Unless the sampling point was located within a regulated area (A2, A4, A5 and C4), access to the rivers was unrestricted. The Umgeni River discharges into the Indian Ocean at sampling point A1 while the Umdloti River discharges into the same ocean at sampling point C1.

2.2.2 Sample collection

Water samples were collected from the above mentioned water resources, in 5 L plastic containers, along the pre-designated collection points. Sampling was conducted every 3 months from October 2008 (spring) to July 2009 (winter), to cover the four seasons of the year, so as to evaluate the possible effects of seasonal variations on the water quality at these sampling stations. The plastic containers were sterilized using 70% (v/v) alcohol and rinsed with the water from the source prior to collection. Each water sample was collected by holding the container by the handle and plunging it 0.5 m below the water surface, opposite to the water current. If there was no current, it was artificially created by pushing the container forward. The container was filled leaving about 30 mm of headspace to allow mixing during laboratory analysis. Collection of water samples from the beaches was along the shoreline. The samples were protected from direct sunlight and transported on gel ice packs to the laboratories in the Department of Microbiology, University of KwaZulu-Natal (Westville campus). All samples were stored at 4°C until further microbiological analysis (Buckalew *et al.*, 2006). Various activities, including illegal dumping; fishing; animal defecation etc., taking place at the sampling points that may have an effect on the water quality were documented and shown in Figs. 2.2 and 2.4. Lower reaches of both rivers were highly affected, compared to the upper parts, as these parts of the rivers flowed through more urbanized areas leading toward the river mouths.

2.2.3 Determination of physico-chemical parameters

Several physico-chemical parameters including, temperature; pH; turbidity; biological oxygen demand; chemical oxygen demand and heavy metal(s) concentrations were measured. The temperatures of the water samples were determined *in situ* using a mercury thermometer, whilst the pH and turbidities was measured immediately upon arrival at the laboratory facility, using a pH meter (Beckman Φ 50 pH meter) and portable 2100P turbidimeter (Hach), respectively. In addition, samples were analyzed for conductivity and inorganic water quality parameters, including total phosphate, nitrate, ammonia and total sulphate concentrations by the Umgeni Water Analytical laboratory (accredited to ISO/IEC 17025:1999).

2.2.3.1 Biological oxygen demand

The biological oxygen demand (BOD_5) of each water sample was determined using the OxiDirect BOD system (Hach) over a 5 day period. The selected BOD_5 range was 0 – 40 mg/L and the corresponding sample volume was used for the analysis. Essential pretreatment steps, if necessary, were carried out prior to analysis, including adjustment of pH for optimum biochemical oxidation; filtering highly turbid samples; homogenizing samples containing fibers and thorough mixing of samples. The analysis was conducted following the manufacturer's instruction and the BOD measured was expressed in mg/L.

2.2.3.2 Chemical oxygen demand

The chemical oxygen demand (COD) of each sample was determined photometrically using the SpectroQuant Nova 60 COD cell test (Merck) measuring in the range of 10 – 150 mg/L COD or O_2. The water sample was oxidized with a hot sulphuric acid solution of potassium dichromate, with silver sulphate as the catalyst. Chloride was masked with mercury sulphate and the concentration of unconsumed $Cr_2O_7^{-2}$ ions was then determined photometrically. Each COD test vial, containing all the required reagents, was vortexed to resuspend the bottom sediment. Three milliliters of each sample was carefully transferred into a reaction cell, capped securely and vigorously mixed. The samples were digested in a thermoreactor (Hach) at 148°C for 2 hrs. Following sample digestion, the reaction cells were allowed to cool for 10 min, gently mixed and then allowed to cool to room temperature before measuring the COD of the sample.

2.2.3.3 Heavy metal concentration

Five heavy metal concentrations; cadmium (Cd^{2+}); copper (Cu^{2+}); aluminium (Al^{3+}); lead (Pb^{2+}) and mercury (Hg^{2+}), were measured in each sample using an inductively coupled plasma – optical emission spectrometer (ICP-OES) 5300 DV and 2100 DV (Perkin-Elmer). Heavy metal standard solutions were prepared in the range of 0.01 parts per million (ppm) to 0.25 parts per million (ppm) from

1000 ppm stock solutions of each metal in a final volume of 25 ml (calibrated volumetric conical flask). Each standard solution (multi-element) was prepared by adding the appropriate amount of each heavy metal from their stock solutions (Merck CertiPUR) to sterile double distilled water in the volumetric flask to achieve the desired concentration followed by the addition of a few drops of nitric acid (HNO_3) for metal preservation. Twenty milliliters of each water sample was filtered through 0.45 µm pore size membrane filters to remove any fibers that may cause a blockage in the autosampler of the ICP-OES and transferred to centrifuge tubes. The prepared heavy metal standard solutions were used to generate calibration curves from which the concentrations of each metal in the water samples were determined.

2.2.4 Enumeration of bacterial indicator organisms

The membrane filtration (MF) technique was employed for the enumeration and isolation of eight indicator organisms from all water samples, according to standard protocol (Standard Methods, 1992). Appropriate dilutions of the water samples were prepared with sterile double distilled water prior to filtration. Fifty milliliter samples were filtered through 0.45 µm pore size GN-6 Metricel membrane filters (Pall, 47 mm), held in a stainless steel filtration unit, which were then transferred to 45 mm petri plates containing various selective media (Appendix A) for recovery of each indicator group (Table 2.1). After the incubation period, all the typical colonies grown on the filters were recorded as presumptive counts for the estimation of colony forming units per milliliter (cfu/ml). Sample blanks were also processed during MF to ensure quality of dilution water (Buckalew *et al*., 2006).

Table 2.1: Overview of selective media and incubation conditions used for the enumeration of the bacterial indicator organisms from river and beach water samples.

Indicator	Selective media	Incubation conditions
Total Heterotrophic Bacteria (THB)	Nutrient agar (NA)	48 hrs at 37°C
Total coliforms (TC)	mEndo agar	24 hrs at 35°C
Faecal coliforms (FC)	m-FC agar	24 hrs at 44.5°C
Enterococci (EC)	Membrane enterococcus agar (mEA) Bile aesculin agar (BAA)	**Presumptive test:** 4 hrs at 37°C followed by 44 hrs at 44°C **Confirmatory test:** 4 hrs at 44°C
Faecal streptococci (FS)	Oxolinic acid aesculin azide agar (OAA)	48 hrs at 42°C
Presumptive *Vibrio cholerae* (VC)	Thiosulphate citrate bile salts sucrose agar (TCBS)	18 – 24 hrs at 37°C
Presumptive *Salmonella spp.* (SAL)	S-S agar	24 hrs at 35°C
Presumptive *Shigella spp.* (SHIG)	S-S agar	24 hrs at 35°C

2.2.5 Statistical analysis

The obtained data was subjected to descriptive statistical analysis (95% confidence limit). The general linearized model (GLM) of SAS (statistical analysis system) was used to generate analysis of variance (ANOVA), means, standard error and range. Pearson product-moment correlation coefficient was used to test differences among all possible pairs of physico-chemical parameters and bacterial indicator organisms. All statistical analysis was performed using SAS (SAS version 8, SAS Institute, Cary, NC).

2.3 Results

2.3.1 Physico-chemical characteristics of water samples

Spatial and seasonal fluctuations of the selected environmental variables of the water samples are presented in Tables 2.2 to 2.5. Temperature profiles varied significantly ($p < 0.05$) and ranged from 13°C (A4 – winter) to 26.5°C (A2/A5 – summer) for the Umgeni River, 13°C (B6 – winter) to 27°C (B6 – summer) for the beaches and 12°C (C3 – winter) to 26°C (C1/C3/C4 – summer) for the Umdloti River. The pH varied significantly ($p < 0.05$) across the four seasons and ranged from 6.30 to 8.45 (Umgeni River), 6.37 to 8.30 (beaches) and 5.96 to 7.94 (Umdloti River). All sampling stations were mostly alkaline except points A4, A5, B1, B6 and C1 during spring and B1, B5 and B6 during summer. Turbidities ranged from 0.53 NTU to 15.6 NTU (Umgeni River); 0.57 NTU to 2.37 NTU (beaches) and 2.23 NTU to 18.8 NTU (Umdloti River) but only varied significantly ($p < 0.05$) for the Umgeni River and the beach water samples. The Umdloti River exhibited high turbidity levels during summer with sampling point C4 having increased turbidity during the summer and winter seasons. Large seasonal variations in turbidity, COD and conductivity levels amongst the sampling points along the Umgeni River were observed, with weak fluctuations in BOD_5 values. BOD_5, COD and conductivity values for the Umgeni River ranged from 0.52 mg/L (A2 – summer) to 3.22 mg/L (A4 – autumn), 10.5 mg/L (A5 – spring) to 63.4 (A4 – spring) and 18.6 mS/m (A4 – autumn) to 5180 (A1 – summer), respectively. Among the beach water samples, significant variations ($p < 0.05$) in BOD_5 and conductivity levels were observed, across the four seasons. Higher BOD_5 values were recorded during spring and summer for the six beaches with values ranging from 1 mg/L (B5 – autumn) to 9.05 mg/L (B6 – summer). COD and conductivity values for the beaches ranged from 43 mg/L (B3 – summer) to 149 mg/L (B6 – summer) and 4730 mS/m (B3 – autumn) to 5190 mS/m (B4 – summer), respectively. BOD_5 values for the Umdloti River ranged from 0.58 mg/L (C4 – spring) to 7.32 mg/L (C1 – spring) while COD ranged from 13.9 mg/L (C2 – spring) to 72.9 mg/L (C1 – spring). Drastic changes in COD values occurred at points C1 and C2 over the seasonal cycle with little or no variations in conductivity values at points C3 and C4. The values for turbidity, BOD_5 and COD at A4, B6 and C1 were consistently higher than those at the other stations.

During spring and summer, all river and beach water samples had < 500 µg/L phosphate concentrations; however, these concentrations increased significantly ($p < 0.05$) during autumn and winter in both Umgeni and Umdloti Rivers with concentrations as high as 2470 µg/L (A3 – winter) and 2590 µg/L (C4 – autumn) been recorded in both rivers. According to the phosphate levels recorded, all water samples from the rivers and beaches, complied with the recommended WHO phosphate limit of 0.5 mg/L (WHO, 1996) during spring and summer; however during autumn and winter all points along the Umgeni and Umdloti Rivers exceeded this limit. Majority of the samples had low concentrations of ammonia and nitrates; however nitrate levels recorded for the beach and Umdloti River samples differed significantly ($p < 0.05$) during spring, summer and winter. Sulphate concentrations varied considerably at points A3, A4, A5 and C1, particularly over spring and winter. Sulphate concentrations for the beach samples varied significantly ($p < 0.05$), ranging from 2355 mg/L (B5 – summer) to 2899 mg/L (B2 – winter) as compared to the Umgeni and Umdloti Rivers which ranged from 3.90 mg/L (A4 – autumn) to 2762 mg/L (A1 – summer) and 4.47 mg/L (C4 – winter) to 168 mg/L (C1 – winter), respectively. Samples collected at the river mouths (A1 and C1), exhibited the highest sulphate concentrations compared to the other sampling points along those rivers.

Random variations among the heavy metal concentrations were recorded for the river and beach water samples. Lead, cadmium and copper concentrations for the Umgeni River varied significantly ($p < 0.05$) while aluminium and copper concentrations for the beaches and the Umdloti River varied significantly ($p < 0.05$), throughout the four seasons. Heavy metal concentrations for the Umgeni River ranged as follows: 0.023 mg/L to 0.082 mg/L for Pb^{2+}; 0.0126 mg/L to 0.1231 mg/L for Hg^{2+}; 0.073 mg/L to 0.416 mg/L for Cd^{2+}; 0.049 mg/L to 0.912 mg/L for Al^{3+} and 0.006 mg/L to 0.108 mg/L for Cu^{2+}. Heavy metal concentrations for the six beaches ranged as follows: 0.049 mg/L to 0.655 mg/L for Pb^{2+}; 0.0121 mg/L to 0.0329 mg/L for Hg^{2+}; 0.088 mg/L to 0.388 mg/L for Cd^{2+}; 0.028 mg/L to 0.303 mg/L for Al^{3+} and 0.006 mg/L to 0.118 mg/L for Cu^{2+}. Finally, heavy metal concentrations for the Umdloti River varied as follows: 0.039 mg/L to 0.135 mg/L for Pb^{2+}; 0.0122 mg/L to 0.1046 mg/L for Hg^{2+}; 0.068 mg/L to 0.283 mg/L for Cd^{2+}; 0.037 mg/L to 1.875 mg/L for Al^{3+} and 0.006 mg/L to 0.144 mg/L for Cu^{2+}. The South African Target Quality Range guidelines for the heavy metals (in surface waters) tested in this study are 0 – 0.01 mg/L for lead; 0 – 5 µg/L for cadmium; 0 – 0.001 mg/L for mercury; 0 – 1 mg/L for copper and 0 – 0.15 mg/L for aluminium (DWAF, 1996). Thus all river and beach water samples exceeded the set limits for Pb^{2+}, Hg^{2+} and Cd^{2+} across all seasons. In contrast, all samples (river and beach) complied with the aluminium guideline during spring and summer, except sampling point C1 (Umdloti River mouth) during summer. In addition, only samples collected at points A4, A5, B4 and B5 complied with the aluminium guideline during autumn, while those from points A3, A4, A5, B2, C2 and C4 complied with this guideline during winter. All water samples complied with the copper guideline throughout the seasons.

Table 2.2: Physico-chemical quality of the water samples collected during the spring season.

Sample location		Physical characteristics						Chemical characteristics				Heavy metal concentrations (mg/L)*				
		T (°C)	pH	Turbidity (NTU)	BOD_5 (mg/L)	COD (mg/L)	Conductivity (mS/m)	Phosphate (µg/L)	Nitrate (soluble) (mg/L)	Ammonia (soluble) (mg/L)	Sulphate (soluble) (mg/L)	Pb^{2+}	Hg^{2+}	Cd^{2+}	Al^{3+}	Cu^{2+}
Umgeni River	A1	21	7.43	0.53	2.09	26.7	5100	< 500	< 0.05	< 0.5	2210	0.082 ± 0.0022	0.0236 ± 0.0027	0.172 ± 0.0002	0.069 ± 0.0029	0.011 ± 0.0001
	A2	23	8.23	3.51	1.20	16.3	188	< 500	4.21	< 0.5	25.3	0.063 ± 0.0002	0.0279 ± 0.0014	0.191 ± 0.0001	0.091 ± 0.0013	0.014 ± 0.0002
	A3	19.5	8.01	4.67	0.83	12.8	53.1	< 500	2.34	1.22	33.7	0.055 ± 0.0002	0.0589 ± 0.0047	0.132 ± 0.0009	0.086 ± 0.0009	0.024 ± 0.0004
	A4	18	6.30	1.64	3.15	63.4	4210	< 500	< 0.05	< 0.5	2159	0.047 ± 0.0003	0.0231 ± 0.0035	0.252 ± 0.0022	0.108 ± 0.0003	0.010 ± 0.0004
	A5	22	6.49	3.65	0.60	10.5	1579	< 500	< 0.05	< 0.5	707	0.064 ± 0.0004	0.0413 ± 0.0021	0.074 ± 0.0001	0.140 ± 0.0022	0.013 ± 0.0002
Beaches	B1	17	6.85	0.68	5.71	90.1	5040	< 500	< 0.05	< 0.5	2659	0.075 ± 0.0004	0.0250 ± 0.0052	0.168 ± 0.0014	0.029 ± 0.0015	0.006 ± 0.0002
	B2	18.5	7.51	0.93	4.35	46.6	5160	< 500	< 0.05	< 0.5	2628	0.088 ± 0.0033	0.0293 ± 0.0043	0.133 ± 0.0011	0.049 ± 0.0024	0.008 ± 0.0002
	B3	19	7.33	2.14	3.70	57.5	5180	< 500	< 0.05	< 0.5	2525	0.091 ± 0.0018	0.0122 ± 0.0019	0.259 ± 0.0002	nd	nd
	B4	18	7.34	1.68	6.45	102	5170	< 500	< 0.05	< 0.5	2485	0.111 ± 0.0001	0.0245 ± 0.0007	0.226 ± 0.0003	0.052 ± 0.0008	0.065 ± 0.0004
	B5	21	7.01	1.37	7.12	85.4	5180	< 500	< 0.05	< 0.5	2687	0.655 ± 0.0122	nd	0.311 ± 0.0010	0.030 ± 0.0025	nd
	B6	20	6.37	2.37	8.53	116	5160	< 500	< 0.05	< 0.5	2417	0.159 ± 0.0004	0.0329 ± 0.0005	0.367 ± 0.0037	0.028 ± 0.0109	0.077 ± 0.0003
Umdloti River	C1	21.5	5.96	5.28	7.32	72.9	132	< 500	< 0.05	< 0.5	19.3	0.077 ± 0.0012	0.0361 ± 0.0017	0.085 ± 0.0030	0.133 ± 0.0013	0.105 ± 0.0002
	C2	20	7.19	4.70	1.02	13.9	23.4	< 500	< 0.05	< 0.5	8.35	0.135 ± 0.0003	0.0155 ± 0.0004	0.071 ± 0.0016	0.039 ± 0.0011	0.087 ± 0.0002
	C3	19.5	7.01	5.67	1.78	24.2	19.2	< 500	< 0.05	< 0.5	8.25	0.050 ± 0.0004	0.0184 ± 0.0014	0.198 ± 0.0001	0.054 ± 0.0012	0.069 ± 0.0004
	C4	23	7.10	4.51	0.58	19.7	16.4	< 500	< 0.05	< 0.5	5.30	0.087 ± 0.0008	0.0311 ± 0.0004	0.283 ± 0.0002	0.157 ± 0.0006	0.069 ± 0.0002

***** Values represent average of results taken from ten replicate analyses ± Standard deviation; nd = not detected.

Table 2.3: Physico-chemical quality of the water samples collected during the summer season.

Sample location		Physical characteristics						Chemical characteristics				Heavy metal concentrations (mg/L)*				
		T (°C)	pH	Turbidity (NTU)	BOD_5 (mg/L)	COD (mg/L)	Conductivity (mS/m)	Phosphate (µg/L)	Nitrate (soluble) (mg/L)	Ammonia (soluble) (mg/L)	Sulphate (soluble) (mg/L)	Pb^{2+}	Hg^{2+}	Cd^{2+}	Al^{3+}	Cu^{2+}
Umgeni River	A1	25.5	7.44	0.64	2.36	40.4	5180	< 500	< 0.05	< 0.5	2762	0.065 ± 0.0002	0.0205 ± 0.0036	0.416 ± 0.0001	0.057 ± 0.0200	0.033 ± 0.0011
	A2	26.5	8.45	3.93	0.52	23.4	49.9	< 500	1.47	< 0.5	20.4	0.081 ± 0.0001	0.0218 ± 0.0003	0.272 ± 0.0001	0.132 ± 0.0004	0.025 ± 0.0002
	A3	25	8.20	6.99	0.81	39.7	51.8	< 500	2.87	< 0.5	27.6	0.059 ± 0.0002	0.0251 ± 0.0019	0.315 ± 0.0003	0.077 ± 0.0053	0.027 ± 0.0001
	A4	20	8.33	15.6	1.30	24.1	132	< 500	2.97	< 0.5	12.3	0.051 ± 0.0002	0.0237 ± 0.0028	0.104 ± 0.0001	0.118 ± 0.0128	0.011 ± 0.0003
	A5	26.5	7.77	9.31	0.67	16.1	58.2	< 500	1.00	< 0.5	17.0	0.062 ± 0.0003	0.0305 ± 0.0052	0.242 ± 0.0006	0.049 ± 0.0003	0.009 ± 0.0002
Beaches	B1	25	6.61	2.21	6.17	141	5160	< 500	< 0.05	< 0.5	2546	0.073 ± 0.0201	0.0283 ± 0.0004	0.259 ± 0.0003	0.074 ± 0.0222	0.061 ± 0.0002
	B2	24	7.28	0.91	6.21	95.8	5170	< 500	< 0.05	< 0.5	2420	0.068 ± 0.0001	0.0236 ± 0.0150	0.380 ± 0.0010	0.037 ± 0.0030	0.019 ± 0.0002
	B3	24.5	7.31	2.30	4.91	43.0	5090	< 500	< 0.05	< 0.5	2391	0.121 ± 0.0015	0.0232 ± 0.0019	0.268 ± 0.0002	0.036 ± 0.0008	0.012 ± 0.0002
	B4	26	7.22	1.72	7.43	134	5190	< 500	< 0.05	< 0.5	2806	0.157 ± 0.0032	0.0305 ± 0.0008	0.110 ± 0.0002	0.050 ± 0.0002	0.008 ± 0.0004
	B5	26	6.95	1.39	3.14	77.2	5130	< 500	< 0.05	< 0.5	2355	0.119 ± 0.0006	0.0261 ± 0.0015	0.192 ± 0.0004	0.041 ± 0.0004	0.009 ± 0.0004
	B6	27	6.51	1.14	9.05	149	5160	< 500	< 0.05	< 0.5	2622	0.099 ± 0.0003	0.0312 ± 0.0012	0.303 ± 0.0025	0.063 ± 0.0090	0.021 ± 0.0002
Umdloti River	C1	26	7.84	2.23	5.11	61.3	55.0	< 500	< 0.05	< 0.5	10.4	0.131 ± 0.0004	0.0175 ± 0.0014	0.127 ± 0.0002	0.170 ± 0.0093	0.114 ± 0.0002
	C2	25	7.94	6.98	1.64	43.0	23.3	< 500	< 0.05	< 0.5	9.15	0.075 ± 0.0005	0.0190 ± 0.0026	0.068 ± 0.0003	0.128 ± 0.0214	0.059 ± 0.0002
	C3	26	7.54	7.87	2.25	39.9	19.2	< 500	< 0.05	< 0.5	7.65	0.104 ± 0.0005	0.0214 ± 0.0002	0.183 ± 0.0003	0.046 ± 0.0043	0.081 ± 0.0001
	C4	26	7.23	14.1	0.89	15.0	16.2	< 500	< 0.05	< 0.5	5.14	0.042 ± 0.0112	0.0182 ± 0.0075	0.145 ± 0.0003	0.037 ± 0.0037	0.076 ± 0.0001

***** Values represent average of results taken from ten replicate analyses ± Standard deviation; nd = not detected.

Table 2.4: Physico-chemical quality of the water samples collected during the autumn season.

Sample location		Physical characteristics						Chemical characteristics				Heavy metal concentrations (mg/L)*				
		T (°C)	pH	Turbidity (NTU)	BOD_5 (mg/L)	COD (mg/L)	Conductivity (mS/m)	Phosphate (µg/L)	Nitrate (soluble) (mg/L)	Ammonia (soluble) (mg/L)	Sulphate (soluble) (mg/L)	Pb^{2+}	Hg^{2+}	Cd^{2+}	Al^{3+}	Cu^{2+}
Umgeni River	A1	20.5	8.03	1.20	1.47	32.5	3880	580	< 2.5	0.82	2259	0.038 ± 0.0003	0.0215 ± 0.0123	0.073 ± 0.0017	0.197 ± 0.0014	0.036 ± 0.0002
	A2	19.5	7.29	2.43	1.66	29	143	1760	0.90	< 0.5	14.2	0.042 ± 0.0004	0.0126 ± 0.0030	0.078 ± 0.0022	0.912 ± 0.0011	0.006 ± 0.0002
	A3	17	7.77	5.61	1.15	23.7	57.3	2460	3.25	< 0.5	34.4	0.050 ± 0.0005	0.0293 ± 0.0015	0.108 ± 0.0120	0.259 ± 0.0001	0.023 ± 0.0001
	A4	19.5	7.14	3.87	3.22	27	18.6	940	2.03	< 0.5	3.90	0.051 ± 0.0005	0.0181 ± 0.0003	0.120 ± 0.0003	0.087 ± 0.0020	0.037 ± 0.0003
	A5	20	7.49	2.80	1.83	40.6	20.8	1020	0.71	< 0.5	11.7	0.047 ± 0.0004	0.1231 ± 0.0001	0.113 ± 0.0002	0.058 ± 0.0015	0.008 ± 0.0019
Beaches	B1	22	8.04	1.53	2.00	98	4910	< 500	< 2.5	< 0.5	2745	0.113 ± 0.0111	0.0233 ± 0.0067	0.362 ± 0.0001	0.173 ± 0.0013	0.049 ± 0.0003
	B2	23.5	8.30	2.13	4.29	113	4920	1700	< 2.5	< 0.5	2834	0.062 ± 0.0006	nd	0.219 ± 0.0001	0.214 ± 0.0003	0.031 ± 0.0003
	B3	21	7.84	1.17	3.89	76.8	4730	< 500	< 2.5	< 0.5	2709	0.093 ± 0.0041	0.0312 ± 0.0025	0.296 ± 0.0004	0.221 ± 0.0003	0.026 ± 0.0007
	B4	22	7.66	0.92	2.46	104	4790	< 500	< 2.5	< 0.5	2607	0.049 ± 0.0018	0.0222 ± 0.0034	0.174 ± 0.0003	0.105 ± 0.0001	0.026 ± 0.0034
	B5	22.5	7.95	0.87	1.00	122	4920	< 500	< 2.5	< 0.5	2755	0.114 ± 0.0001	0.0121 ± 0.0001	0.388 ± 0.0011	0.130 ± 0.0004	0.024 ± 0.0004
	B6	19.5	8.01	2.09	1.20	105	4760	< 500	< 2.5	< 0.5	2686	0.103 ± 0.0002	0.0216 ± 0.0001	0.298 ± 0.0028	0.204 ± 0.0017	0.029 ± 0.0005
Umdloti River	C1	20.5	7.56	2.84	0.71	32.3	572	1530	< 0.05	< 0.5	168	0.069 ± 0.0007	0.0122 ± 0.0008	0.169 ± 0.0022	0.385 ± 0.0011	0.046 ± 0.0005
	C2	18.5	7.13	2.72	2.60	30.4	40.6	2100	0.46	< 0.5	8.18	0.060 ± 0.0001	0.0207 ± 0.0002	0.176 ± 0.0002	0.600 ± 0.0002	0.012 ± 0.0002
	C3	20.5	7.40	3.05	0.76	31	20.6	2170	< 0.05	< 0.5	7.68	0.039 ± 0.0002	0.0204 ± 0.0002	0.114 ± 0.0003	0.930 ± 0.0001	0.006 ± 0.0001
	C4	20	7.50	6.51	3.00	20.9	15.8	2590	0.20	< 0.5	4.60	0.042 ± 0.0002	0.0139 ± 0.0002	0.122 ± 0.0002	1.875 ± 0.0001	0.007 ± 0.0006

***** Values represent average of results taken from ten replicate analyses ± Standard deviation; nd = not detected.

Table 2.5: Physico-chemical quality of the water samples collected during the winter season.

Sample location		Physical characteristics						Chemical characteristics				Heavy metal concentrations (mg/L)*				
		T (°C)	pH	Turbidity (NTU)	BOD_5 (mg/L)	COD (mg/L)	Conductivity (mS/m)	Phosphate (µg/L)	Nitrate (soluble) (mg/L)	Ammonia (soluble) (mg/L)	Sulphate (soluble) (mg/L)	Pb^{2+}	Hg^{2+}	Cd^{2+}	Al^{3+}	Cu^{2+}
Umgeni River	A1	17	7.77	2.63	1.62	53.1	3880	740	< 2.5	1.08	2482	0.027 ± 0.0012	0.0163 ± 0.0001	0.263 ± 0.0019	0.162 ± 0.0032	0.061 ± 0.0027
	A2	17	8.37	7.42	1.48	38	164	1710	0.99	< 0.5	14.9	0.032 ± 0.0003	0.0134 ± 0.0001	0.132 ± 0.0010	0.278 ± 0.0018	0.093 ± 0.0014
	A3	16	8.00	4.82	0.77	32.6	539	2470	1.48	< 0.5	236	0.023 ± 0.0005	0.0191 ± 0.0031	0.097 ± 0.0006	0.140 ± 0.0016	nd
	A4	13	8.20	4.58	0.93	28	32.3	1170	2.18	0.5	4.87	0.061 ± 0.0023	0.1022 ± 0.0008	0.105 ± 0.0018	0.112 ± 0.0011	0.108 ± 0.0021
	A5	15	7.81	1.64	1.35	11.4	21.3	1000	0.65	< 0.5	11.3	0.038 ± 0.0026	0.0202 ± 0.0005	0.207 ± 0.0020	0.149 ± 0.0013	0.075 ± 0.0002
Beaches	B1	14	8.09	1.17	2.61	112	4900	< 500	< 2.5	< 0.5	2761	0.055 ± 0.0003	0.0281 ± 0.0001	0.088 ± 0.0020	0.201 ± 0.0013	0.064 ± 0.0054
	B2	13.5	8.06	0.57	2.82	87.9	4950	< 500	< 2.5	< 0.5	2899	0.083 ± 0.0002	nd	0.222 ± 0.0002	0.136 ± 0.0002	0.118 ± 0.0049
	B3	15	8.01	1.21	3.05	93	4740	6230	< 2.5	< 0.5	2648	0.057 ± 0.0004	0.0253 ± 0.0002	0.249 ± 0.0001	0.194 ± 0.0037	0.101 ± 0.0005
	B4	16	7.99	0.78	1.11	110	4790	< 500	< 2.5	< 0.5	2789	0.106 ± 0.0002	0.0306 ± 0.0002	0.312 ± 0.0011	0.267 ± 0.0024	0.039 ± 0.0015
	B5	14.5	8.05	0.63	3.25	115	4940	< 500	< 2.5	< 0.5	2618	0.133 ± 0.0001	0.0300 ± 0.0003	0.190 ± 0.0028	0.301 ± 0.0021	0.086 ± 0.0022
	B6	13	8.05	1.53	3.01	91.6	4810	< 500	< 2.5	< 0.5	2671	0.073 ± 0.0011	nd	0.381 ± 0.0003	0.303 ± 0.0014	0.080 ± 0.0039
Umdloti River	C1	14.5	7.49	5.39	1.79	16.7	440	2000	0.41	< 0.5	161	0.061 ± 0.0001	0.0240 ± 0.0026	0.213 ± 0.0008	0.183 ± 0.0010	0.084 ± 0.0018
	C2	12.5	7.79	4.63	2.14	15.3	52.2	2050	0.65	< 0.5	10.6	0.048 ± 0.0008	0.0122 ± 0.0001	0.173 ± 0.0009	0.109 ± 0.0006	0.049 ± 0.0011
	C3	12	7.67	3.44	2.53	21.2	19.3	1990	0.14	< 0.5	7.73	0.080 ± 0.0002	0.1046 ± 0.0003	0.126 ± 0.0002	0.222 ± 0.0002	0.103 ± 0.0010
	C4	13	7.66	18.8	2.08	18.4	15.6	2560	0.21	< 0.5	4.47	0.059 ± 0.0014	0.0189 ± 0.0040	0.184 ± 0.0002	0.072 ± 0.0001	0.144 ± 0.0003

***** Values represent average of results taken from ten replicate analyses ± Standard deviation; nd = not detected.

The correlation matrices of the physico-chemical properties of the water samples are presented in Tables 2.6 to 2.8 (physical parameters), Tables 2.9 to 2.10 (chemical parameters) and Table 2.11 to 2.12 (heavy metal concentrations); however insignificant matrices were not shown. The sampling stations belonging to a particular resource (Umgeni River, beaches or Umdloti River) were combined to calculate the correlation matrix. The correlation coefficients should therefore be interpreted with caution as they are affected simultaneously by spatial and temporal variations. Nevertheless clear relationships can be readily inferred. In general, the water temperature of the Umgeni River samples showed weak correlations with pH (r = 0.021), turbidity (r = 0.085) and conductivity (r = 0.107) and anti-correlated with BOD_5 (r = - 0.143) and COD (r = - 0.144) (Table 2.6). Positive correlations were observed between BOD_5 and conductivity (r = 0.490; p = 0.028) and COD and conductivity (r = 0.508; p = 0.022) (Table 2.6). Significant anti-correlations were indicated by pH and BOD_5 (r = - 0.562; p = 0.010); turbidity and conductivity (r = - 0.531; p = 0.016) and BOD_5 and COD (r = - 0.556; p = 0.011). As shown in Table 2.7, the temperature of the beach water samples was positively correlated to turbidity (r = 0.558; p = 0.011) and exhibited a negative correlation with pH (r = - 0.476; p = 0.034). pH showed strong, negative correlations to BOD_5 (r = - 0.691; p = 0.001) and conductivity (r = - 0.739; $p < 0.01$) while BOD_5 showed a strong positive correlation to conductivity (r = 0.713; $p < 0.01$). COD formed weak, insignificant correlations with temperature (r = 0.071), pH (r = 0.109), turbidity (r = - 0.070), BOD_5 (r = 0.011) and conductivity (r = - 0.165) (Table 2.7). As shown in Table 2.8, the BOD_5 parameter of the Umdloti River samples strongly correlated with COD (r = 0.786; $p < 0.01$). In addition, water temperature of the Umdloti River samples exhibited negative correlations with pH (r = - 0.138), turbidity (r = - 0.052) and conductivity (r = - 0.151) while turbidity made insignificant anti-correlations with BOD_5 (r = - 0.136), COD (r = - 0.278) and conductivity (r = - 0.238) (Table 2.8).

Table 2.6: Correlation matrix of physical characteristics for the Umgeni River.

	Temperature	pH	Turbidity	BOD_5	COD	Conductivity
Temperature	1					
pH	0.021	1				
Turbidity	0.085	0.435	1			
BOD_5	- 0.143	- 0.562[b]	- 0.375	1		
COD	- 0.144	- 0.242	- 0.215	- 0.556[a]	1	
Conductivity	0.107	- 0.422	- 0.531[a]	0.490[a]	0.508[a]	1

[a] Correlation is significant at the 0.05 level (2-tailed)
[b] Correlation is significant at the 0.01 level (2-tailed)

Table 2.7: Correlation matrix of physical characteristics for the beaches.

	Temperature	pH	Turbidity	BOD_5	COD	Conductivity
Temperature	1					
pH	- 0.476[a]	1				
Turbidity	0.558[a]	- 0.308	1			
BOD_5	0.360	- 0.691[b]	0.387	1		
COD	0.071	0.109	- 0.070	0.011	1	
Conductivity	0.401	- 0.739[b]	0.400	0.713[b]	- 0.165	1

[a] Correlation is significant at the 0.05 level (2-tailed)
[b] Correlation is significant at the 0.01 level (2-tailed)

Table 2.8: Correlation matrix of physical characteristics for the Umdloti River.

	Temperature	pH	Turbidity	BOD_5	COD	Conductivity
Temperature	1					
pH	- 0.138	1				
Turbidity	- 0.052	0.071	1			
BOD_5	0.072	- 0.447	- 0.136	1		
COD	0.481	- 0.344	- 0.278	0.786[a]	1	
Conductivity	- 0.151	0.014	- 0.238	- 0.088	0.037	1

[a] Correlation is significant at the 0.01 level (2-tailed)

Furthermore, nitrate concentrations in the beach water samples showed a strong correlation to sulphate (r = 0.628; p = 0.003) (Table 2.9) while phosphate concentrations in the Umdloti River samples had a positive correlation to nitrate (r = 0.605; p = 0.013) (Table 2.10). Table 2.11 indicates a positive correlation between aluminium and copper concentrations (r = 0.532; p = 0.019) for the beaches while Table 2.12 indicates a negative correlation between aluminium and copper concentrations (r = - 0.691; p = 0.003) for the Umdloti River samples.

Table 2.9: Correlation matrix of chemical characteristics for the beaches.

	Phosphate	Nitrate	Ammonia	Sulphate
Phosphate	1			
Nitrate	0.274	1		
Ammonia	*b*	*b*	1	
Sulphate	0.068	0.628[a]	*b*	1

[a] Correlation is significant at the 0.01 level (2-tailed)
[b] Cannot be computed because at least one of the variables is constant

Table 2.10: Correlation matrix of chemical characteristics for the Umdloti River.

	Phosphate	Nitrate	Ammonia	Sulphate
Phosphate	1			
Nitrate	0.605[a]	1		
Ammonia	*b*	*b*	1	
Sulphate	0.180	0.137	*b*	1

[a] Correlation is significant at the 0.05 level (2-tailed)
[b] Cannot be computed because at least one of the variables is constant

Table 2.11: Correlation matrix of heavy metal concentrations for the beaches.

	Lead	Mercury	Cadmium	Aluminium	Copper
Lead	1				
Mercury	0.094	1			
Cadmium	0.203	- 0.431	1		
Aluminium	- 0.261	0.276	0.064	1	
Copper	- 0.316	0.131	- 0.052	0.532[a]	1

[a] Correlation is significant at the 0.05 level (2-tailed)

Table 2.12: Correlation matrix of heavy metal concentrations for the Umdloti River.

	Lead	Mercury	Cadmium	Aluminium	Copper
Lead	1				
Mercury	0.095	1			
Cadmium	- 0.198	- 0.067	1		
Aluminium	- 0.405	- 0.120	- 0.160	1	
Copper	0.484	0.299	0.020	- 0.691[a]	1

[a] Correlation is significant at the 0.01 level (2-tailed)

2.3.2 Enumeration of bacterial indicator organisms of the water samples

Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for the Umgeni River over the four seasons are presented in Figure 2.5, while the Total Heterotrophic Bacterial (THB) population is presented in Figure 2.6 and presumptive enterococci (EC) and faecal streptococci (FS) levels for the river are presented in Figure 2.7, respectively. TC and FC counts varied significantly ($p < 0.05$) and ranged from 1.805 x 10^3 cfu/100ml (A3 – winter) to 8.60 x 10^3 cfu/100ml (A2 – summer) and 0.82 x 10^3 cfu/100ml (A3/A4 – winter) to 3.70 x 10^3 cfu/100ml (A2 – summer), respectively (Fig. 2.5). VC, SAL and SHIG populations were detected at all sampling points along the river throughout the seasons. VC, SAL and SHIG concentrations for the Umgeni River ranged from 2.168 x 10^3 cfu/100ml (A4 – winter) to 10.6 x 10^3 cfu/100ml (A2 – summer), 0.245 x 10^3 cfu/100ml (A3 – spring) to 2.45 x 10^3 cfu/100ml (A2 – summer) and 0.605 x 10^3 cfu/100ml (A4 – winter) to 2.725 x 10^3 cfu/100ml (A3 – autumn), respectively (Fig. 2.5). The highest population of TC, FC, VC and SAL was observed in the water sample collected at point A2 during summer, compared to the other sampling points along the Umgeni River while sampling site A4 had the lowest FC, VC and SHIG concentrations during winter.

With respect to THB counts, the general trend observed was a significant increase ($p < 0.05$) in the levels of THB at all sampling points along the Umgeni River, during summer followed by a gradual decline through autumn and winter ($p < 0.05$). Minimum THB counts were recorded during spring while the maximum values were recorded during summer and ranged as follows: 1.575 x 10^6 cfu/100ml (A5 – spring) to 21.55 10^6 cfu/100ml (A1 – summer) (Fig. 2.6). The Umgeni River mouth (sampling point A1) consistently had the highest THB counts during spring, summer and autumn compared to the other sampling sites along the river during those seasons.

EC and FS concentrations ranged from 0.5 x 10^2 cfu/100ml (A1 – spring) to 4.8 x 10^2 cfu/100ml (A2 – summer) and 1.67 x 10^2 cfu/100ml (A4 – autumn) to 7.40 x 10^2 cfu/100ml (A2 – spring), respectively (Fig. 2.7). Increased EC concentrations were recorded in water samples collected from points A1 and A2 during the summer period while a reduction in EC levels at these sampling sites was documented during the autumn and winter seasons. In contrast, an increase in EC levels was observed in water samples collected from points A3, A4 and A5 from summer to autumn and winter. In addition, no EC was detected at sampling point A3 during the spring season.

Correlation matrices (significant at the 0.05 level) between the bacterial indicator organisms for Umgeni River during the study period are shown in Table 2.13 while those matrices between the bacterial indicator organisms and selected physico-chemical parameters are shown in Appendix B.

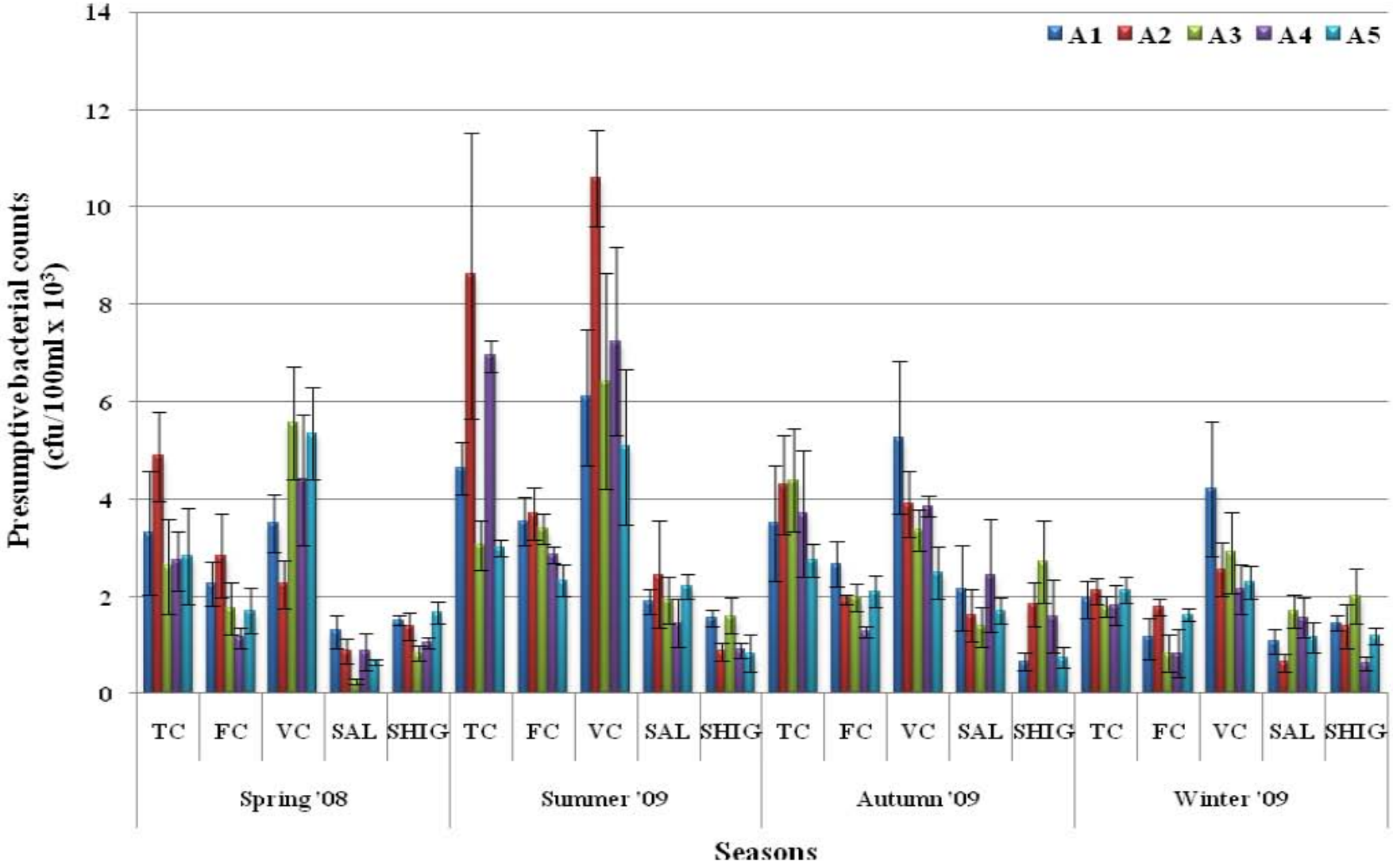


Fig. 2.5: Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for Umgeni River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (n = 4 or 5) while the *error bars* show the standard deviation.

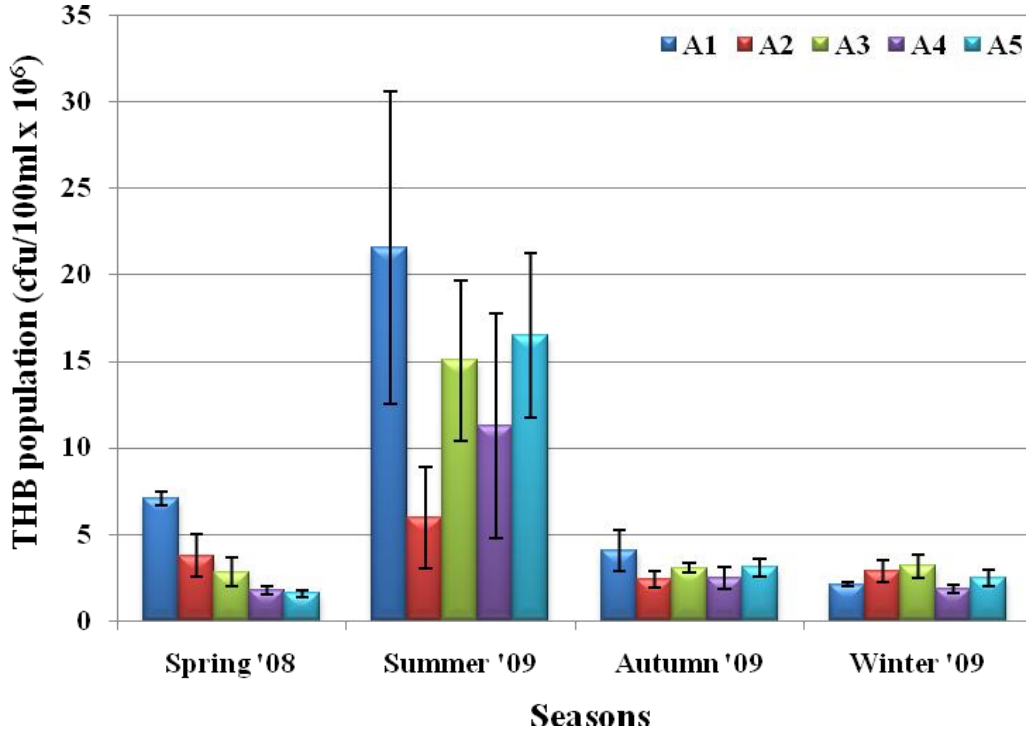


Fig. 2.6: Total Heterotrophic Bacterial (THB) populations for Umgeni River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (*n* = 4 or 5) while the *error bars* show the standard deviation.

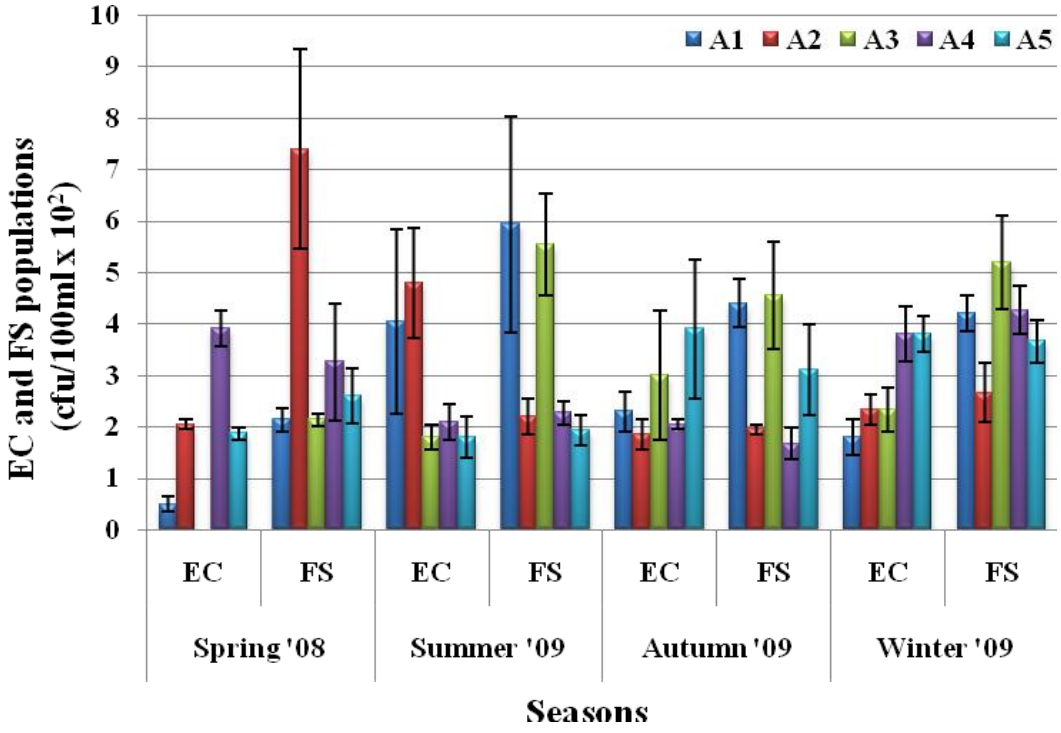


Fig. 2.7: Presumptive enterococci (EC) and faecal streptococci (FS) populations for Umgeni River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (*n* = 3 or 4) while the *error bars* show the standard deviation.

Positive, significant correlations between the indicator organisms for the Umgeni River include: TC and FC (r = 0.394), TC and VC (r = 0.294), VC and FC (r = 0.557), FC and SAL (r = 0.342) and VC and SAL (r = 0.370) with no significant correlations between EC, FS and SHIG populations with the other indicators during the four seasons. Strong, significant correlations were observed between water temperature and TC (r = 0.549), FC (r = 0.767), VC (r = 0.599) and SAL (r = 0.598) counts while TC correlated with turbidity (r = 0.483), over the study period. THB displayed high, significant correlations with FC (r = 0.575), VC (r = 0.459), SAL (r = 0.361) and temperature (r = 0.706).

Table 2.13: Correlation matrix of the bacterial indicator organisms for the Umgeni River during the study period.

	THB	**TC**	**FC**	**EC**	**FS**	**VC**	**SAL**	**SHIG**
THB	1							
TC	0.023	1						
FC	0.575[a]	0.394[a]	1					
EC	0.066	0.113	0.086	1				
FS	0.209	0.035	0.181	0.197	1			
VC	0.459[a]	0.294[a]	0.557[a]	0.060	- 0.206	1		
SAL	0.361[a]	0.213	0.342[a]	0.205	- 0.048	0.370[a]	1	
SHIG	- 0.074	0.106	- 0.081	- 0.214	0.180	- 0.173	- 0.010	1

[a] Correlation is significant at the 0.05 level (2-tailed)
THB, total heterotrophic bacterial; TC, total coliforms; FC, faecal coliforms; EC, enterococci; FS, faecal streptococci; VC, *Vibrio cholerae*; SAL, *Salmonella spp.*; SHIG, *Shigella spp.*

Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for the different beaches over the four seasons are presented in Figure 2.8, while the Total Heterotrophic Bacterial (THB) population is presented in Figure 2.9 and presumptive enterococci (EC) and faecal streptococci (FS) levels are presented in Figure 2.10, respectively. TC and FC counts varied significantly ($p < 0.05$) and ranged from 1.468 x 10^2 cfu/100ml (B4 – winter) to 6.40 x 10^2 cfu/100ml (B2 – summer) and 0.61 x 10^2 cfu/100ml (B5 – winter) to 3.37 x 10^2 cfu/100ml (B6 – autumn), respectively (Fig. 2.8). VC, SAL and SHIG populations were detected at all beaches on all sampling occasions and concentrations of these indicators varied significantly ($p < 0.05$) across the seasonal cycle. VC, SAL and SHIG counts for the beaches ranged from 1.364 x 10^2 cfu/100ml (B5 – winter) to 4.44 x 10^2 cfu/100ml (B6 – summer), 0.6 x 10^2 cfu/100ml (B3 – spring) to 3.075 x 10^2 cfu/100ml (B5 – summer) and 0.844 x 10^2 cfu/100ml (B3 – winter) to 3.775 x 10^2 cfu/100ml (B4 – spring), respectively (Fig. 2.8). In general, the lowest TC, FC and VC counts were recorded during winter

among the beaches whilst the lowest SAL and SHIG counts were recorded during spring. TC, FC, VC and SAL indicator concentrations peaked during summer and autumn. Overall, Addington beach (B6) had the highest FC and VC levels compared to the other beaches; however Beachwood beach (B2) consistently had the highest FC counts during spring summer and winter and the highest TC counts during summer, autumn and winter compared to the other beaches while Virginia beach (B1) had the highest VC counts during autumn and winter.

A significant increase ($p < 0.05$) in THB population was observed for all beach water samples during the summer season with beaches B4, B5 and B6 having the upper THB levels compared to beaches B1 to B3 (Fig. 2.9). During spring and winter, Virginia beach (B1) had the highest THB counts whereas during summer and autumn Addington beach (B6) had the highest THB counts. THB counts for the beaches ranged as follows: 1.296×10^4 cfu/100ml (B4 – spring) to 5.385×10^4 cfu/100ml (B6 – summer).

EC concentrations for the beach water samples varied from 0.16×10^2 cfu/100ml (B5 – autumn) to 1.90×10^2 cfu/100ml (B4 – winter) (Fig. 2.10). High EC levels were detected in beach water samples B3 and B6 during spring and summer followed by a reduction in these levels over the autumn and winter seasons. No EC was detected at sampling sites B3 and B5 during winter; however a drastic increase in the EC concentration at sampling site B4 was observed during the same season compared to the previous seasons. FS counts varied significantly ($p < 0.05$) among the four seasons and ranged from 0.1133×10^2 cfu/100ml (B2 – autumn) to 0.6067×10^2 cfu/100ml (B2 – spring). Beach water sample, B2, had the lowest FS counts during summer and autumn and the highest FS counts during spring and winter.

Correlation matrices (significant at the 0.05 level) between the bacterial indicator organisms for the beaches during the study period are shown in Table 2.14 while correlation matrices (significant at the 0.05 level) between the bacterial indicator organisms and selected physico-chemical parameters are shown in Appendix B. TC showed positive, significant correlations with FC ($r = 0.700$), VC ($r = 0.229$), SHIG ($r = 0.449$), temperature ($r = 0.668$) and BOD_5 ($r = 0.588$) while FC exhibited positive, significant correlations with SAL ($r = 0.373$), SHIG ($r = 0.284$) and temperature ($r = 0.754$). FS showed weak to fair correlations with VC ($r = 0.337$), SAL ($r = 0.274$) and SHIG ($r = 0.325$) while VC weakly correlated with SHIG ($r = 0.221$). In addition, strong, significant correlations were observed between VC and turbidity ($r = 0.545$), VC and BOD_5 ($r = 0.459$), SAL and temperature ($r = 0.545$), SHIG and temperature ($r = 0.600$) and SHIG and BOD_5 ($r = 0.492$). pH displayed negative correlations with TC ($r = - 0.558$), VC ($r = - 0.542$), SAL ($r = - 0.538$) and SHIG ($r = - 0.609$).

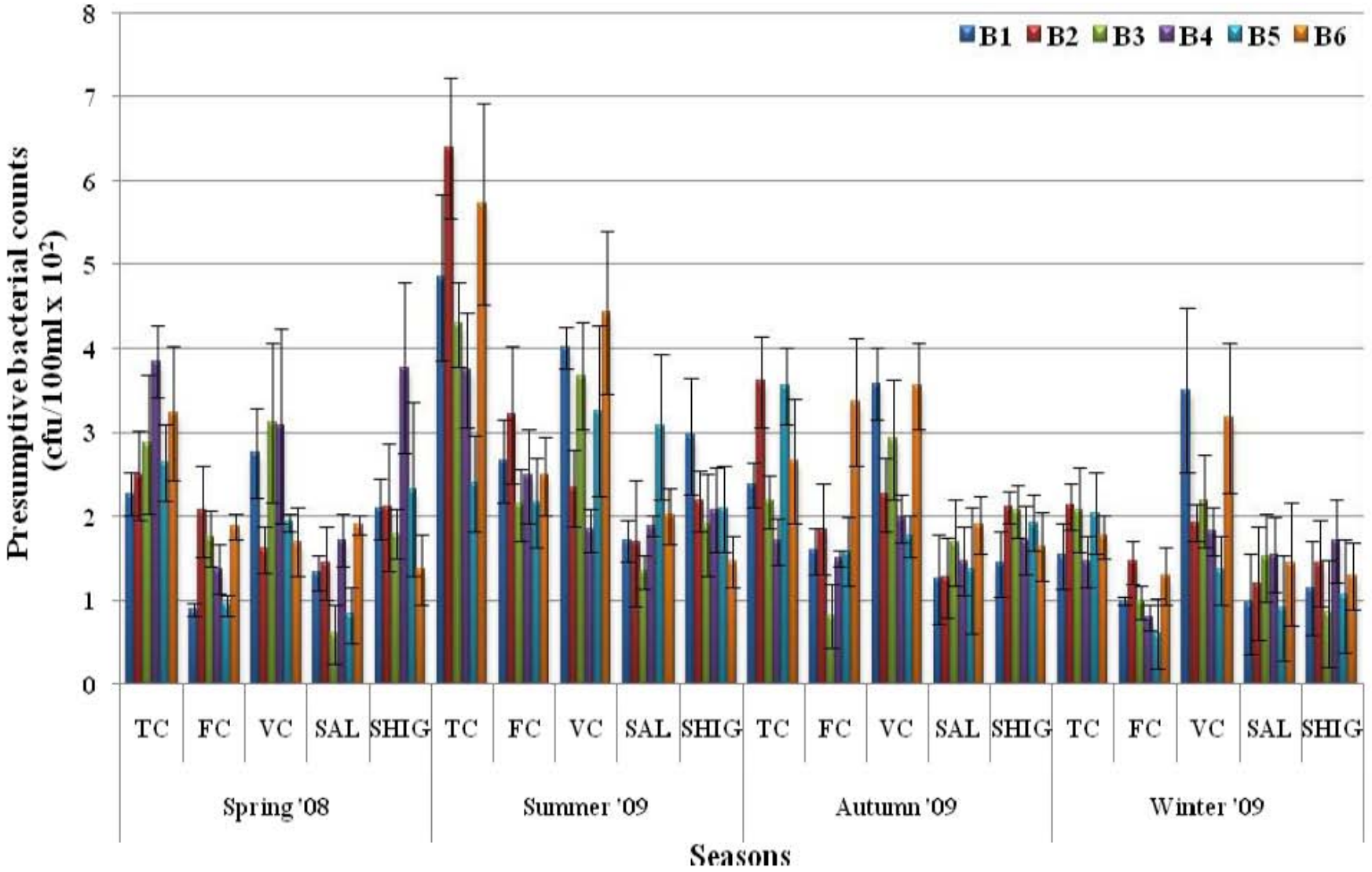


Fig. 2.8: Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for the different beaches over the different seasons. *Bars* indicate the average of replicate samples (n = 4 or 5) while the *error bars* show the standard deviation.

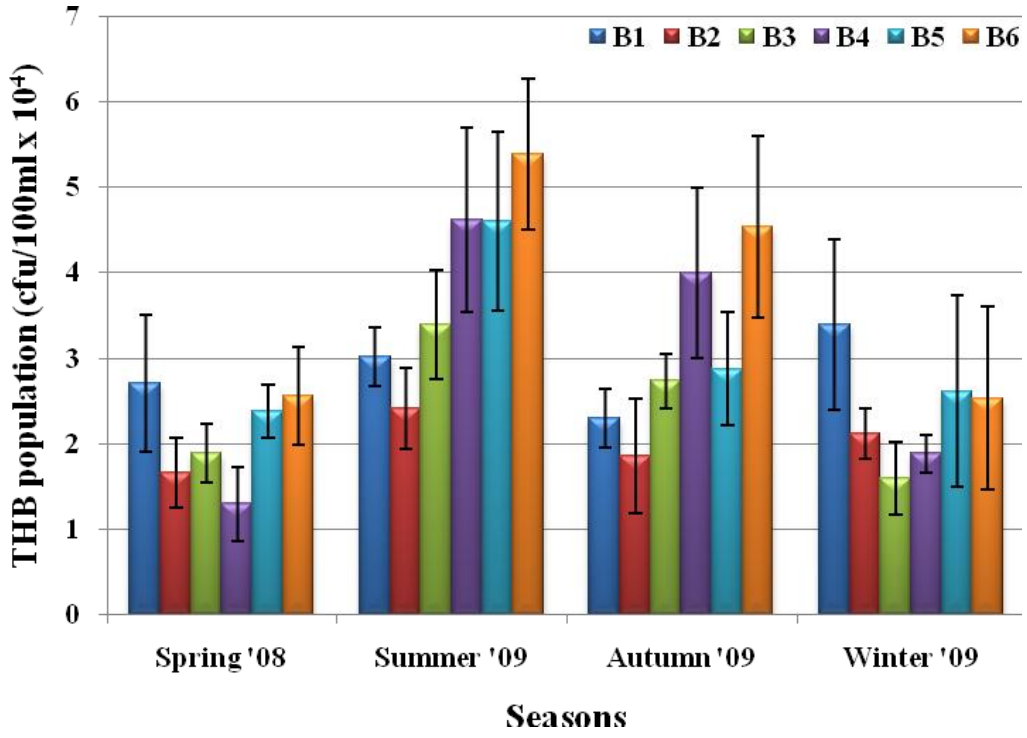


Fig. 2.9: Total Heterotrophic Bacterial (THB) populations for the different beaches over the different seasons. *Bars* indicate the average of replicate samples (*n* = 4 or 5) while the *error bars* show the standard deviation.

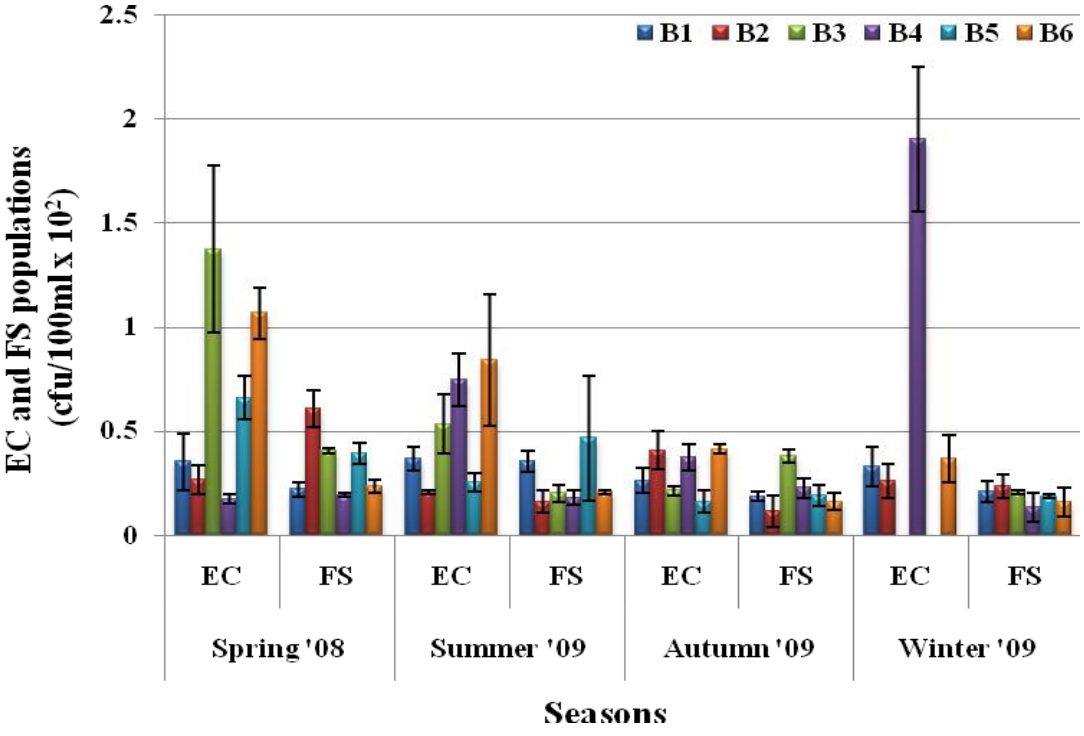


Fig. 2.10: Presumptive enterococci (EC) and faecal streptococci (FS) populations for the different beaches over the different seasons. *Bars* indicate the average of replicate samples (*n* = 3 or 4) while the *error bars* show the standard deviation.

Table 2.14: Correlation matrix of the bacterial indicator organisms for the beaches during the study period.

	THB	TC	FC	EC	FS	VC	SAL	SHIG
THB	1							
TC	- 0.029	1						
FC	0.195	0.700[a]	1					
EC	- 0.047	- 0.139	- 0.064	1				
FS	0.105	- 0.021	0.010	- 0.013	1			
VC	0.132	0.229[a]	0.153	0.024	0.337[a]	1		
SAL	0.385[a]	0.190	0.373[a]	- 0.124	0.274[a]	0.183	1	
SHIG	- 0.060	0.449[a]	0.284[a]	0.025	0.325[a]	0.221[a]	0.422[a]	1

[a] Correlation is significant at the 0.05 level (2-tailed)

THB, total heterotrophic bacterial; TC, total coliforms; FC, faecal coliforms; EC, enterococci; FS, faecal streptococci; VC, *Vibrio cholerae*; SAL, *Salmonella spp.*; SHIG, *Shigella spp.*

Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for the Umdloti River over the four seasons are presented in Figure 2.11, while the Total Heterotrophic Bacterial (THB) population is presented in Figure 2.12 and presumptive enterococci (EC) and faecal streptococci (FS) levels are presented in Figure 2.13, respectively. TC and FC concentrations varied significantly ($p < 0.05$) over the seasonal periods with sampling points C2 and C3 having the highest TC counts during spring and summer and autumn and winter, respectively. TC counts ranged from 1.604 x 10^3 cfu/100ml (C4 – winter) to 4.155 x 10^3 cfu/100ml (C2 – summer) and FC counts ranged from 0.74 x 10^3 cfu/100ml (C3 – spring) to 2.328 x 10^3 cfu/100ml (C1 – summer) (Fig. 2.11). Hazelmere dam (C4) consistently had the lowest TC counts during all four seasons compared to the other points along the Umdloti River. The Umdloti River mouth (C1) had the highest FC counts during spring and summer while sampling points C2 and C3 had the highest FC counts during autumn and winter, respectively. VC, SAL and SHIG populations for the Umdloti River showed significant ($p < 0.05$) variations across the seasons and ranged from 1.28 x 10^3 cfu/100ml (C3 – winter) to 3.165 x 10^3 cfu/100ml (C1 – spring), 0.575 x 10^3 cfu/100ml (C2 – winter) to 3.04 x 10^3 cfu/100ml (C1 – summer) and 0.5 x 10^3 cfu/100ml (C1 – winter and C4 – autumn) to 2.132 x 10^3 cfu/100ml (C2 – summer), in that order (Fig. 2.11). The sampling point C2 (Conco Bridge – Verulam) had the highest VC population from spring till winter and the highest SHIG population during summer, compared to the other points along the river. The highest VC population during the spring season was enumerated in the water sample collected from the mouth (C1) of the Umdloti River. In addition, the highest SAL counts during summer and autumn were enumerated from the same sampling site, i.e. C1.

No significant variations occurred with the THB counts for the Umdloti River, which ranged from 1.535 x 10^6 cfu/100ml (C4 – spring) to 4.585 x 10^6 cfu/100ml (C1 – summer). According to Fig. 2.12, Hazelmere dam (C4) had the lowest microbial load during spring, autumn and winter compared to the other sampling stations and the THB counts for all sampling points along the river, except C3, peaked during summer. EC and FS counts differed significantly ($p < 0.05$) over the four seasons and varied from 0.12 x 10^3 cfu/100ml (C1 – winter) to 0.82 x 10^3 cfu/100ml (C1 – spring) and 0.05 x 10^3 cfu/100ml (C3 – winter) to 1.755 x 10^3 cfu/100ml (C1 – spring), respectively. The highest EC counts during spring, summer and autumn were recorded for sampling site C1, while the highest FS counts during summer and winter were recorded for sampling site C2.

Correlation matrices (significant at the 0.05 level) between the bacterial indicator organisms for the Umdloti River during the study period are shown in Table 2.15 while correlation matrices (significant at the 0.05 level) between the bacterial indicator organisms and selected physico-chemical parameters are shown in Appendix B. Positive correlations were observed between THB and TC ($r = 0.333$), FC ($r = 0.347$), SAL ($r = 0.430$) and SHIG ($r = 0.285$). Weak to fair correlations were observed between TC and FC ($r = 0.286$), TC and SAL ($r = 0.414$), TC and SHIG ($r = 0.405$) and FC and SAL ($r = 0.380$). FS correlated significantly with EC ($r = 0.460$), VC ($r = 0.544$) and SHIG ($r = 0.405$) while SHIG showed weak, significant relationships to VC ($r = 0.251$) and SAL ($r = 0.308$). Temperature exhibited positive, significant correlations to TC ($r = 0.615$) and SHIG ($r = 0.580$) while pH anti-correlated with FS ($r = -0.819$) and EC ($r = -0.615$).

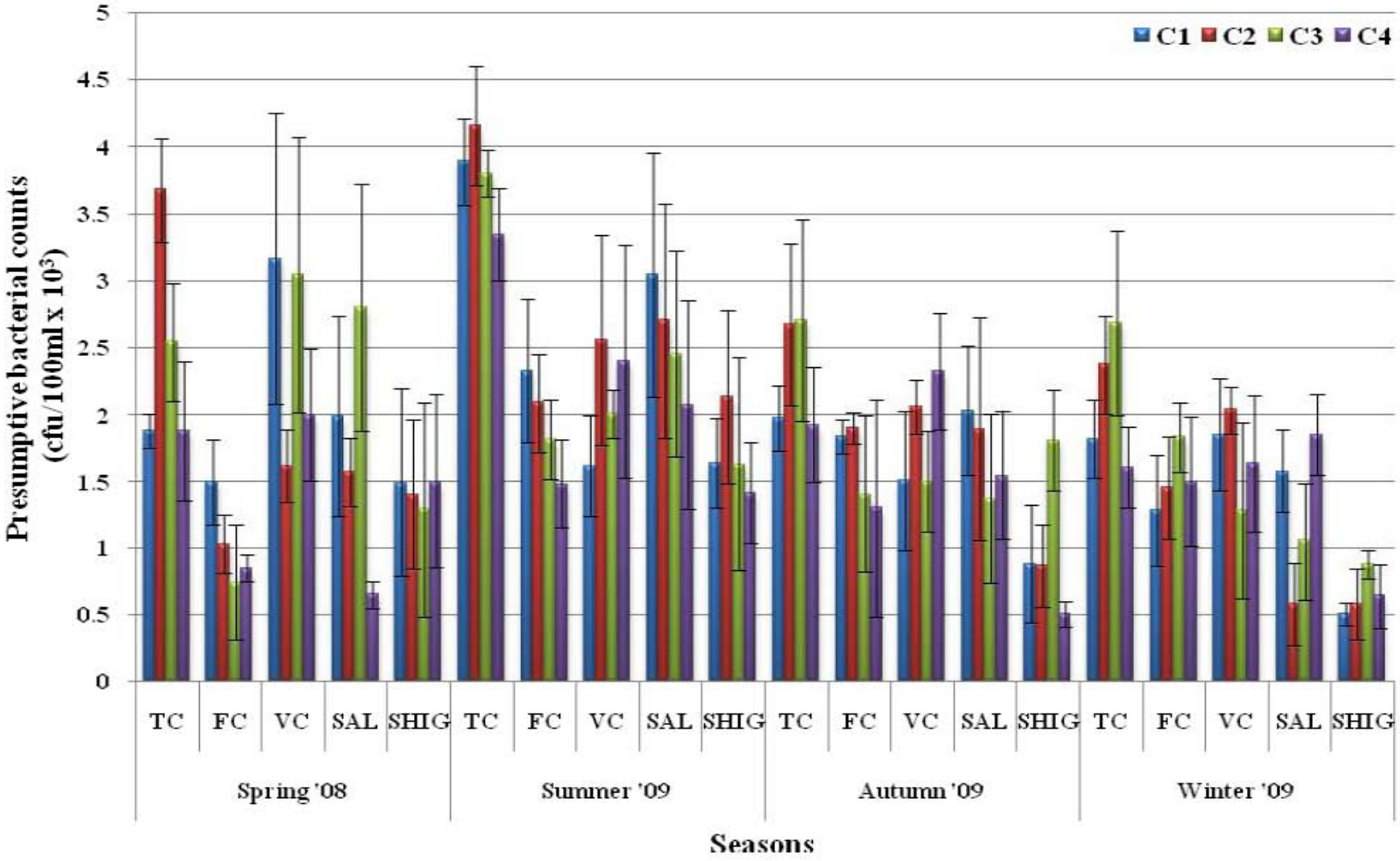


Fig. 2.11: Presumptive total coliform (TC), faecal coliform (FC), *V. cholerae* (VC), *Salmonella spp.* (SAL) and *Shigella spp.* (SHIG) populations for Umdloti River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (n = 4 or 5) while the *error bars* show the standard deviation.

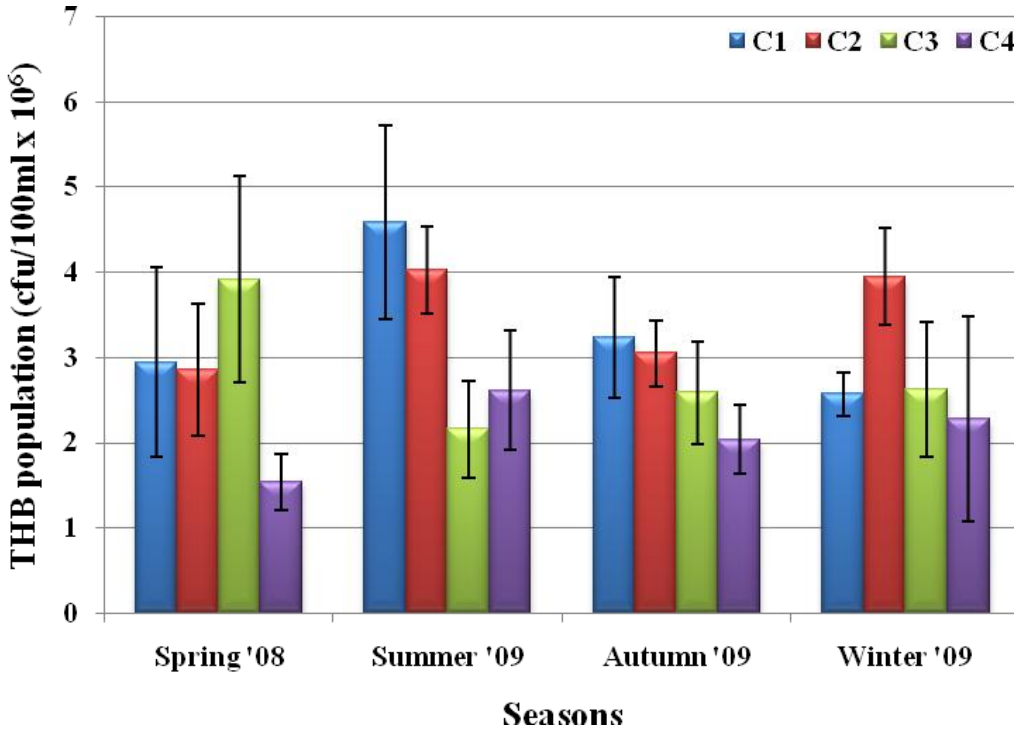


Fig. 2.12: Total Heterotrophic Bacterial (THB) populations for Umdloti River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (*n* = 4 or 5) while the *error bars* show the standard deviation.

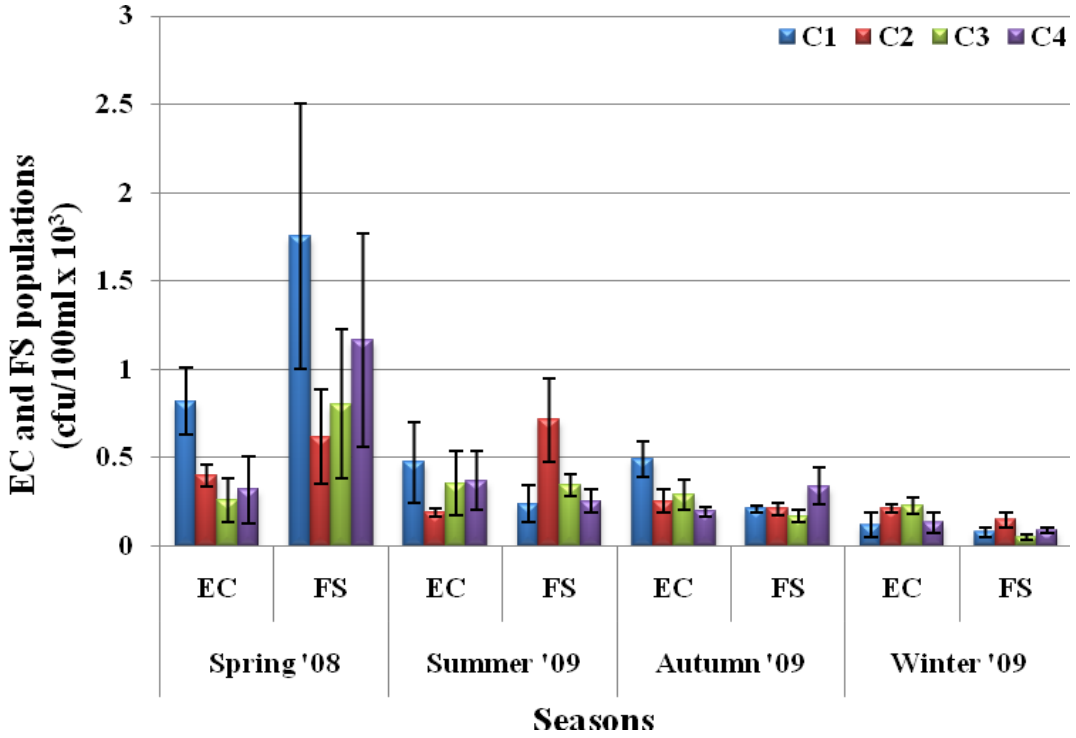


Fig. 2.13: Presumptive enterococci (EC) and faecal streptococci (FS) populations for Umdloti River at the different sampling points over the different seasons. *Bars* indicate the average of replicate samples (*n* = 3 or 4) while the *error bars* show the standard deviation.

Table 2.15: Correlation matrix of the bacterial indicator organisms for the Umdloti River during the study period.

	THB	TC	FC	EC	FS	VC	SAL	SHIG
THB	1							
TC	0.333[a]	1						
FC	0.347[a]	0.286[a]	1					
EC	0.202	0.081	0.154	1				
FS	0.053	- 0.177	- 0.216	0.460[a]	1			
VC	0.195	0.007	- 0.115	0.208	0.544[a]	1		
SAL	0.430[a]	0.414[a]	0.380[a]	0.164	0.034	0.210	1	
SHIG	0.285[a]	0.405[a]	0.152	0.212	0.405[a]	0.251[a]	0.308[a]	1

[a] Correlation is significant at the 0.05 level (2-tailed)

THB, total heterotrophic bacterial; TC, total coliforms; FC, faecal coliforms; EC, enterococci; FS, faecal streptococci; VC, *Vibrio cholerae*; SAL, *Salmonella spp.*; SHIG, *Shigella spp.*

2.4 Discussion

The physical and chemical characteristics of surface water resources under natural conditions are influenced by a number of factors, including topography, geology and climate (Perona *et al.,* 1999). Solute content and composition varies greatly in space depending on the particular sources, processes and pathways that dominate in a given water ecosystem. The extent of variation and the controlling factors also depend on the geographical location and scale of the area under consideration (Perona *et al.,* 1999). Recent climate change has given rise to changes in hydrologic patterns in both fresh- and marine waters, whose irregular water regimes have been additionally influenced by anthropogenic factors due to pollution and other human interference which gives rise to specific problems (Pradhan *et al.,* 2009). In this study, seasonal variability in physico-chemical and microbiological characteristics of two vital rivers and six recreational beaches in Durban, were investigated.

The little variation in water temperature within a season, at the sampling points belonging to a particular resource observed, could be attributed to the fact that these samples were collected within a 3 – 4 hr duration on the same day. However, the seasonal variation in temperature at these sampling sites showed a typical trend, with peak values recorded in summer and the lowest in winter. All river and beach water samples were below 25°C during spring, autumn and winter, which is the recommended limit for no risk according to the South African water quality guidelines for domestic use (DWAF, 1995). However, during summer this guideline was only met by samples from points A4, B2 and B3. Temperature has a marked influence on the chemical and biochemical reactions that occur in water bodies. High temperature for instance, increases the toxicity of many substances such as heavy metals and pesticides. It also increases the sensitivity of living organisms to toxic substances (Momba *et al.,* 2006). The fact that sampling point A4 is located within the KrantzKloof Nature Reserve and surrounded by extensive natural vegetation could have probably contributed to the low water temperature recorded at this point during summer as compared to the other points along the Umgeni River within the same season. Similar findings were also documented by Da Silva and Sacomani (2001). Generally, the pH regimes fell within the South African water quality pH protection limit of 5 – 9 (DWAF, 1996). The Blue Drop report that was released in early 2009, suggested that pH values lower than 7 were probably due to leachates and rain runoff waters from abandoned mines. The generally higher pH of the beach waters during the dry season could be ascribed to increased photosynthetic assimilation of dissolved inorganic carbon by planktons (Iqbal *et al.,* 2004). A similar effect could also be produced by water evaporation through the loss of half bound CO_2 and precipitation of mono-carbonate (Wang *et al.,* 2007). Turbidity profiles of all samples across all seasons, exceeded the South African guideline limit of 0 – 1 NTU for domestic and recreational use (DWAF, 1996), which disqualifies these surface water resources for direct domestic use. Turbidity is caused by the presence of suspended matter, which usually consists of a mixture of inorganic matter, such as clay and soil particles, and organic matter (Momba *et al.,* 2006). The variety of sources,

character and size of suspended solids means that the measurement of turbidity gives only an indication of the extent of pollution. High turbidity therefore indicates the presence of organic suspended material which promotes the growth of microorganisms (Momba *et al.*, 2006). High turbidity values during the summer period could be attributed to increased surface runoff and erosion, through summer rains. Excessive turbidity in surface waters, destined for human consumption can cause potential problems for water purification processes such as flocculation and filtration, which may increase treatment costs (Igbinosa and Okoh, 2009). There may also be a tendency for an increase in trihalomethane (THM) precursors, when highly turbid waters are chlorinated. Anderson *et al.* (2005) demonstrated that particulate matter can play a major role in the survival and distribution of bacteria as they can adsorb to particulates and settle at the bottom when introduced into the water column. DiDonato *et al.* (2009) further reiterated that faecal organisms can accumulate in sediments and become concentrated relative to the water and re-contaminate the water column through bio-turbation and storm water flows. BOD_5 is used to indicate the extent of organic pollution in aquatic systems, which adversely affects water quality. All Umgeni River samples fell within the universal water quality index of 3 mg/L BOD (Boyacioglu, 2007) over the different seasons, except for point A4 which exceeded this standard during spring and autumn. The beaches failed to meet this guideline limit during spring and summer with all points along the Umdloti River complying with the guideline for BOD, except for point C1 which exceeded the universal water quality index over the spring and summer months. Decreased BOD_5 values during autumn and winter correlated well with the findings of Ouyang *et al.* (2006), who found a good correlation between temperature and BOD_5 during winter. A decrease in water temperature during autumn and winter increases the solubility of oxygen in water. Dissolved oxygen partially oxidizes organic matter, therefore, the increase in oxygen concentration in the water results in a decrease in organic matter concentration and hence oxygen demand. Although no COD limits for aquatic systems are stipulated in the South African water quality guidelines, high COD values obtained in this study are alarming and suggests that both organic and inorganic substances as well as organic contaminants from municipal sewage treatment plants and other industries are entering these water systems. This is undesirable as continuous discharge of effluent can negatively impact the quality of these receiving water bodies to some extent and subsequently can cause harm to aquatic life. Several factors including temperature, ionic mobility and ionic valencies influence water conductivity (Iqbal *et al.*, 2004). In turn, conductivity qualitatively reflects the status of inorganic pollution and is a measure of total dissolved solids and ionized species in the water. The electrical conductivities of the water samples varied for Umgeni River during the study period, ranging from 18.6 mS/m (A4 – autumn) to 5180 mS/m (A1 – summer). Conductance levels across the beaches were fairly similar with little to no variation across seasons. The conductivity of the water sample collected at the mouth of the Umdloti River (C1) decreased during summer while conductivities of the water samples from points C2, C3 and C4 remained relatively unchanged. In addition, the high

concentrations of electrical conductance values recorded in the water samples collected from the river mouths (A1 and C1) depend on the high or low tide of the ocean i.e. the impact of seawater.

The chemical composition within a water system is based on an interaction between various factors, including the weathering of rocks, intensity and composition of the rainfall in that area, chemical reactions that occur between the water and soil/sediment and pollution from varying sources (Da Silva and Sacomani, 2001). Rivers in watersheds with substantial agricultural and urban land use experience increased inputs and varying compositions of organic matter (Sickman *et al.*, 2007) and excessive concentrations of nutrients from fertilizer application and watershed releases (Easton *et al.*, 2007) which eventually discharges into oceans. Photosynthesis and respiration play an important role in the self-purification of natural waters. However, disturbance to the balance between these delicate processes leads to chemical and biological changes that reflect pollution. High levels of these species increases the growth of vegetation in water systems which leads to increases in oxygen demand (Jonnalagadda and Mhere, 2001). Elevated phosphate levels in the river water samples, during autumn and winter, could be attributed to the decreased flux in the river systems during these dry seasons whereas the low concentrations detected in spring and summer could be a consequence of the dilution effect. This trend of fluctuating phosphate levels during the wet and dry seasons was also observed in four major rivers investigated in Nigeria (Ololade and Ajayi, 2009). Other investigators have pointed out that eutrophication-related problems in temperate zones of aquatic systems begin to increase at ambient total P concentrations exceeding 0.035 mg/L. Nitrate, the most highly oxidized form of nitrogen compounds is commonly present in surface water because it is the end product of aerobic decomposition of organic nitrogenous matter. However, unpolluted natural waters usually contain only minute amounts of nitrate (Jaji *et al.*, 2007). Fortunately, all the samples tested in this study fell below the recommended WHO nitrate limit of 50 mg/L (WHO, 1996) for all seasons. Additionally, the phosphate levels obtained in this report are exceedingly too high for aquatic life, irrigation purposes, and livestock watering and recreational activities with a guideline value < 0.05 mg/L (FEPA, 1991). The Umgeni River water samples exceeded the nitrates guideline value of < 0.5 mg/L making this river unsuitable for aquatic life and irrigation purposes. However, all river and beach water samples are utilizable for livestock watering and recreational activities as they complied with the < 10 mg/L guideline for nitrates across all seasons (FEPA, 1991; USEPA, 2004). Ammonia, nitrates and phosphates are essential nutrients to plant life, but when found in excessive quantities; they can stimulate excessive and undesirable plant growth such as algal blooms. Eutrophication could adversely affect the use of rivers and dams for recreational purposes as the covering of large areas by blue-green algae and/or macrophytes that can release toxic substances (cyanotoxins) could prevent access to waterways (Igbinosa and Okoh, 2009). This problem was clearly seen during the collection of water from sampling point A2 (Umgeni River) during autumn where a high density of green algae plagued the water (Fig. 2.2). This observation corresponded with the nutrient levels

detected at this sampling point. Sulphate concentrations in rivers tend to reach extremes of 270 to 1300 mg/L with normal amplitudes of 2.2 to 48 mg/L, the most common natural concentration being 4.8 mg/L while the standard value according to the WHO for drinking water is 250 mg/L. Sulphate concentrations at sampling points A3, C1, C2 and C3 increased during autumn and winter. The observed trend could be attributed to the evaporation of water from rivers during the dry seasons and subsequent dilution due to precipitation and run-off from the catchment areas during the wet season (Radhika and Gangaderr, 2004).

Heavy metals are among the most common environmental pollutants, and their occurrence in waters indicates the presence of natural or anthropogenic sources. The main natural sources of metals in waters are chemical weathering of minerals and soil leaching while the anthropogenic sources are mainly associated with industrial and domestic effluents, urban storm, water runoff, landfill leachate, mining of coal and ore and atmospheric sources (Papafilippaki *et al.,* 2008). All heavy metals in surface waters exist in colloidal, particulate, and dissolved phases. Rivers are a dominant pathway for metal transport with many reports on temporal changes, especially seasonal variations, in heavy metal concentrations in river waters (Iwashita and Shimamura, 2003). The solubility of trace metals in surface water is predominantly controlled by water pH, water temperature, the river flow and the redox environment of the river system. A lower pH increases the competition between metal and hydrogen ions for binding sites whereas a decrease in pH may also dissolve metal-carbonate complexes, releasing free metal ions into the water column (Papafilippaki *et al.*, 2008). This effect was noticeable at sampling points A2 – A5 and C1 – C3 where a decrease in water pH from summer to autumn resulted in increased concentrations of the different heavy metals. During autumn, cadmium concentrations increased at sites A4, C1 and C2; mercury concentrations increased at sites A3, A5 and C2 and aluminium concentrations increased at sites A2, A3, A5, C1 – C3. Higher metal concentrations during the dry seasons compared to the wet seasons could also be due to water evaporation during the dry season and dilution due to precipitation and run-off during the rainy season. These observations have been reported in other studies (Singh *et al.,* 2008). The presence of cadmium in the aquatic environment is of concern because it bioaccumulates. At elevated concentrations cadmium is acutely toxic and can cause severe renal damage with renal failure (Paasivirta, 2007). The major adverse effects of aluminium in water used for domestic purposes are aesthetic, although chronic human health effects at high concentrations can occur. Aluminium is also used in water treatment processes, which may result in increased concentrations of aluminium in the final water (DWAF, 1996). At neutral and alkaline pH, the concentration of copper in surface waters is usually low, typically, 0.003 mg/L, whereas in acidic waters, copper readily dissolves, and substantially higher concentrations may occur (DWAF, 1996). Although mercury is unlikely to occur naturally in surface waters at concentrations which are of concern to human health, it may occur at high concentrations in water bodies subject to industrial pollution. Mercury serves no known beneficial physiological function in humans, and is a

chronic neurotoxin. Poisoning takes the form of neurological (organic mercury) and renal (inorganic mercury) disturbances (Guzzi and La Porta, 2008; Paasivirta, 2007).

Total coliforms (TC) are frequently used to assess the general hygienic quality of water. In some instances they may indicate the presence of pathogens responsible for the transmission of infectious diseases such as gastroenteritis, salmonellosis, dysentery, cholera and typhoid fever. The TC group includes bacteria of faecal origin and indicates the possible presence of bacterial pathogens such as *Salmonella spp*., *Shigella spp*., *Vibrio cholerae, Campylobacter jejuni, Yersinia enterocolitica* and pathogenic *E. coli*, especially when detected in conjunction with other FC (Ashbolt *et al.,* 2001). Several limitations associated with the application of coliforms as an indicator of water quality in aquatic environments include: short survival in water body, non-faecal source, ability to multiply after release into water column, great weakness to the disinfection process, inability to identify the source of faecal contamination (point and non-point), low levels of correlation with the presence of pathogens and low sensitivity of detection methods have been reported (Savichtcheva and Okabe, 2006). With this in mind, the use of TC and FC as indicators of the microbiological quality of the water samples collected in this study have proved to be useful. According to DWAF, the maximum limit for no risk (domestic and recreational use) for TC and FC is 10 cfu/100ml and 0 cfu/100ml, respectively (DWAF, 1996). All points along both rivers and the six beaches grossly violated both microbiological water quality guidelines with the highest TC indicator concentrations being recorded during the summer months and lowest during winter. During late spring to early autumn and winter, many of the smaller tributaries can experience low and even stagnant waters, a factor that could affect microbial ecology in the water courses (Romaní *et al.,* 2006). In contrast, material in the water courses such as sediment and vegetative matter that may harbour bacteria can be re-mobilized during wet seasons even in low water velocity systems (Droppo *et al.,* 2006) resulting in the higher levels of these indicators during summer. In addition, the FC levels for both rivers across all seasons did not meet the South African guidelines of 0 – 130 cfu/100ml set for full-contact recreation and 0 – 200 cfu/100ml set for livestock watering. This could pose a health risk, of contracting gastrointestinal illnesses, to both humans and animals that use these water bodies. The Virginia (B1) and Sunkist (B5) beaches complied with the South African Target Quality range of 0 – 130 cfu/100ml for FC (full-contact recreational waters) (DWAF, 1996) during spring and winter whereas only the Umgeni South (B3) beach fell within this range during autumn. This indicates that the risk of gastrointestinal effects is possible in the swimmer and bather population. All beaches complied with the South African Target Quality range of 0 – 1000 cfu/100ml for FC (intermediate contact recreational waters) (DWAF, 1996) over the four seasons. According to the USEPA criteria for recreational water use for FC (< 200 cfu/100ml) (USEPA, 2004), all beaches fell below this guideline during winter, 83.3% of the beaches complied during spring and autumn whereas all beaches failed to comply during the summer season. Environmental bodies of water used for recreation are usually not treated. The quality of these waters

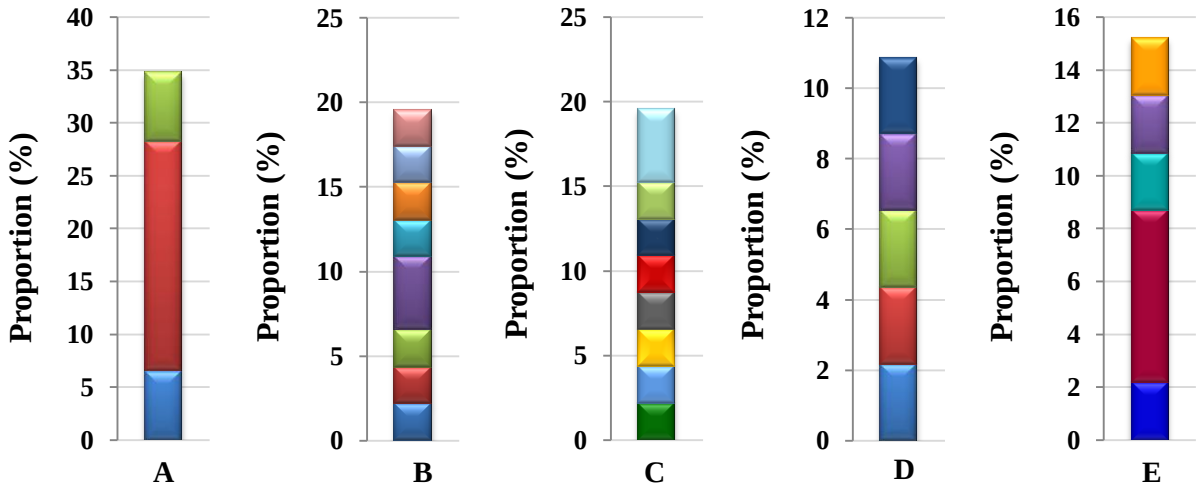
primarily depends on pollution control. The objectives of monitoring the quality of the water are primarily to caution users about risks of infection, to locate sources of pollution, and to take steps for control of the pollution. Overall data emphasizes that the concentration of indicator organisms in recreational marine waters is highly variable in time and space. Shibata *et al.* (2004) demonstrated that the shoreline of beaches and subsequently the wash zone was associated with the highest concentrations of indicator organisms due to the various direct and indirect contributions. Other studies have shown how indicator organisms are capable of multiplying in beach sand, in particular within soils subjected to tidal action (Byappanahalli *et al.,* 2003). In addition, moisture was found to be a key factor in controlling the regrowth of the indicator microbes as the sand within the wash zone between low and high tide is characterized by a gradient of moisture content, with the highest moisture content near the ocean side and the lowest moisture near the beach side. It is very possible for optimum moisture conditions to occur within this zone thus allowing proliferation of these indicators (Shibata *et al.*, 2004).

Total Heterotrophic Bacterial counts indicate the general microbial quality of water and are used to detect a wide range of bacteria which are omnipresent in nature. Pollution of water such as high nutrient concentrations and high turbidity, promotes bacterial growth thus resulting in a substantial increase of these naturally-occurring organisms. According to the South African Bureau of Standards (SABS), the stipulated recommended limit for THB population is 100 cfu/ml with all samples failing to adhere to this standard (SABS, 2001). According to the consolidated rainfall data retrieved from the South African Weather Services for the summer period (December 2008 – February 2009), KwaZulu-Natal received very high rainfall levels (300 – 500 mm) in comparison to the other provinces around the country. This rainy period could have contributed to increased runoff from agricultural lands, undeveloped human settlements etc. carrying with it high microbial loads and excess nutrients into these surface waters. Overall, Umgeni River was more contaminated than the Umdloti River, as this river flows through the more urbanized areas of Durban and is subject to higher surface runoff than the Umdloti River (Olaniran *et al.,* 2009). It is interesting to note that both the Inanda dam (A5) and Hazelmere dam (C4) had the lowest THB levels over the four seasons compared to the other sampling stations along those rivers. This was probably due to the surrounding thicket and bushland that enclosed these dams, which serve as vegetated pervious buffers that act as passive treatment systems, allowing agricultural and storm water runoff to reach the dams in a less impaired state. In addition, observed increases in THB concentrations from the dams to the river mouths suggest that land use changes associated with coastal development, particularly in watershed impervious cover, affect water quality. These results are consistent with previous studies that documented elevated indicator organism levels along gradients of coastal urbanization (Mallin *et al.,* 2000). Beach water samples from points B4 – B6 were the most contaminated as evidenced by the high THB levels during summer and autumn, compared to the other beaches, which is of concern as these beaches serve as major tourist attractions along the infamous golden

mile of Durban. It can be speculated that informal settlements and trading activities in the storm water drain catchment areas located between points B3 and B6 could be important sources of microbial contamination resulting in these beaches having a higher distribution pattern of THB counts compared to those beaches lying further north.

The South African water quality guidelines only consider *E. coli* as an indicator of pathogenic pollution in marine waters, even though the ability of the EC group to survive longer in the environment than FC makes them more reliable indicators of contamination and potential health risk (DWAF, 1996). According to the USEPA criteria for EC counts (<33 cfu/100ml for freshwater; <35 cfu/100ml for recreational water use) (USEPA, 2004), all points along the rivers exceeded the first guideline except for position A3 where no EC was detected during spring and 33% of the beach samples satisfied the second guideline during spring and summer, while 50% and 67% of the beach samples complied during autumn and winter, respectively. DWAF specifies two guidelines for FS levels: (1) 0 – 30 cfu/100ml for full contact recreation and (2) 0 – 230 cfu/100ml for intermediate contact recreation. All beaches satisfied the second guideline with only beaches B4 and B6 complying with the first guideline, throughout the four seasons. According to Mardon and Stretch (2004), discharges from the Umgeni River and six storm water drains, located between beaches B3 and B6, are the main sources of pollution to the beaches especially near shore pollution. Battery beach (B4) has a particularly serious water quality problem and specific causes for this problem have been identified by the local municipality, including leakages from sewage systems into the storm water systems which could have a knockdown effect on adjacent beaches (Mardon and Stretch, 2004; Schuster-Wallace *et al.,* 2008). High EC counts, especially during summer, should be a concern as designated bathing beaches that do not meet local and international guidelines are unsafe for users as they are used intensively during the summer holiday period.

In summary, this study demonstrated the spatial and seasonal fluctuations of the physical and chemical environmental variables among the investigated water samples indicating the dynamic nature of surface water resources and consequently the challenges in monitoring and, therefore, managing these water bodies. In addition, the study reinforced the contention that no one bacterial indicator organism or simple physical/chemical characteristic can be used to monitor water quality or predict contamination. The study also highlights the poor microbiological quality of rivers and beaches in Durban, South Africa which raises concerns regarding public health. Therefore, when selecting water quality parameters for implementing environmental monitoring plans in river basins and beaches, the seasonal variations of parameters must be considered.



A: Six- and seven-drug patterns

- RP/T/PG/E/TM/FC/CD
- RP/AP/PG/E/TM/FC/CD
- RP/PG/E/TM/FC/CD

B: Eight-drug patterns

- TS/RP/T/PG/E/TM/FC/CD
- RP/S/AP/PG/E/TM/FC/CD
- C/RP/AP/PG/E/TM/FC/CD
- RP/AK/AP/PG/E/TM/FC/CD
- TS/RP/S/PG/E/TM/FC/CD
- RP/AP/PG/AUG/E/TM/FC/CD
- RP/T/AP/PG/E/TM/FC/CD
- RP/CXM/AP/PG/E/TM/FC/CD

C: Nine-drug patterns

- RP/AK/S/AP/PG/E/TM/FC/CD
- C/TS/RP/T/PG/E/TM/FC/CD
- RP/S/T/AP/PG/E/TM/FC/CD
- RP/AK/T/AP/PG/E/TM/FC/CD
- TS/RP/T/AP/PG/E/TM/FC/CD
- C/RP/AK/AP/PG/E/TM/FC/CD
- CIP/RP/NA/S/PG/E/TM/FC/CD
- RP/T/AP/PG/AUG/E/TM/FC/CD

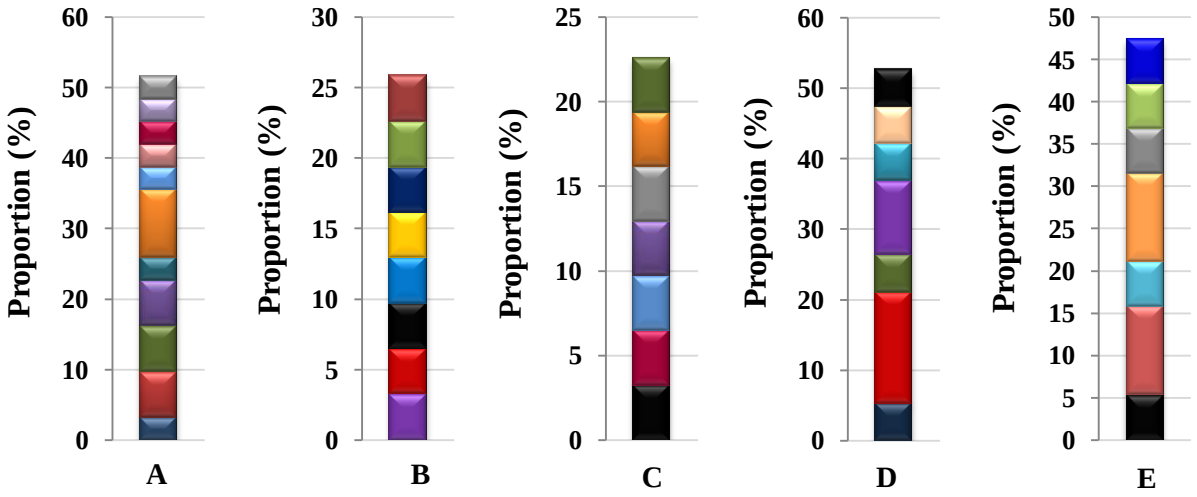
D: Ten-drug patterns

- TS/RP/S/AP/PG/AUG/E/TM/FC/CD
- TS/RP/AK/S/AP/PG/E/TM/FC/CD
- TS/RP/CXM/T/AP/PG/E/TM/FC/CD
- C/TS/RP/NA/T/PG/E/TM/FC/CD
- C/TS/RP/T/PG/AUG/E/TM/FC/CD

E: Eleven- and twelve-drug patterns

- TS/RP/CXM/AK/S/T/AP/PG/E/TM/FC/CD
- TS/RP/CXM/AK/T/AP/PG/E/TM/FC/CD
- C/TS/RP/S/T/PG/AUG/E/TM/FC/CD
- RP/CXM/FOX/CTX/CRO/PG/AUG/E/TM/FC/CD
- TS/RP/AK/S/T/AP/PG/E/TM/FC/CD

Fig. 3.1: Resistance spectra of *E. coli* isolates, from the Umgeni River, against the 21 antibiotics tested. CIP, ciprofloxacin; C, chloramphenicol; TS, cotrimoxazole; RP, rifampicin; NA, nalidixic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CRO, ceftriaxone; AK, amikacin; S, streptomycin; T, tetracycline; AP, ampicillin; PG, penicillin G; AUG, augmentin; E, erythromycin; TM, trimethoprin; FC, fusidic acid; CD, clindamycin. Multiple antibiotic resistance (MAR) indices were calculated for the above drug patterns: six drugs (0.29), seven drugs (0.33), eight drugs (0.38), nine drugs (0.43), ten drugs (0.48), eleven drugs (0.52) and twelve drugs (0.57).



A: Seven- and eight-drug patterns

- TS/RP/PG/AUG/E/TM/FC/CD
- RP/TN/T/PG/E/TM/FC/CD
- RP/AP/PG/AUG/E/TM/FC/CD
- RP/S/PG/AUG/E/TM/FC/CD
- RP/CXM/S/PG/E/TM/FC/CD
- RP/T/AP/PG/E/TM/FC/CD
- TS/RP/AP/PG/E/TM/FC/CD
- RP/S/T/PG/E/TM/FC/CD
- RP/AK/PG/E/TM/FC/CD
- RP/AP/PG/E/TM/FC/CD
- RP/T/PG/E/TM/FC/CD

B: Nine-drug patterns

- RP/GM/T/AP/PG/E/TM/FC/CD
- RP/NA/S/T/PG/E/TM/FC/CD
- RP/S/T/PG/AUG/E/TM/FC/CD
- RP/S/AP/PG/AUG/E/TM/FC/CD
- TS/RP/CXM/AK/PG/E/TM/FC/CD
- TS/RP/T/PG/AUG/E/TM/FC/CD
- RP/AK/S/AP/PG/AUG/E/TM/FC/CD
- RP/CXM/S/AP/PG/AUG/E/TM/FC/CD

C: Ten- and eleven-drug patterns

- C/RP/NA/T/AP/PG/AUG/E/TM/FC/CD
- TS/RP/S/T/AP/PG/AUG/E/TM/FC/CD
- RP/NA/AK/S/AP/PG/AUG/E/TM/FC/CD
- RP/NA/AK/T/AP/PG/E/TM/FC/CD
- TS/RP/NA/AK/AP/PG/E/TM/FC/CD
- TS/RP/AK/S/AP/PG/E/TM/FC/CD
- TS/RP/AK/AP/PG/AUG/E/TM/FC/CD

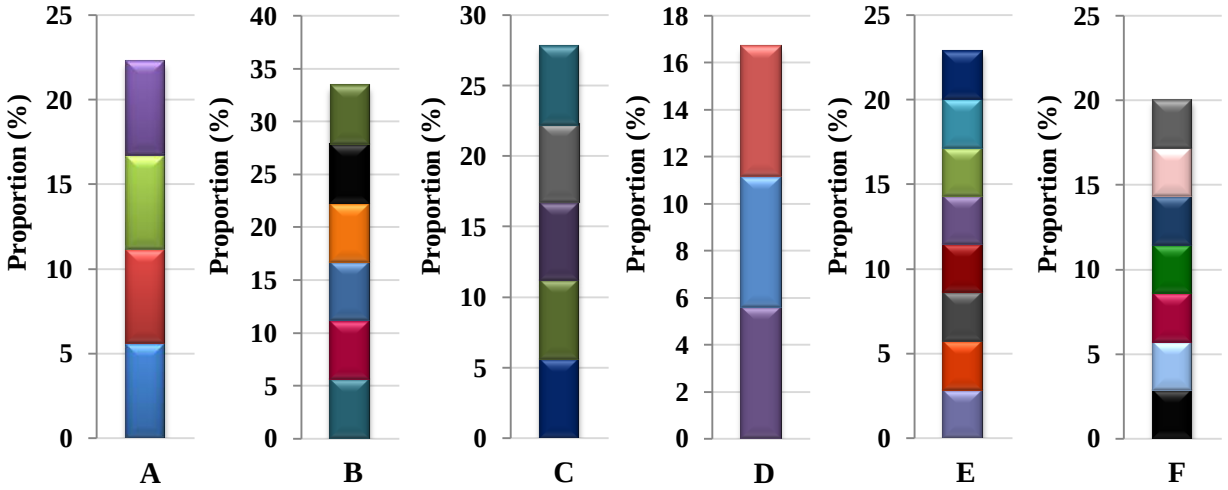
D: Seven- and eight-drug patterns

- TS/RP/AP/PG/E/TM/FC/CD
- RP/AK/T/PG/E/TM/FC/CD
- RP/AK/AP/PG/E/TM/FC/CD
- RP/PG/AUG/E/TM/FC/CD
- RP/CXM/ PG/E/TM/FC/CD
- RP/AK/PG/E/TM/FC/CD
- RP/T/PG/E/TM/FC/CD

E: Nine- and eleven-drug patterns

- RP/AK/S/T/AP/PG/AUG/E/TM/FC/CD
- RP/CXM/S/PG/AUG/E/TM/FC/CD
- RP/T/AP/PG/AUG/E/TM/FC/CD
- TS/RP/AP/PG/AUG/E/TM/FC/CD
- RP/NA/AK/S/ PG/E/TM/FC/CD
- TS/RP/AK/PG/AUG/E/TM/FC/CD
- RP/CXM/AK/AP/PG/E/TM/FC/CD

Fig. 3.2: Resistance spectra of *E. coli* isolates, from the beaches (A – C) and the Umdloti River (D – E), against the 21 antibiotics tested. C, chloramphenicol; TS, cotrimoxazole; RP, rifampicin; NA, nalidixic acid; CXM, cefuroxime; GM, gentamicin; TN, tobramycin; AK, amikacin; S, streptomycin; T, tetracycline; AP, ampicillin; PG, penicillin G; AUG, augmentin; E, erythromycin; TM, trimethoprin; FC, fusidic acid; CD, clindamycin. Multiple antibiotic resistance (MAR) indices were calculated for the above drug patterns: seven drugs (0.33), eight drugs (0.38), nine drugs (0.43), ten drugs (0.48) and eleven drugs (0.52).



A: Six-, seven- and eight-drug patterns

- C/TS/RP/CXM/T/PG/AUG/E
- C/TS/CTX/S/PG/AUG/E
- TS/RP/S/AP/PG/AUG/TM
- TS/NA/S/PG/AUG/TM

B: Nine- and ten-drug patterns

- C/TS/RP/S/T/AP/PG/AUG/E/TM
- TS/RP/NA/AK/S/T/AP/PG/AUG/TM
- C/TS/FOX/GM/AK/PG/E/MUP/TM
- C/TS/RP/S/T/AP/PG/E/MUP
- RP/CXM/FOX/CTX/S/T/PG/MUP/TM
- C/TS/RP/CXM/FOX/S/PG/E/TM

C: Eleven- and twelve-drug patterns

- C/TS/RP/CXM/FOX/GM/S/T/AP/PG/E/MUP
- C/TS/TN/AK/S/T/AP/PG/AUG/E/TM
- C/TS/RP/FOX/TN/T/PG/AUG/E/MUP/TM
- C/TS/RP/CXM/FOX/AK/AP/PG/AUG/E/TM
- C/TS/RP/FOX/TN/S/PG/AUG/E/MUP/TM

D: Thirteen- and fourteen-drug patterns

- C/TS/CXM/FOX/TN/AK/S/T/AP/PG/AUG/E/MUP/TM
- C/TS/RP/CXM/FOX/TN/AK/S/T/AP/PG/AUG/E/TM
- TS/RP/NA/CXM/TN/AK/S/T/PG/AUG/E/MUP/TM

E: Five-, seven- and eight-drug patterns

- TS/RP/CXM/S/AP/PG/E/TM
- C/TS/FOX/S/PG/AUG/MUP/TM
- C/S/T/AP/PG/AUG/E/TM
- TS/FOX/CTX/S/AP/PG/MUP/TM
- C/TS/RP/CXM/AK/PG/MUP/TM
- C/TS/RP/S/PG/E/TM
- C/TS/S/AP/PG
- NA/AK/PG/E/TM

F: Nine-drug patterns

- C/NA/FOX/AK/S/PG/AUG/MUP/TM
- TS/RP/CTX/GM/AK/S/AP/PG/TM
- C/RP/GM/TN/AK/PG/AUG/E/TM
- C/TS/CRO/TN/AK/PG/AUG/MUP/TM
- C/TS/CXM/AK/S/PG/E/MUP/TM
- C/TS/FOX/TN/S/AP/PG/E/TM
- C/TS/RP/FOX/GM/TN/S/PG/TM

Fig. 3.3: Resistance spectra of *V. cholerae* isolates, from the Umgeni River (A – D) and the beaches (E – F), against the 20 antibiotics tested. C, chloramphenicol; TS, cotrimoxazole; RP, rifampicin; NA, nalidixic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CRO, ceftriaxone; GM, gentamicin; TN, tobramycin; AK, amikacin; S, streptomycin; T, tetracycline; AP, ampicillin; PG, penicillin G; AUG, augmentin; E, erythromycin; MUP, mupirocin; TM, trimethoprim. Multiple antibiotic resistance (MAR) indices were calculated for the above drug patterns: five drugs (0.25), six drugs (0.30), seven drugs (0.35), eight drugs (0.40), nine drugs (0.45), ten drugs (0.50), eleven drugs (0.55), twelve drugs (0.60), thirteen drugs (0.65) and fourteen drugs (0.70).

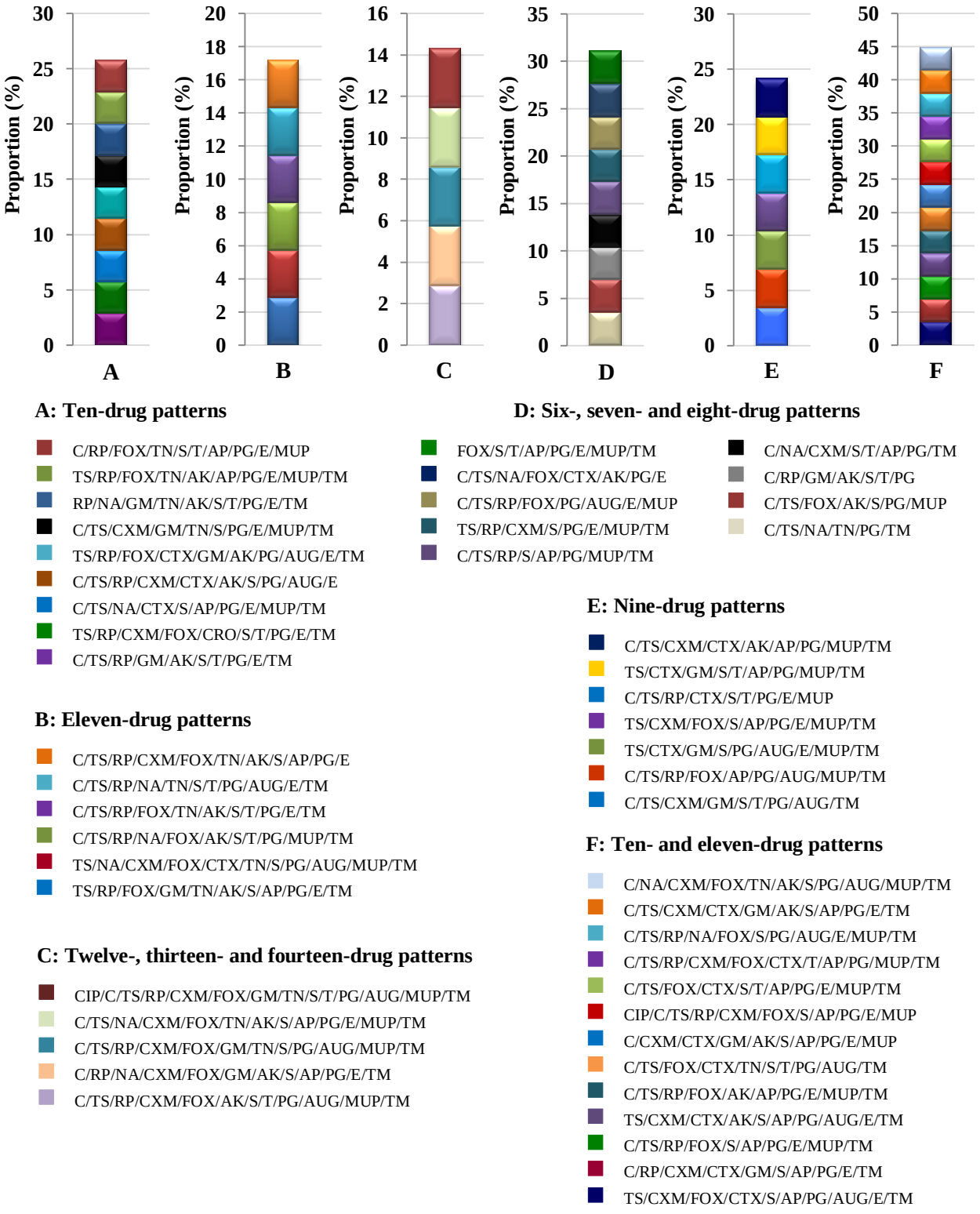


Fig. 3.4: Resistance spectra of *V. cholerae* isolates, from the beaches (A – C) and the Umdloti River (D – E), against the 20 antibiotics tested. C, chloramphenicol; TS, cotrimoxazole; RP, rifampicin; NA, nalidixic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; GM, gentamicin; TN, tobramycin; AK, amikacin; S, streptomycin; T, tetracycline; AP, ampicillin; PG, penicillin G; AUG, augmentin; E, erythromycin; MUP, mupirocin; TM, trimethoprim. Multiple antibiotic resistance (MAR) indices were calculated for the above drug patterns: six drugs (0.30), seven drugs (0.35), eight drugs (0.40), nine drugs (0.45), ten drugs (0.50), eleven drugs (0.55), twelve drugs (0.60), thirteen drugs (0.65) and fourteen drugs (0.70).

3.1 Introduction

Antibiotic resistance is considered one of the most critical human health challenges of the century and heralded the need for "a global strategy to contain resistance" (Pruden *et al.,* 2006). The uses and abuses of antibiotics include the treatment of infectious disease in humans and animals, preventative applications in farming operations, and growth promotion of livestock (Chee-Sanford *et al.,* 2009). This widespread use of antibiotics has resulted in the extensive contamination of surface water resources (McEwen and Fedorka-Cray 2002). Majority of these antibiotics are released into the environment in an unaltered state and this raises many concerns over the potential impact these antibiotic residues may have on receiving water environments (Zhang *et al.,* 2009). Furthermore, antibiotics can have a direct biological action on microorganisms and can hinder crucial processes (denitrification, nitrogen fixation and organic breakdown etc.) that largely control aquatic systems (Martínez, 2008). Antibiotics may even act as persistent pollutants as they are continuously being released into the environment although some have high degradation rates (Costanzo *et al.,* 2005). The indiscriminate use of these compounds, several of which are non-biodegradable increases antibiotic selective pressure in water, facilitates the transfer of antibiotic-resistance determinants between aquatic bacteria, fish and human pathogens, and allows the presence of residual antibiotics in commercialized fish and shellfish products (Baquero *et al.,* 2008). Apart from their polluting effects, the immoderate use of antibiotics has accelerated the development of bacterial antibiotic resistance and proliferation of resistant pathogens (Kemper, 2008). Due to the capacious nature of this problem, it has also been suggested that antibiotic resistance genes themselves could be considered as environmental pollutants, as their geographical distribution has been indicated in various studies worldwide (Pei *et al.,* 2006). These include the frequent detection of many types of antibiotic resistance genes in aquatic environments across Europe (Caplin *et al.,* 2008; Heuer *et al.,* 2002; Messi *et al.,* 2006; Moura *et al.,* 2007; Nikolakopoulou *et al.,* 2005; Schmidt *et al.,* 2001a, b; Tennstedt *et al.,* 2005), North America (Auerbach *et al.,* 2007; Macauley *et al.,* 2007; Poppe *et al.,* 2006), Asia (Dang *et al.,* 2007; Kim *et al.,* 2004; Mohapatra *et al.,* 2008; Rahman *et al.,* 2008; Suzuki *et al.,* 2008), Australia (Akinbowale *et al.,* 2007) and Africa (Jacobs and Chenia, 2007; Taviani *et al.,* 2008).

Antibiotic resistant bacterial strains are an increasing threat to human and animal health, with resistance mechanisms having been identified and elucidated for all known antimicrobials currently available for clinical use (Chen, 2004). Several pathogens have been shown to demonstrate a significant increase in resistance to specific antibiotics over a short period of time, either as a result of selective pressure, antibiotic abuse by humans or overuse in animals (Olaniran *et al.,* 2009). Previous studies indicate a direct correlation between antimicrobial use and the extent of antimicrobial resistance (Mellon *et al.* 2001). Presently, the increase in multi-drug resistance patterns and range of bacterial pathogens displaying resistance to an expanding number of clinically important drugs is threatening the success of medical treatment and renders therapy precarious and costly (Heuer *et al.,* 2002; Okesola and Oni, 2009).

Notable global examples of multiple resistant organisms include, hospital and community multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis, Enterococcus faecium, Enterobacter cloacae, Klebsiella pnuemoniae, Staphylococcus aureus, Acinetobacter baumanii and Pseudomonas aeruginosa* (Levy and Marshall, 2004). Furthermore, in developing countries, MDR enteric disease agents such as *Escherichia coli, Salmonella enteritidis, Shigella flexneri and Vibrio cholerae* threaten and circumvent public health measures. Multiple antibiotic resistances may arise from a complex multi-factorial process confounded by a panoply of mobile genetic elements, including plasmids, transposons, and integrons that contain and transfer resistance determinants and may confer resistance to numerous antimicrobials (Miriagou *et al.,* 2006; Obi *et al.*, 2004).

The health consequences associated with diarrheagenic *E. coli* infection has been worsened by the emergence of multi-drug resistant *E. coli* (MDREC). Resistance to antibiotics recommended by the WHO as first-line treatment for diarrhoea including ampicillin, tetracycline, sulphamethoxazole-trimethoprim, chloramphenicol and augmentin has increased among diarrhoeagenic *E. coli* (Bii *et al.,* 2005; Chandran *et al.,* 2008). Several reports also demonstrated that *V. cholerae* O139 isolates, recovered during 1992 to 1993, were sensitive to tetracycline and resistant to trimethoprim-sulfamethoxazole (SXT) and streptomycin, because they harboured a chromosomally encoded integrating conjugative element, termed the SXT element (Basu *et al.,* 2000; Dalsgaard *et al.,* 2001; Faruque *et al.,* 2003). Subsequently, resistance to other drugs commonly used for prophylaxis and clinical therapy, such as tetracycline, ampicillin, chloramphenicol, gentamicin, furazolidone, ciprofloxacin, and others, has been reported at different rates among O139 strains with MDR O139 strains been frequently isolated (Pan *et al.,* 2008). It was believed that the bacterial mobile genetic elements harbouring resistance genes, including conjugative plasmids, integrating conjugative elements, and integrons, were largely responsible for the shift of drug resistance patterns among both the O1 and O139 serogroups (Pan *et al.*, 2008).

Now that antibiotic contamination in the environment has been confirmed, a growing concern is that the release of antibiotics into the environment may contribute to the emergence of strains of disease-causing bacteria that are resistant to even high doses of these drugs. Therefore the main objective of this chapter was to determine the prevalence and distribution of antimicrobial resistance among the *E. coli* and *V. cholerae* isolates obtained from two river basins and six different beaches in Durban, South Africa. This study was based on the phenotypic resistance testing of twenty four and twenty antibiotics against *E. coli* and *V. cholerae* isolates, respectively. The determination of antibiotic resistant profiles of *E. coli* and *V. cholerae* isolates in river and beach water can provide a prototypical view of the effects of antibiotic use on the bacterial populations.

3.2 Materials and methods

3.2.1 Sample collection

Water samples were collected as described in section 2.2.2 of Chapter two.

3.2.2 Isolation of presumptive *E. coli* and *V. cholerae*

Presumptive *E. coli* was isolated from the selective media (m-FC) used during the membrane filtration (MF) technique, as described in section 2.2.4 of Chapter two. Typical blue colonies on the m-FC plates, indicative of faecal coliforms, were streaked onto eosin methylene blue (EMB) agar for initial identification. Presumptive *E. coli* isolates that grew as dark violet colonies with a distinct green metallic sheen on the EMB medium were selected and purified on nutrient agar (NA) and incubated at 37°C for 18 – 24 hrs. Presumptive *V. cholerae* was isolated by filtering 250 ml of the water samples through 0.45 µm pore size GN-6 Metricel (Pall) membrane filters. This was followed by incubation of the membranes in an enrichment medium consisting of 100 ml of alkaline peptone water (APW, pH 8.6), overnight at 30°C (Choopun *et al.*, 2002). The enrichment medium was incubated at a lower temperature than that generally used for clinical samples in order to accommodate and reduce the stress for environmental isolates that inhabit lower-temperature environments. Following incubation, a loopful from the surface layer of the APW homogenates was streaked onto TCBS agar and incubated at 37°C for 18 – 24 hrs. Typical yellow, smooth, 1 to 3 mm diameter colonies were selected and purified on Luria-Bertani (LB) agar and incubated at 37°C for 18 – 24 hrs. The composition and preparation of the enrichment media used is detailed in Appendix A.

3.2.3 Presumptive bacterial identification

Gram stain was first performed on 18 – 24 hr cultures of the isolates to determine if the purified isolates were Gram negative/positive. The Gram negative, non-spore forming rods were then identified using standard biochemical reactions (Standard Methods, 1992). All isolates were subjected to the oxidase and ONPG (2-Nitrophenyl β-*D*-galactopyranoside) tests prior to biochemical testing. For the oxidase test, bacterial colonies were picked using pre-sterilized disposable loops and streaked on filter paper saturated with 0.5% tetramethyl-*p*-phenylenediamine hydrochloride (Sigma). The rapid appearance of a dark purple colour was considered a positive reaction. The ONPG test required emulsification of the bacterial colony in a microcentrifuge tube containing 0.1 ml of sterile 0.85% (w/v) sodium chloride solution (physiological saline) and one ONPG disk (Fluka). Following incubation at 35°C, colour development was recorded every hour for 6 hrs to detect active lactose fermenters. Late lactose fermenters are detected after a 24 hr incubation period.

The biochemical tests used for primary identification of the presumptive *E. coli* isolates, included, the triple sugar iron (TSI) slant test and the indole; methyl-red; voges-proskauer and the citrate utilization

tests, all collectively known as the IMViC test (Standard Methods, 1992). Indole production test was performed by inoculating a 1% tryptone broth medium and incubation at 37°C for 48 hrs. The presence of indole was detected by the addition of 10 drops of Kovac's reagent to the medium. If indole was produced, it will be extracted from the medium into the reagent layer by the acidified butanol component and forms a complex with ρ-dimethylaminobenzaldehyde, yielding a cherry red ring indicating a positive reaction. The methyl-red (MR) and voges-proskauer (VP) tests were performed simultaneously by inoculating MRVP broth medium and incubated at 37°C for 48 hrs. Following the incubation period, one-third of the culture broth was aseptically transferred to a clean, sterilized test tube for the VP test. To detect glucose metabolism and subsequent production and stabilization of high concentration acid end products (MR), 5 drops of the methyl red pH indicator was added to the MR culture broth aliquot. The broth changes red (positive reaction) at a pH of 4 and yellow (negative reaction) at a pH of 6. To detect the production of non-acidic or neutral end products from the organic acids that result from glucose metabolism (VP), 10 drops of Barritt's A followed by 10 drops of Barritt's B solutions were added to the VP culture broth aliquot and shaken every 5 min with colour changes recorded after 15 min. Development of a deep rose colour of the broth was indicative of a positive reaction while no colour change was regarded as negative. Lastly, citrate utilization was detected by inoculation of Simmon's citrate agar slants, incubation at 37°C for 48 hrs and recording colour change of medium after the incubation period. A change in the colour of the slants from green to blue was taken as a positive result whereas no colour change in the medium was recorded as a negative reaction.

The biochemical tests used for primary identification of the presumptive *V. cholerae* isolates, included, TSI slant test; kligler iron agar (KIA) slant test; arginine dihydrolase test and gelatinase production (Choopun *et al.,* 2000). TSI and KIA slants were stabbed and streaked and incubated with loosened caps at 37°C for 24 hrs. Arginine dihydrolase test medium was prepared as LB broth containing 1% (w/v) L-arginine (pH 6.8) and phenol red powder as an indicator (Thornley, 1960). After inoculation, the medium was covered with sterile mineral oil (Oxoid) and incubated at 37°C for 24 hrs. Gelatin agar butts were stabbed and incubated at 37°C for 24 hrs followed by a 30 min incubation at 4°C. The composition and preparation of the presumptive biochemical test media and reagents used is detailed in Appendix A.

3.2.4 Confirmation of bacterial identification

The identification of the presumptive *E. coli* isolates were further confirmed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (MS) (Bruker Daltonik, Germany) (Smole *et al.,* 2002). All chemicals and reagents (analytical grade) were purchased from Bruker Daltonik (Bremen, Germany). The working matrix solution was freshly prepared each time as a saturated solution of the matrix material, α-cyano-4-hydroxy cinnamic acid (α-HCCA) in 50% acetonitrile

(ACN) and 2.5% trifluoroacetic acid (TFA) in Eppendorf microcentrifuge tubes, which were stored (maximum of one week) and used at room temperature. Samples were prepared prior to protein extraction using the ethanol/formic acid extraction method, according to the manufacturer's instructions. Briefly, the bacterial isolates were grown overnight on NA at 37°C after which single colonies were resuspended in 300 µl of sterile double distilled water in microcentrifuge tubes followed by the addition of 900 µl of absolute ethanol. The samples were mixed by vortexing vigorously for 1 min and centrifuged at 13 200 rpm for 2 min to harvest the cells. The supernatant was carefully decanted to not disturb the bacterial pellet, and the step repeated to remove any residual ethanol. The pellet was allowed to air-dry for a few minutes after which 50 µl of 70% formic acid was added to the pellet and mixed thoroughly by vortexing to resolve the pellet. Fifty microliters of pure ACN was added to the mixture and mixed carefully. The suspension was centrifuged at 13 200 rpm for 2 min and the supernatant was used for further analysis. Equal volumes of matrix and bacterial solution (1µl:1µl) was spotted onto the target plate (MTP 384 target ground steel TF) and allowed to air-dry completely before analysis. The optimal number of peaks were produced with a Bruker Daltonics Ultraflex instrument (Bruker Daltonik, Bremen, Germany) using the following parameters: linear acquisition mode, PIE delay of 350 nsec, positive ion mode, ion source voltage of 125 kV, 223.40 kV ion source, 6 kV ion source lens voltage, laser repetition rate in Hz 50 psec, linear detector voltage of 1.65 kV, and 100 laser shots. The resulting spectra were processed using the flexAnalysis program and bacterial identification was determined.

Confirmatory identification of the *V. cholerae* isolates was performed using additional biochemical tests, including the esculin hydrolysis test; sodium ion requirement (0%; 6%; 8%); temperature tolerance (42°C); mannitol and arabinose production tests; MRVP tests; catalase test; autoagglutination; motility test and urease production (Bag *et al.*, 2008; Choopun *et al.*, 2002; Janda *et al.*, 1988; Noguerola and Blanch, 2008). The ability of the presumptive *V. cholerae* isolates to hydrolyze esculin was determined by stab inoculation of prepared heart infusion agar butts containing 0.1% esculin and 0.05% ferric chloride followed by incubation at 30 to 37°C for up to 3 days. Thereafter the butts were examined for colour change and decrease in fluorescence. Salt tolerance was tested by growing the isolates in nutrient broth supplemented with 0, 6, and 8% (w/v) NaCl to determine the requirement of sodium ions (Na^+). The prepared medium was inoculated and incubated at 30 or 37°C for up to 7 days after which tubes were examined for turbidity. Trypticase soy broth was inoculated and incubated overnight at 42°C to determine if these isolates were able to grow at high temperatures, indicated by the appearance of turbidity. Carbohydrate utilization tests involving acid production from 1% mannitol and 1% arabinose were determined using purple broth base (pH 6.8) amended with the individual carbohydrates. Following inoculation and incubation at 37°C for no more than 24 hrs, a change in the colour of the medium was recorded. Catalase production was determined by preparing a smear of each isolate on a glass slide and saturating the slide with 3% hydrogen peroxide, where vigorous bubbling

within 10 – 20 sec indicated a positive reaction. For the autoagglutination test, strains were grown in LB (pH 6.5) supplemented with 1% (w/v) NaCl and incubated at 37°C with aeration for 18 hrs. A visible clumping of bacteria indicated a positive result. Motility butts were heavily inoculated by stabbing and incubated at 37°C for 24 – 48 hrs. The ability of the presumptive *V. cholerae* isolates to hydrolyze urea was tested by heavily inoculating Christensen's urea agar slants containing 40% filter sterilized urea solution. The inoculated slants were incubated at 37°C for 24 hrs to record any colour change in the medium.

Following proper identification of both bacterial groups, the isolates were maintained on NA at 4°C (subcultured every 2 – 3 weeks) and strains were stored as glycerol stocks (LB broth containing 25% glycerol) at –70°C (Edward and Ewing, 1972). The composition and preparation of the confirmatory biochemical test media and reagents used is detailed in Appendix A.

3.2.5 Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method (CLSI, 2009) was used to determine the antibiotic sensitivity of the identified isolates. All isolates were grown in tryptic soy broth at 35°C for 18 – 24 hrs after which the inoculum density of the actively growing broth cultures were adjusted with sterile broth medium to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. The cartridges containing the commercially prepared antibiotic discs (Mast Diagnostics) specifically for susceptibility testing were stored in vacuum sealed plastic bags at –20°C and resealed after use, whilst the working supply was stored at 4°C. The antibiotics were allowed to equilibrate to room temperature prior to use to reduce condensation. The antibiotics employed in this study are listed in Table 3.1. All antibiotics were tested against the *E. coli* isolates except mupirocin. In addition, all antibiotics were tested against the *V. cholerae* isolates except fusidic acid and clindamycin.

3.2.5.1 Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a barium sulphate ($BaSO_4$) turbidity standard, equivalent to a 0.5 McFarland standard was freshly prepared. A 0.5 ml aliquot of 0.048 mol/L $BaCl_2$ [1.175% (w/v) $BaCl_2.2H_2O$] was added to 99.5 ml of 0.18 mol/l H_2SO_4 [1% (v/v)] with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified using a spectrophotometer with a 1 cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard. The $BaSO_4$ suspension was aliquoted into screw-cap tubes, tightly sealed and stored in the dark at room temperature. The turbidity standard was thoroughly vortexed before each use and replaced if densities varied.

3.2.5.2 Inoculation of the susceptibility test media

After proper standardization of each isolate, a sterile swab was submerged into the broth and used to inoculate Müeller Hinton agar plates by swabbing the entire surface of the test medium. The inoculated agar plates were left to dry for 2 hrs at room temperature. The antibiotic discs were then placed at equi-distances (5 discs on each plate) on the agar surface using sterile forceps and tapped gently to ensure each disc had adhered properly to the agar surface. The plates containing the discs were incubated at 35°C for 16 to 18 hrs after which the diameter of the zone of inhibition for each antibiotic disc was measured with a ruler against a dark background. The isolates were then classified as sensitive, intermediate or resistant to a particular antibiotic based on the CLSI standards (Appendix B). *Escherichia coli* ATCC 25922 (beta-lactamase negative) was included as the MIC quality control in accordance with the CLSI standards. The Multiple Antibiotic Resistance (MAR) index, defined as *a/b*, where *a* represents the number of antibiotics to which the isolate was resistant, and *b* represents the number of antibiotics to which the isolate was exposed, was calculated for each isolate (Krumperman, 1983).

Table 3.1: Antibiotics employed in this study.

Class of antibiotic	Antibiotic	Level (µg)	Symbol
Fluoroquinolones (2nd generation)	Ciprofloxacin	5	CIP
Phenicols	Chloramphenicol	30	C
Folate Inhibitors	Cotrimoxazole	25	TS
Ansamycins	Rifampicin	5	RP
Quinolones (1st generation)	Nalidixic acid	30	NA
Cephalosporins (2nd generation)	Cefuroxime	30	CXM
	Cefoxitin	30	FOX
Cephalosporins (3rd generation)	Cefotaxime	30	CTX
	Ceftriaxone	30	CRO
Aminoglycosides	Gentamicin	10	GM
	Tobramycin	10	TN
	Amikacin	30	AK
	Streptomycin	25	S
Tetracyclines	Tetracycline	30	T
β-lactam Penicillins (extended spectrum)	Ampicillin	10	AP
	Penicillin G	10	PG
β-lactams (combination)	Augmentin	30	AUG
Macrolides	Erythromycin	15	E
Epoxides	Mupirocin	5	MUP
Antifolates	Trimethoprim	5	TM
Steroids	Fusidic acid	10	FC
Lincosamides	Clindamycin	2	CD

3.3 Results

3.3.1 Isolation and identification of environmental *E. coli* and *V. cholerae* strains

A total of 96 *E. coli* and 82 *V. cholerae* strains retrieved from the respective sources, as indicated in Table 3.2 and identified based on the several presumptive and confirmatory tests were tested for antibiotic resistance profiling. The different biochemical profiles used for the identification of the *E. coli* and *V. cholerae* strains are shown in Tables 3.3 and 3.4, respectively.

Table 3.2: Number of positively identified *E. coli* and *V. cholerae* strains isolated from the respective sources.

E. coli		***V. cholerae***	
Source	**No. of isolates**	**Source**	**No. of isolates**
Umgeni River	46	Umgeni River	18
Beaches	31	Beaches	35
Umdloti River	19	Umdloti River	29
Total	**96**	**Total**	**82**

Table 3.3: Summary of the typical phenotypic profiles used for primary and confirmatory identification of presumptive *E. coli* isolates.

Biochemical test	**Characteristics**
Isolation	
EMB	Dark violet colonies with a distinct green metallic sheen
Primary identification	
Gram stain	Gram-negative; non-spore forming; straight rod
Oxidase	Negative: no appearance of dark purple on filter paper
ONPG	Positive: development of yellow colour within 6 hrs of incubation indicating active lactose fermenter
Triple sugar iron	Acid slant (yellow) and acid butt (yellow); gas production with no H_2S production
Indole production	Positive: cherry red ring produced when Kovac's reagent is added
Methyl red	Positive: medium becomes red upon addition of methyl red indicator
Voges-Proskauer	Negative: no change in medium colour (copper-like)
Citrate utilization	Negative: no change in medium colour (blue-green)
Confirmatory identification	
MALDI-ToF	Bacterial identification

Table 3.4: Summary of the typical phenotypic profiles used for primary and confirmatory identification of presumptive *V. cholerae* isolates.

Biochemical test	Characteristics
Isolation	
TCBS	Yellow smooth colonies; 1 - 3mm diameter; center of colonies are opaque with slightly translucent edges
Primary identification	
Gram stain	Gram-negative; non-spore forming; straight or curved rod
Oxidase	Positive: rapid appearance (>20 seconds) of dark purple on filter paper
ONPG	Positive: development of yellow colour within 6 hrs of incubation indicating active lactose fermenter
Triple sugar iron	Acid slant (occasionally alkaline) and acid butt; no gas or H_2S production
Kligler iron agar	Alkaline slant (purple) and acid butt (yellow); no gas or H_2S production
Arginine dihydrolase	Negative: medium changes from red to yellow
Gelatin agar	Positive: medium remains a liquid after incubation and does not solidify at 4°C
Confirmatory identification	
Esculin hydrolysis	Negative: medium does not turn black and no loss of fluorescence
Salt tolerance	Positive (0% NaCl): medium was turbid after incubation period Variable (6% NaCl): select isolates grew at this salt concentration Negative (8% NaCl): no growth as medium did not become turbid after incubation
Temperature tolerance	Positive: growth was indicated by turbidity
Mannitol/arabinose production	Positive (mannitol): medium changes from purple to yellow Negative (arabinose): no change in medium colour
Methyl red	Variable: upon addition of methyl red indicator, medium turned red (positive) or yellow (negative)
Voges-Proskauer	Positive (El Tor biotype): medium turns pink-burgundy Negative (Classical biotype): medium remains unchanged
Catalase	Positive: vigorous bubbling upon addition of H_2O_2
Autoagglutination	Positive: visible clumping of bacteria
Motility	Positive: growth diverted from point of stab inoculation
Urease	Negative: no change in medium colour following incubation

3.3.2 Antibiotic resistance patterns of *E. coli* isolates

The antibiotic resistance profiles (ARPs) of the *E. coli* isolates from the river and beach water samples, to the individual antibiotics, is presented in Table 3.5 while the resistance spectra/pattern(s) of the isolates against the 24 antibiotics are illustrated in Figs. 3.1 and 3.2. Resistance to antimicrobial agent was detected in all 96 isolates from both rivers and the different beaches. All isolates from the Umgeni River, beaches and Umdloti River were resistant (100%) to rifampicin, penicillin G, erythromycin, trimethoprim, fusidic acid and clindamycin (Table 3.5).

High prevalence of resistance to ampicillin [70% (A1), 83.3% (A2), 83.3% (A3), 62.5% (A5)] was encountered among the Umgeni River isolates, followed by tetracycline [50% (A1), 60% (A4), 50% (A5)], streptomycin [66.7% (A2)], amikacin [50% (A2)], cotrimoxazole [40% (A1), 50% (A4)] and augmentin [40% (A4)]. All Umgeni River isolates were sensitive to ciprofloxacin except one isolate (12.5%) from sampling site A5. In addition, all Umgeni River isolates were susceptible to cefoxitin, cefotaxime, ceftriaxone, gentamicin and tobramycin; however one isolate from sampling point A4 was resistant to the cefoxitin, cefotaxime and ceftriaxone antibiotics. Low resistance rates were observed against chloramphenicol [10% (A1), 16.7% (A2)], nalidixic acid [10% (A4), 12.5% (A5)] and cefuroxime [8.33% (A3), 10% (A4), 12.5 % (A5)], among the isolates. The antibiotic resistance index (ARI) of the isolates from the Umgeni River samples were as follows: 0.390 (A1), 0.688 (A2), 0.321 (A3), 0.438 (A4) and 0.499 (A5). Among the *E. coli* isolates obtained from the different beaches, high resistance frequencies were observed against tetracycline (88.9%, B1), ampicillin (80%, B2 and B6), streptomycin (66.7%, B3) and amikacin (60%, B5). All *E. coli* isolates from the beach water samples were susceptible to ciprofloxacin, cefoxitin, cefotaxime and ceftriaxone. In addition, all isolates were sensitive to chloramphenicol, gentamicin and tobramycin; however, two isolates from sampling site B1 were resistant to chloramphenicol and gentamicin, respectively with one isolate from sampling site B5 being resistant to tobramycin. The least resistance was detected against cotrimoxazole [22.2% (B1), 20% (B2 and B5), 33.3% (B3), 25% (B4)], nalidixic acid [22.2% (B1), 20% (B5)] and cefuroxime [20% (B2 and B6), 25% (B4)]. The ARI of the isolates from the beach water samples ranged from 0.470 to 1.322 for all the sampling points. Similar susceptibility patterns were observed among the Umdloti River *E. coli* isolates with all isolates from all points along the river being sensitive to chloramphenicol, ciprofloxacin, cefoxitin, cefotaxime, ceftriaxone, gentamicin and tobramycin antibiotics. These isolates demonstrated predominant resistance to augmentin [40% (C1), 75% (C3), 50% (C4)], ampicillin [60% (C1), 75% (C3)], amikacin [80% (C1), 75% (C4)] and cotrimoxazole [40% (C1), 50% (C3)] with high tetracycline resistance (50%) among the isolates from sampling point C3. According to the ARI of the sampling stations along the river, the *E. coli* isolates obtained from C2 exhibited the lowest level of resistance (0.431) against the tested antibiotics whereas the ARI for the other sampling points ranged from 0.582 to 1.042.

Table 3.5: Antibiotic resistance profile of *E. coli* isolates from the different sampling sites to the individual antibiotics.

	Umgeni River					**Beaches**						**Umdloti River**			
	A1 ***n*** **= 10**	**A2** ***n*** **= 6**	**A3** ***n*** **= 12**	**A4** ***n*** **= 10**	**A5** ***n*** **= 8**	**B1** ***n*** **= 9**	**B2** ***n*** **= 5**	**B3** ***n*** **= 3**	**B4** ***n*** **= 4**	**B5** ***n*** **= 5**	**B6** ***n*** **= 5**	**C1** ***n*** **= 5**	**C2** ***n*** **= 6**	**C3** ***n*** **= 4**	**C4** ***n*** **= 4**
Antibiotic Resistance Index (ARI)[a]	0.390	0.688	0.321	0.438	0.499	0.470	0.857	1.322	0.952	0.819	0.800	0.838	0.582	1.042	0.982
% Resistance to:															
Ciprofloxacin	0	0	0	0	12.5	0	0	0	0	0	0	0	0	0	0
Chloramphenicol	10.0	0	16.7	30.0	0	11.1	0	0	0	0	0	0	0	0	0
Cotrimoxazole	40.0	16.7	33.3	50.0	25.0	22.2	20.0	33.3	25.0	20.0	40.0	40.0	0	50.0	25.0
Rifampicin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Nalidixic acid	0	0	0	10.0	12.5	22.2	40.0	0	0	20.0	0	0	16.7	0	0
Cefuroxime	20.0	0	8.33	10.0	12.5	0	20.0	0	25.0	0	20.0	20.0	16.7	0	25.0
Cefoxitin	0	0	0	10.0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	10.0	0	0	0	0	0	0	0	0	0	0	0
Ceftriaxone	0	0	0	10.0	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	11.1	0	0	0	0	0	0	0	0	0
Tobramycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amikacin	20.0	50.0	25.0	20.0	12.5	0	40.0	33.3	50.0	60.0	20.0	80.0	33.3	25.0	75.0
Streptomycin	10.0	66.7	25.0	30.0	37.5	55.6	40.0	66.7	0	40.0	20.0	20.0	16.7	0	25.0
Tetracycline	50.0	33.3	16.7	60.0	50.0	88.9	40.0	0	25.0	40.0	20.0	20.0	16.7	50.0	0
Ampicillin	70.0	83.3	83.3	40.0	62.5	44.4	80.0	66.7	50.0	40.0	80.0	60.0	0	75.0	25.0
Penicillin G	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Augmentin	0	16.7	0	40.0	12.5	33.3	20.0	33.3	25.0	40.0	40.0	40.0	33.3	75.0	50.0
Erythromycin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Trimethoprim	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Fusidic acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Clindamycin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

[a] The antibiotic resistance index (ARI) of the samples were calculated by the equation $a/(b \times c)$, where a is the aggregate antibiotic resistance score of all isolates from the sample, b is the number of antibiotics, and c is the number of isolates from the sample (Krumperman, 1983).

According to Fig. 3.1, twenty nine different resistance patterns (one six-drug, two seven-drug, eight eight-drug, eight nine-drug, five ten-drug, four eleven-drug and one twelve-drug) were observed among the Umgeni River *E. coli* isolates, with the proportions of 6.52% (A), 28.26% (A), 19.57% (B), 19.57% (C), 10.87% (D), 13.04% (E) and 2.17% (E), respectively. Among these multi-resistant isolates, the most common resistance pattern was RP/AP/PG/E/TM/FC/CD which accounted for 21.74% of the 46 resistant isolates, followed by RP/PG/E/TM/FC/CD (6.52%),RP/T/PG/E/TM/FC/CD (6.52%), TS/RP/AK /S/T/AP/PG/E/TM/FC/CD (6.52%), TS/RP/S/PG/E/TM/FC/CD (4.35%) and RP/AK/S/AP/PG/E/TM/FC/ CD (4.35%). The MAR index values of the isolates ranged from 0.29 (for isolates resistant to six out of the 21 antibiotics tested) to 0.57 (for resistance to twelve out of the 21 antibiotics). Thirteen of the 46 *E. coli* isolates recovered from this river were resistant to seven antibiotics with six of the thirteen isolates been recovered from sampling point A1.

As shown in Fig. 3.2 (A – C), twenty six different resistance patterns (three seven-drug, eight eight-drug, eight nine-drug, four ten-drug and three eleven-drug) were observed among the *E. coli* isolates obtained from the beach water samples, with the proportions of 16.13% (A), 35.48% (A), 25.81% (B), 12.90% (C) and 9.68% (C), respectively. Three of the 31 *E. coli* isolates obtained from the beach water samples exhibited the most frequently encountered resistance pattern of RP/T/AP/PG/E/TM/FC/CD (9.68%) while RP/AP/PG/E/TM/FC/CD (6.45%) and RP/AK/PG/E/TM/FC/CD (6.45%) were the second most common resistance patterns demonstrated by the isolates. Analysis of the antibiotic resistance spectra revealed that all beach water isolates were multi-antibiotic resistant with the MAR indices ranging from 0.33 (seven drugs) to 0.52 (eleven drugs).

Fourteen different resistance patterns (four seven-drug, three eight-drug, six nine-drug and one eleven-drug) were demonstrated by the Umdloti River *E. coli* isolates with the proportional resistances of 36.84% (D), 15.79% (D), 42.11% (E) and 5.26% (E), respectively [Fig. 3.2 (D – E)]. The most common resistance pattern encountered among these multi-antibiotic resistant isolates, was RP/AK/PG/E/TM/FC/ CD (15.79%), followed by RP/AUG/PG/E/TM/FC/CD (10.53%), RP/CXM/PG/E/TM/FC/CD (10.53%), TS/RP/AK/AUG/PG/E/TM/FC/CD (10.53%) and RP/NA/AK/S/PG/E/TM/FC/CD (10.53%). One isolate (0.053%) was resistant to eleven of the 21 antibiotics that were tested and revealed a resistance pattern of RP/AK/S/T/AP/AUG/PG/E/TM/FC/CD. Of the six *E. coli* isolates recovered from sampling point C2 of the Umdloti River, five (83.3%) of the isolates were resistant to seven antibiotics while three (75%) of the four isolates recovered from sampling point C3 were resistant to nine antibiotics.

3.3.3 Antibiotic resistance patterns of *V. cholerae* isolates

The antibiotic resistance profiles (ARPs) of the *V. cholerae* isolates, to the individual antibiotics, from the river and beach water samples is presented in Table 3.6 while the resistance spectra/pattern(s) of the isolates against the 20 antibiotics are illustrated in Figs. 3.3 and 3.4. Antimicrobial agent resistance was detected in all 82 isolates from both rivers and the different beaches.

All Umgeni River isolates were sensitive to ciprofloxacin and ceftriaxone while all isolates were resistant to penicillin G. All isolates recovered from sampling points A1 – A3 were resistant to chloramphenicol, cotrimoxazole and erythromycin while all isolates recovered from points A4 and A5 were inhibited by streptomycin. Overall, the most frequently encountered form of resistance in all *V. cholerae* isolates from the Umgeni River samples was resistance to cotrimoxazole (93.34%), followed by resistance to erythromycin (78.66%), trimethoprim and streptomycin (75%), augmentin (72.68%), rifampicin (70%), tetracycline (63%), cefoxitin (51.32%), ampicillin (49.68%), cefuroxime (46.34%) and mupirocin (44.66%). Tobramycin and amikacin resistance was identified among 33% and 38% of the total number of Umgeni River *V. cholerae* isolates, respectively. Moreover, low prevalence of resistance to nalidixic acid (14.66%), cefotaxime (13.34%) and gentamicin (10%) was observed among the isolates. The calculated ARI of the isolates from the Umgeni River samples ranged between 0.462 (A3) to 0.508 (A4). Similarly, 95.83% and 93.33% of the *V. cholerae* isolates obtained from the beach water samples were sensitive to ciprofloxacin and ceftriaxone, while 96.67% of the isolates were resistant to penicillin G. Overall, more than 50% of the isolates obtained from the beach water samples were resistant to trimethoprim (87.50%), streptomycin (84%), cotrimoxazole (80.67%), chloramphenicol (71.38%), erythromycin (66.05%), rifampicin (64.53%), cefoxitin and amikacin (52.72%). In addition, lower antibiotic resistance levels were also detected against gentamicin (31.15%), augmentin (35.20%), tetracycline (30.47%), ampicillin (39.63%), cefuroxime (37.57%), cefotaxime (17.92%) and nalidixic acid (24.88%). The ARI of the isolates from the beach water samples ranged between 0.458 (B4) to 0.500 (B1). All Umdloti River isolates were sensitive to ciprofloxacin (except one isolate obtained from sampling point C4) and ceftriaxone. Overall, the most frequently encountered form of resistance in all isolates from the Umdloti River samples was resistance to cotrimoxazole (81.88%), followed by streptomycin (79.58%), trimethoprim (77.70%), chloramphenicol (68.75%), mupirocin (67.30%), erythromycin (63.33%), ampicillin (60.42%), cefoxitin (48.95%), rifampicin (46.88%), cefuroxime (45.63%), amikacin (45.20%) and cefotaxime (45%). Furthermore, more than 20% of the isolates retrieved from these river water samples were resistant to gentamicin (24.58%), tetracycline (29.18%) and augmentin (30.83%) while 15.63% and 13.75% of isolates were resistant to nalidixic acid and tobramycin, respectively. The ARI values for the Umdloti River isolates varied from 0.492 (C1), 0.431 (C2), 0.490 (C3) to 0.469 (C4).

Table 3.6: Antibiotic resistance profile of *V. cholerae* isolates from the different sampling sites to the individual antibiotics.

	Umgeni River					**Beaches**						**Umdloti River**			
	A1 ***n*** **= 4**	**A2** ***n*** **= 3**	**A3** ***n*** **= 3**	**A4** ***n*** **= 3**	**A5** ***n*** **= 5**	**B1** ***n*** **= 6**	**B2** ***n*** **= 5**	**B3** ***n*** **= 8**	**B4** ***n*** **= 4**	**B5** ***n*** **= 5**	**B6** ***n*** **= 7**	**C1** ***n*** **= 10**	**C2** ***n*** **= 6**	**C3** ***n*** **= 5**	**C4** ***n*** **= 8**
Antibiotic Resistance Index (ARI)[a]	0.466	0.486	0.462	0.508	0.474	0.500	0.500	0.472	0.458	0.492	0.475	0.492	0.431	0.490	0.469
% Resistance to:															
Ciprofloxacin	0	0	0	0	0	0	0	0	25.0	0	0	0	0	0	12.5
Chloramphenicol	100	100	100	33.3	40.0	83.3	60.0	50.0	75.0	60.0	100	90.0	50.0	60.0	75.0
Cotrimoxazole	100	100	100	66.7	100	83.3	80.0	75.0	100	60.0	85.7	60.0	100	80.0	87.5
Rifampicin	50.0	66.7	100	33.3	100	83.3	80.0	37.5	75.0	40.0	71.4	20.0	50.0	80.0	37.5
Nalidixic acid	0	0	0	33.3	40.0	0	60.0	50.0	25.0	0	14.3	30.0	0	20.0	12.5
Cefuroxime	25.0	66.7	66.7	33.3	40.0	33.3	40.0	25.0	50.0	20.0	57.1	30.0	50.0	40.0	62.5
Cefoxitin	50.0	100	33.3	33.3	40.0	66.7	40.0	62.5	50.0	40.0	57.1	60.0	33.3	40.0	62.5
Cefotaxime	0	0	0	66.7	0	0	0	37.5	50.0	20.0	0	40.0	50.0	40.0	50.0
Ceftriaxone	0	0	0	0	0	0	0	0	0	40.0	0	0	0	0	0
Gentamicin	50.0	0	0	0	0	16.7	20.0	37.5	50.0	20.0	42.7	20.0	33.3	20.0	25.0
Tobramycin	25.0	100	0	0	40.0	66.7	80.0	25.0	50.0	40.0	28.6	30.0	0	0	25.0
Amikacin	50.0	66.7	33.3	0	40.0	33.3	60.0	62.5	50.0	40.0	71.4	70.0	33.3	40.0	37.5
Streptomycin	75.0	66.7	33.3	100	100	83.3	100	75.0	100	60.0	85.7	60.0	83.3	100	75.0
Tetracycline	75.0	100	66.7	33.3	40.0	16.7	60.0	12.5	25.0	40.0	28.6	30.0	16.7	20.0	50.0
Ampicillin	75.0	66.7	66.7	0	40.0	66.7	40.0	12.5	50.0	40.0	28.6	40.0	66.7	60.0	75.0
Penicillin G	100	100	100	100	100	100	80.0	100	100	100	100	100	100	100	100
Augmentin	50.0	100	66.7	66.7	80.0	16.7	20.0	50.0	50.0	60.0	14.3	20.0	33.3	20.0	50.0
Erythromycin	100	100	100	33.3	60.0	66.7	100	37.5	75.0	60.0	57.1	40.0	83.3	80.0	50.0
Mupirocin	50.0	66.7	33.3	33.3	40.0	50.0	20.0	62.5	50.0	40.0	42.9	60.0	66.7	80.0	62.5
Trimethoprim	75.0	100	33.3	66.7	100	50.0	100	100	75.0	100	100	60.0	83.3	80.0	87.5

[a] The antibiotic resistance index (ARI) of the samples were calculated by the equation $a/(b \times c)$, where a is the aggregate antibiotic resistance score of all isolates from the sample, b is the number of antibiotics, and c is the number of isolates from the sample (Krumperman, 1983).

Eighteen different resistance patterns (one six-drug, two seven-drug, one eight-drug, four nine-drug, two ten-drug, four eleven drug, one twelve-drug, one thirteen-drug and two fourteen-drug) were observed among the Umgeni River *V. cholerae* isolates, which indicates that all isolates obtained from this river source did not share common resistance patterns [Fig. 3.3 (A – D)]. With regard to multi-drug resistance, 22.2% of the isolates from the Umgeni River were resistant to nine drugs producing four different drug patterns (C/TS/FOX/GM/AK/PG/E/MUP/TM, C/TS/RP/S/T/AP/PG/E/MUP, RP/CXM/FOX/CTX/S/T/PG/MUP/TM and C/TS/RP/CXM/FOX/S/PG/E/TM) while another four isolates (22.2%) were resistant to eleven antibiotics producing four different drug patterns (C/TS/TN/AK/S/T/AP/PG/AUG/E/TM, C/TS/RP/FOX/TN/T/PG/AUG/E/MUP/TM, C/TS/RP/CXM/FOX/AK/AP/PG/AUG/E/TM and C/TS/RP/FOX/TN/S/PG/AUG/E/MUP/TM). Two of the 18 isolates (11.1%) were resistant to ten drugs (C/TS/RP/S/T/AP/PG/AUG/E/TM), one (5.56%) to twelve drugs (C/TS/RP/CXM/FOX/GM/S/T/AP/PG/E/MUP) and one (5.56%) to a total of thirteen drugs (TS/RP/NA/CXM/TN/AK/S/T/PG/AUG/E/MUP/TM). Two of the isolates recovered from sampling point A2 along the river were resistant to fourteen out of the 20 tested antibiotics following two different patterns (C/TS/CXM/FOX/TN/AK/S/T/AP/PG/AUG/E/MUP/TM and C/TS/RP/CXM/FOX/TN/AK/S/T/AP/PG/AUG/E/TM).

The *V. cholerae* isolates recovered from the beach water samples produced thirty five different resistance patterns with no two isolates sharing the same pattern [Fig. 3.3 (E – F) and Fig. 3.4 (A – C)]. Multiple antimicrobial resistances among these isolates occurred as follows: five isolates (14.29%) were resistant to eight antibiotics, seven isolates (20%) to nine antibiotics, nine isolates (25.71%) to ten drugs and six isolates (17.14%) to eleven drugs. In addition, three isolates (8.57%) were resistant to twelve drugs (C/TS/RP/CXM/FOX/GM/TN/S/PG/AUG/MUP/TM, C/RP/NA/CXM/FOX/GM/AK/S/AP/PG/E/TM and C/TS/RP/CXM/FOX/AK/S/T/PG/AUG/MUP/TM), one isolate (2.86%) was resistant to thirteen drugs out of the 20 tested antibiotics (C/TS/NA/CXM/FOX/TN/AK/S/AP/PG/E/MUP/TM) while one isolate (2.86%) was resistant to an array of fourteen drugs (CIP/C/TS/RP/CXM/FOX/GM/TN/S/T/PG/AUG/MUP/TM).

Twenty nine different resistance patterns were observed among the *V. cholerae* isolates from the Umdloti River, which indicates that all isolates obtained from this river source did not share common resistance patterns [Fig. 3.4 (D – E)]. Seven (24.14%) of the 29 isolates were resistant to nine antibiotics and produced seven different antimicrobial resistance patterns as follows: C/TS/CXM/CTX/AK/AP/PG/MUP/TM, TS/CTX/GM/S/T/AP/PG/MUP/TM, C/TS/RP/CTX/S/T/PG/E/MUP, TS/CXM/FOX/S/AP/PG/E/MUP/TM, TS/CTX/GM/S/PG/AUG/E/MUP/TM, C/TS/RP/FOX/AP/PG/AUG/MUP/TM and C/TS/CXM/GM/S/T/PG/AUG/TM. A further seven more *V. cholerae* isolates were resistant to ten antibiotics with the resultant resistance patterns: C/CXM/CTX/GM/AK/S/AP/PG/E/MUP, C/TS/FOX/CTX/TN/S/T/PG/AUG/TM, C/TS/RP/FOX/AK/AP/PG/E/MUP/TM, TS/CXM/CTX/AK/S/AP/PG/AUG/E/TM, C/TS/RP/FOX/S/AP/PG/E/MUP/TM, C/RP/CXM/CTX/GM/S/AP/PG/E/TM and TS/CXM/FOX/CTX/AP/PG

/AUG/E/TM. In addition, six *V. cholerae* isolates (20.69%) were resistant to eight antibiotics while a further six more isolates were resistant to eleven antibiotics.

The multiple antibiotic resistance (MAR) indices calculated for the *V. cholerae* isolates obtained from the river and beach water sources and their resultant drug patterns are as follows: five drugs (0.25), six drugs (0.30), seven drugs (0.35), eight drugs (0.40), nine drugs (0.45), ten drugs (0.50), eleven drugs (0.55), twelve drugs (0.60), thirteen drugs (0.65) and fourteen drugs (0.70).

Resistance of the total number of *E. coli* and *V. cholerae* isolates from the different water sources to different classes of antibiotics ranged from six antibiotic classes to eleven classes (Table 3.7) and four antibiotic classes to eleven classes (Table 3.8), respectively. Moreover, the high level of multi-antibiotic resistance demonstrated by these environmental isolates is evidenced by the number of antibiotic classes these isolates were resistant to. The ARPs of the *E. coli* isolates revealed that 13 (28.3%) of the 46 *E. coli* isolates obtained from the Umgeni River were resistant to six antibiotic classes while 10 (32.3%) of the 31 isolates recovered from the beach water samples were resistant to eight antibiotic classes. Almost half of the Umdloti River *E. coli* isolates were resistant to seven antibiotic classes (47.4%). One *E. coli* isolate, recovered from the Umgeni River, was resistant to an astounding 11 antibiotic classes. The ansamycin, macrolide, antifolate, steroid and lincosamide antibiotic classes exhibited the lowest activity against the *E. coli* isolates, as all the isolates retrieved from both rivers and the beaches were resistant to the antimicrobial agents belonging to these classes. Among the aminoglycosides, gentamicin and tobramycin showed the highest activity toward the isolates as only one isolate from sampling point B1 was resistant to gentamicin and one isolate obtained from sampling point B5 was resistance to tobramycin. On the other hand, amikacin and streptomycin proved to be weak inhibitory agents as the isolates demonstrated high levels of resistance to these drugs. The second generation fluoroquinolones, phenicols and third generation cephalosporins displayed excellent inhibitory properties as evidenced by high susceptibility levels of the isolates to these antibiotics. One of the *E. coli* isolates obtained from the Umgeni river water sample, A5, was resistant to twelve classes of antibiotics and was the only isolate resistant to ciprofloxacin.

The ARPs of the *V. cholerae* isolates revealed that 7 (38.9%) of the 18 isolates obtained from the Umgeni River were resistant to eight antibiotic classes while 10 (28.57%) of the 18 isolates recovered from the beach water samples were resistant to eight antibiotic classes and 12 (41.38%) of the 29 *V. cholerae* isolates from the Umdloti River isolates were resistant to eight antibiotic classes. Only the second generation fluoroquinolones and third generation cephalosporins (ceftriaxone only) exhibited good inhibitory activity against the isolates; however the other antibiotic classes showed poor activity toward the isolates as multiple resistances against these antibiotics were frequently observed. One *V. cholerae* isolate obtained from the beach water sample, B4, was resistant to eleven classes of antibiotics and was the only beach water isolate resistant to ciprofloxacin.

Table 3.7: Resistance of total *E. coli* isolates to the different classes of antibiotics.

No. of antibiotic classes	Resistant strains (%)		
	Umgeni river (*n* = 46)	**Beaches (*n* = 31)**	**Umdloti river (*n* = 19)**
6	28.3 (13)	6.45 (2)	0 (0)
7	23.9 (11)	29.03 (9)	47.4 (9)
8	19.6 (9)	32.3 (10)	31.6 (6)
9	17.4 (8)	25.8 (8)	21.1 (4)
10	8.70 (4)	6.45 (2)	0 (0)
11	2.17 (1)	0 (0)	0 (0)

Values in parenthesis represent the number of total *E. coli* isolates resistant to the indicated numbers of antibiotic class

Table 3.8: Resistance of total *V. cholerae* isolates to the different classes of antibiotics.

No. of antibiotic classes	Resistant strains (%)		
	Umgeni river (*n* = 18)	**Beaches (*n* = 35)**	**Umdloti river (*n* = 29)**
4	0 (0)	2.86 (1)	0 (0)
5	0 (0)	2.86 (1)	3.45 (1)
6	11.1 (2)	2.86 (1)	6.90 (2)
7	5.56 (1)	28.57 (10)	20.69 (6)
8	38.9 (7)	28.57 (10)	41.38 (12)
9	16.7 (3)	22.86 (8)	20.69 (6)
10	16.7 (3)	8.57 (3)	3.45 (1)
11	11.1 (2)	2.86 (1)	3.45 (1)

Values in parenthesis represent the number of total *V. cholerae* isolates resistant to the indicated numbers of antibiotic class

3.4 Discussion

The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern (Tjaniadi *et al.,* 2003). Increased introduction of antimicrobial agents into the environment via medical therapy, agriculture and animal husbandry has resulted in selective pressures on bacterial populations (Martínez, 2008). Antibiotic resistance has been detected in various aquatic environments including rivers, sewage, ocean water and drinking water (Ozgumus *et al.,* 2007). Bacterial pathogens of animal and human origin are becoming increasingly resistant to most frontline antimicrobials, including expanded-spectrum cephalosporins, aminoglycosides, and fluoroquinolones which has severe implications for future treatment and prevention of infectious diseases in animals and humans (Chen, 2004). This study investigated the patterns of antimicrobial resistance among *E. coli* and *V. cholerae* isolates obtained from river and beach water samples.

Low prevalence of resistance was detected among the *E. coli* isolates from the river and beach water samples, to ciprofloxacin, gentamicin, tobramycin, chloramphenicol, nalidixic acid and the cephalosporins. The introduction of enrofloxacin (belonging to the fluoroquinolone class of antibiotics) into poultry production in Europe saw the rapid emergence of resistance to this class of antibiotics which resulted in the restricted use of fluoroquinolones since the 1990s (Park *et al.,* 2003). In addition, fluoroquinolones were introduced into clinical medicine only 20 years ago, making them relatively new antimicrobial agents, and animal populations do not have a long history of exposure to these drugs compared to the history of exposure to other agents, such as penicillin or tetracycline (Alhaj *et al.,* 2007). This could probably explain the low level of resistance of the *E. coli* isolates tested, in this study, against the ciprofloxacin and nalidixic acid. *E. coli* is generally susceptible to narrow-spectrum cephalosporins (Österblad *et al.,* 2000), which was confirmed in this study. The observed high frequencies of resistance to ampicillin, tetracycline, streptomycin, amikacin, cotrimoxazole and augmentin exhibited by the *E. coli* isolates, recovered from river and beach water samples, in this study is alarming. This occurrence of multi-antibiotic resistance is probably due to the extensive use of chemotherapeutic drugs in medicine, veterinary and agricultural practices. It has been documented that *E. coli* isolates that acquired resistance against ciprofloxacin were also resistant to multiple antimicrobial agents (Alam *et al.,* 2006; Karlowsky *et al.,* 2006). The antibiotic resistance frequencies of isolates documented in this study show good corroboration with previous studies that have reported similar resistances to these drugs (Chandran *et al.,* 2008; Khan *et al.,* 2002; Nyberg *et al.,* 2007; Okesola and Oni, 2009; Ram *et al.,* 2009; Sáenz *et al.,* 2001; Sayah *et al.,* 2005). Chandran *et al.* (2008) revealed that more than 95% of *E. coli* isolates retrieved from a tropical estuary in India were multi-antibiotic resistant with 93%, 83%, 80%, 62% and 50% of the isolates resistant to vancomycin, tetracycline, streptomycin, ampicillin and amikacin, respectively. Overall, tetracycline [42% (Umgeni River), 35.65% (beaches), 59.25% (Umdloti River)] and streptomycin [33.84% (Umgeni River), 37.05% (beaches), 15.43% (Umdloti River)] resistance was lower

among the *E. coli* isolates in this study compared to those reported by Chandran and colleagues. Furthermore, overall ampicillin [67.82% (Umgeni River), 60.18% (beaches), 40% (Umdloti River)] and amikacin [25.5% (Umgeni River), 33.88% (beaches), 53.33% (Umdloti River)] resistance reported here were similar to the resistance levels of the *E. coli* isolates recovered from the tropical estuary, to these antibiotics. One possible explanation for the wide variation in ampicillin resistance among *E. coli* isolates from different studies may be due to the composition of bacterial species in different environments and the exchange of R factors. For example, the species composition of a sample has been shown to be influenced by the frequency of faecal input, type and proportion of input, and type of recipient water (Parveen *et al.,* 1997). Alhaj *et al.* (2007) demonstrated high resistances of *E. coli* isolates, recovered from different sources in Malaysia, to tetracycline (81.4%), chloramphenicol (75.7%), gentamicin (74.3%), cefoxitin (44.3%) and ciprofloxacin (24.3%); however in this study, resistance to tetracycline was frequently encountered among the isolates, with a lower proportional resistance detected. Moreover, it is interesting to note that in the present study, chloramphenicol, gentamicin, cefoxitin and ciprofloxacin resistance among the *E. coli* isolates was very low or absent compared to the very high resistances reported by Alhaj and colleagues. Higher resistance levels to ampicillin [67.82% (Umgeni River), 60.18% (beaches)], tetracycline [42% (Umgeni River), 35.65% (beaches), 21.68% (Umdloti River)], amikacin [25.5% (Umgeni River), 33.88% (beaches), 53.33% (Umdloti River)] and chloramphenicol [11.34% (Umgeni River)] was observed among the *E. coli* isolates in this study compared to the resistance levels to ampicillin (47%), tetracycline (12.8%), amikacin (17.9%) and chloramphenicol (2.5%) reported by Ozgumus *et al.* (2007).

V. cholerae isolates recovered from the same water samples exhibited low prevalence of resistance to ciprofloxacin, gentamicin, tobramycin, nalidixic acid and third generation cephalosporins. Chander *et al.* (2009) consistently showed the sensitivity of *V. cholerae* isolates, obtained from Chandigarh, north India to gentamicin and cefotaxime over a period of nine years, as well as, high resistance to cotrimoxazole. Resistance of *V. cholerae* to ciprofloxacin observed by Chander *et al.* (2009) was attributed to extensive and injudicious use of the antibiotic for the empirical treatment of diarrhoeal infections. In contrast, this study showed higher levels of chloramphenicol [74.66% (Umgeni River), 71.38% (beaches), 68.75% (Umdloti River)] and tetracycline resistance [63% (Umgeni River), 30.47% (beaches), 29.18% (Umdloti River)] of the *V. cholerae* isolates compared to the low resistance levels previously reported (Kaistha *et al.*, 2005; Sharma *et al.*, 2007; Taneja *et al.,* 2003). However, there have been reports of tetracycline resistant *V. cholerae* strains responsible for major epidemics of cholera in Latin America, Tanzania, Bangladesh and Zaire (Chander *et al.,* 2009). The resistance profile of *V. cholerae* is known to vary, depending on the local antibiotic over use/abuse at that period of time (Sack *et al.*, 2001). Hence, even a low level of resistance is significant and requires monitoring. The observed erythromycin resistance patterns [78.66% (Umgeni River), 66.05% (beaches), 63.33% (Umdloti River)]

among the *V. cholerae* isolates obtained in this study is similar to that reported by Kumar *et al.* (2009). However, a higher resistance to chloramphenicol, streptomycin [75% (Umgeni River), 84% (beaches), 79.58% (Umdloti River)] and penicillin G [100% (Umgeni River), 96.7% (beaches), 100% (Umdloti River)] and a lower ampicillin [49.68% (Umgeni River), 39.63% (beaches), 60.43% (Umdloti River)] resistance level was observed in the isolates investigated in the current study.

Resistance to antimicrobial agents in bacteria is mediated by several mechanisms, including 1) changes of bacterial cell wall permeability, 2) energy-dependent removal of antimicrobial agents via membrane bound efflux pumps, 3) modification of the site of drug action, and 4) destruction or inactivation of antimicrobial agents (Barbosa and Levy, 2000; Schwarz and Chaslus-Dancla. 2001). Apart from all 96 *E. coli* isolates been resistant to erythromycin (macrolides), trimethoprim (antifolates), fusidic acid (steroids) and clindamycin (lincosamides), the antibiograms of these isolates also revealed high resistances toward the aminoglycosides, tetracyclines and extended spectrum β-lactam class of antibiotics. The *V. cholerae* isolates were most resistant toward the phenicols, aminoglycosides, folate inhibitors, ansamycins, macrolides, antifolates and extended spectrum β-lactam class of antibiotics. The most successful antimicrobial agents are those whose targets are anatomic structures or biosynthetic functions unique to microorganisms (Barbosa and Levy, 2000).

Aminoglycosides inhibit bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosome subunits (Chen and Kaye, 2009). The aminoglycoside-bound bacterial ribosome becomes unavailable for translation of mRNA during protein synthesis thereby causing bactericidal effects (Chen and Kaye, 2009; Le Goffic *et al.,* 1979). The most common mechanism of aminoglycoside resistance is antibiotic inactivation by plasmid- and transposons-encoded modifying enzymes (Shaw *et al.,* 1993). More than 50 modification enzymes have been found so far (Ramón-García *et al.,* 2006; Vakulenko and Mobashery, 2003). Three classes of aminoglycoside-modifying enzymes: acetyltransferases, adenylytransferases and phosphotransferases are encoded by three types of genes, namely, *aac*, *ant* (*aad*) and *aph*, respectively. The aminoglycoside resistance genes (*aac*, *aph*, and *ant* genes) are widely distributed in various genera including *Aeromonas*, *Escherichia*, *Vibrio*, *Salmonella*, and *Listeria spp*. isolated from polluted or natural water environments (Zhang *et al.,* 2009). This might explain the high resistances against the aminoglycosides among the *E. coli* and *V. cholerae* isolates obtained in this study.

Tetracyclines act by inhibiting protein synthesis and enter bacteria by an energy-dependent process and bind reversibly to the 30S ribosomal subunits of the bacteria (Copra *et al.,* 1992). This process blocks the access of aminoacyl-tRNA to the RNA-ribosome complex, preventing bacterial polypeptide synthesis (Copra *et al.,* 1992). Tetracycline resistance is the most common antibiotic resistance encountered in nature. Resistance to this drug is plasmid mediated, with a wide variety of genetic determinants (Chopra and Roberts, 2001). Since tetracycline is a naturally derived compound, bacteria can be exposed to this agent in nature and outside any human use for disease treatment, for

prophylaxis, or for livestock growth promotion. Tetracycline is a commonly used first-line antibiotic for many different species of domestic animals and is often used before the resistance of a pathogen to the antimicrobial agent, is determined (Alekshun and Levy, 2007). Resistance to tetracycline may be conserved in bacterial populations over time, regardless of selection pressure, which might result in an overall increase in resistance (Alhaj *et al.,* 2007). Additionally, most multi-drug resistant *E. coli* isolates in this study were resistant to a combination of antimicrobial agents that included tetracycline, which may suggest that *E. coli* strains that are tetracycline resistant are also at increased risk of becoming resistant to additional antimicrobial agents. This could have resulted from independent and simultaneous development of resistance to different agents or co-selection of resistance determinants (Ram *et al.,* 2009). Other studies have also reported the occurrence of resistance to antimicrobials of the tetracycline class in surface water *E. coli* isolates (Begum *et al.,* 2005; Obi *et al.,* 2004; Qadri *et al.,* 2005; Sayah *et al.,* 2005). To date, at least 38 different tetracycline resistance (*tet*) genes have been characterized (Roberts 2005; Thompson *et al.,* 2007), of which 23 of these genes encode for efflux proteins (efflux pump mechanism), 11 genes for ribosomal protection proteins (target modification mechanism), 3 genes for an inactivating enzyme and 1 gene with an unknown resistance mechanism (Levy *et al.,* 1999; Roberts 2005). Among them, more than 22 *tet* genes have been found in bacterial isolates from water environments (Mackie *et al.,* 2006; Zhang *et al.,* 2009). Agersø and Petersen (2007) have found that the *tetE* gene which encodes for an efflux protein is often located on large plasmids of *Aeromonas spp.* and have proved the gene to be capable of interspecies transfer to *E. coli*.

β-Lactams are the most widely used antibiotics, and resistance to these antibiotics is a cause for serious concern because they have low toxicity and are used to treat a broad range of infections (Livermore, 1996). This class of antibiotics inhibits a number of bacterial enzymes, namely, penicillin-binding proteins (PBPs), which are essential for peptidoglycan synthesis, thereby interfering with synthesis of peptidoglycan of the cell wall (Walsh, 2000; Waxman and Strominger, 1983). Of the various mechanisms of acquired resistance to β-lactam antibiotics, resistance due to production of β-lactamase by the cell is the most prevalent (Bush *et al.,* 1995; Livermore, 1995). β-Lactam antibiotic resistance genes (*bla*) often co-exist with other antimicrobial resistance determinants and can also be associated with mobile genetic elements, increasing the possibility of multi-drug resistance and environmental dissemination (Schlüter *et al.,* 2007; Tennstedt *et al.,* 2003; Weldhagen, 2004). This was evident as all *E. coli* and *V. cholerae* strains that were resistant to the different β-lactam antibiotics tested, was also multi-drug resistant.

Trimethoprim is a pyrimidine analog that inhibits the enzyme dihydrofolate reductase (DHFR), thus interfering with folic acid metabolism, ultimately preventing the synthesis of bacterial DNA (Projan, 2002; Rubin and Swartz, 1980). The most widespread trimethoprim resistance mechanism is the replacement of a trimethoprim-sensitive DHFR by a plasmid-, transposon-, or cassette-borne

trimethoprim-resistant DHFR (Blahna *et al.,* 2006; Sköld, 2001). Resistances to trimethoprim are often encoded by mutations located on highly conserved areas of DHFR genes (*dfr*) (Sköld, 2001) with more than 25 different resistant *dfr* genes been identified (Džidić *et al.,* 2008; Kehrenberg and Schwa 2005). The environmental habitats of these genes include urban wastewater treatment plants (Da Silva *et al.,* 2007), aquaculture systems (Jacobs and Chenia, 2007) and river water (Mohapatra *et al.,* 2008; Mukherjee and Chakraborty, 2006; Park *et al.,* 2003). Ampicillin resistant isolates are often also resistant to other antimicrobials such as trimethoprim and sulfamethoxazole (Kahlmeter and Menday, 2003). The combination of resistance to ampicillin, trimethoprim, tetracycline and chloramphenicol is not uncommon, since the encoding genes are located on the same plasmid (Gulay *et al.,* 2000; Sherley *et al.,* 2004). One (3.23%) *E. coli* isolate obtained from a beach water sample was resistant to ampicillin, trimethoprim, tetracycline and chloramphenicol while five (27.8%), one (2.86%) and three (10.34%) *V. cholerae* isolates obtained from the Umgeni River, beaches and Umdloti River, respectively, were also resistant to these four antibiotics.

Webster *et al.* (2004) demonstrated that *E. coli* isolates from urban areas or point source have resistance to more antibiotics than rural or nonpoint source isolates possibly because of greater exposure to antibiotics. Multi-antimicrobial resistant *E. coli* have been detected in surface water samples of developing and developed countries (Edge and Hill, 2005; Hamelin *et al.,* 2006; Hu *et al.,* 2008; Kaneene *et al.,* 2007; Qadri *et al.,* 2005; Ram and Shanker, 2005). Furthermore, several researchers have indicated multi-antimicrobial resistance among *E. coli* from groundwater (McKeon *et al.,* 1995), urban and rural water (Kaspar *et al.,* 1990), municipal waste (Parveen *et al.,* 1997), tap and spring water (Ozgumus *et al.,* 2007), river water (Olaniran *et al.,* 2009; Ram *et al.,* 2009; Toroglu *et al.,* 2005), tropical estuary (Chandran *et al.,* 2008) and marine water (Alhaj *et al.,* 2007). Since 1977, outbreaks of cholera caused by resistant *V. cholerae* strains have been reported in Africa, Asia, and America (Binsztein *et al.,* 2004; Falbo *et al.,* 1999; Jenkins, 2000; Rivera *et al.,* 2003; Thompson *et al.,* 2008). The therapeutic and prophylactic treatment of cholera with antibiotics like tetracycline has probably contributed to the sporadic appearance of drug-resistant strains in different geographical areas (Falbo *et al.,* 1999; Taneja *et al.,* 2009). Multiple-antibiotic resistance in *V. cholerae* has been described, frequently upon the acquisition of R plasmids belonging to the conjugative group C (Falbo *et al.,* 1999). Chakraborty *et al.* (2001) suggested that the drug resistance patterns of clinical and environmental *V. cholerae* strains show remarkable differences, with clinical strains being resistant to more antibiotics and exhibiting multi-drug resistance compared to their environmental counterparts. It was suggested that the presence of plasmids might explain differences in the frequency of resistant strains. The multi-drug resistance patterns of clinical isolates may also suggest that these strains are exposed to high concentrations of antibiotics, therefore resistant mutants are generated and selected. This is a matter of concern as the emergence of decreasing levels of susceptibility among surface water isolates of *E. coli* and *V. cholerae* to a wide

spectrum of antimicrobial agents, raises fears for availability of limited antimicrobial agents for use in clinical management of future water-borne outbreaks (Ram *et al.*, 2009).

In summary, results from this study identified a spectrum of multi-drug resistances among environmental *E. coli* and *V. cholerae* isolates obtained from two rivers and six different beaches located in Durban, South Africa. The high level of multi-antibiotic resistances demonstrated by these isolates is evidenced by the number of antibiotics and antibiotic classes these isolates were resistant to. Upon antibiotic use, resistant bacteria may rapidly appear in the environment and persist, even when the selecting antibiotic is absent (Levy and Marshall, 2004). Additionally, horizontally transferrable plasmids may carry resistance genes that are often linked with genes specifying resistance to other antimicrobials or toxic substances (Levy and Marshall, 2004). These findings confirm the need to institute continuous monitoring of the changing trends in antimicrobial resistance patterns, as the emergence of such resistance amongst *E. coli* and *V. cholerae* may significantly influence the control strategies in future disease outbreaks. This is especially true as the rate of discovery and development of new antibiotics is continually declining while the corresponding development and spread of resistance is occurring at a rapid pace (Pruden *et al.*, 2006). Strong provincial commitment to surveillance and preparedness for outbreaks should be maintained and timely information should be given to the health authorities as well as to the public. In conclusion, the current study highlights the necessity for continuous monitoring of antibiotic resistance in diarrhoeal-related bacterial pathogens.

4.1 Introduction

Microorganisms numerically and biochemically dominate all surface water habitats with some representing key players in biogeochemical processes that are crucial for the functioning of entire ecosystems (Øvreås, 2000). Owing to the rapidly increasing importance of sustainable management of surface water resources, detailed knowledge on the diversity, specific functions and ecology of microorganisms inhabiting these ecosystems is needed (Hahn, 2006). Therefore, the ability to characterize and differentiate microbial communities of surface water resources in a single fingerprint might not only facilitate the mitigation of microbial pollution, but can also address the impact of environmental perturbations (i.e. pollution runoff, agricultural practices and land use changes) and seasonal, spatial and geographic variation on pathogen indicators (Esseili *et al.*, 2008; Wei *et al.*, 2008).

In the past, detection and analysis of environmental microorganisms were based on culture-dependent methods, however the vast majority (> 99%) of bacterial cells from aquatic systems is not culturable and this hampered the investigation of the biodiversity and taxonomic structure of bacteria until culture-independent methods were developed (Hahn, 2006). Also, due to the intrinsic selectivity of different cultivation techniques, the growth of specific members is enhanced, decreased or even inhibited (Schwieger and Tebbe, 1998). Thus, culturing techniques grossly underestimate the actual *in situ* diversity (species richness and abundance) of natural environments as cultured microorganisms represent only a small fraction of natural microbial communities. With advancing technologies for analyzing environmental samples, it is now possible to circumvent the challenge in studying non-culturable environmental bacteria (Ercolini, 2004; Hahn, 2006). Several nucleic-acid-based methods are now available to investigate the community structure of bacteria (Kemp and Aller, 2004; Weinbauer *et al.*, 2002). These new methods, despite their individual limitations, collectively reveal the power of modern tools to demonstrate changes, both temporarily and spatially, in microbial communities (Øvreås, 2000).

Evaluation of changes in the microbial community structure or diversity has now become easier with the introduction of culture-independent techniques. The shortage of knowledge regarding the natural conditions and habitats under which most bacteria proliferate in and the difficulty in developing artificial media that resemble these natural conditions, has resulted in the widespread use of these culture-independent techniques. Over the last decade, powerful molecular tools using 16S rDNA-based approaches have been developed to elucidate bacterial diversity of complex microbial assemblages residing in various aquatic environments. Today, genetic fingerprinting is considered an appropriate tool for rapid and comparative analysis of natural communities (Lyautey *et al.*, 2005). One such approach, denaturing gradient gel electrophoresis (DGGE) of 16S rDNA genes, allows rapid qualitative analysis of the phylogenetic structure of bacterial communities in aquatic systems, associated with environmental perturbations or seasonal, spatial and geographical variability (Hollibaugh *et al.*, 2000; Konopka *et al.*, 1999; Lebaron *et al.*, 1999; Lindström, 1998, 2000; Riemann *et al.*, 2000; Riemann and Winding, 2001; Zwisler *et al.*, 2003). This powerful technique is attractive as it allows for simultaneous analysis of

multiple samples. Consequently, increased appreciation of microbial diversity and dynamics in environmental settings has been realized (Scanlan *et al.,* 2006). DGGE is an electrophoretic separation procedure based on sequence differences that result in differential denaturing (melting) profiles of double-stranded DNA fragments (PCR products) (Fisher and Lermann, 1979; Muyzer and Smalla, 1998). During the electrophoresis, the PCR products encounter increasingly higher concentrations of the denaturant (urea and formamide) as they migrate through a polyacrylamide gel (Fisher and Lermann, 1979). Upon reaching a threshold denaturant concentration, the double-stranded PCR product will begin to partially melt/denature in discrete regions called melting domains at which time migration slows dramatically (Fisher and Lermann, 1979). The melting temperature (*Tm*) of these domains is sequence-specific and once the *Tm* of the lowest melting domain is reached, that part of the fragment becomes partially melted, creating branched ‘‘breaking’’ molecules (Myers *et al.,* 1985). This behaviour reduces the DNA mobility in the polyacrylamide gel. Differing sequences of DNA will show a different response to the denaturing gradient resulting in a pattern of bands (Muyzer *et al.,* 1997), with each band theoretically representing a different bacterial population present in the community (Ercolini, 2004). Once generated, fingerprints can be uploaded into databases in which fingerprint similarity can be assessed to determine microbial structural differences between environments (Ercolini, 2004). The most recent applications of this technique revealed stable seasonal as well as pronounced temporal and spatial variations of bacterial communities from soil (Avrahami *et al.,* 2003; Nicol *et al.,* 2003; Norris *et al.,* 2002), seawater (Bano and Hollibaugh, 2002), river water (Lyautey *et al.,* 2003; Sekiguchi *et al.,* 2002; Selje and Simon, 2003), ground water (Watanabe *et al.,* 2001) and lake waters (Crump *et al.,* 2003; Wei *et al.,* 2008; Zwart *et al.,* 2002; Zwisler *et al.,* 2003); gastrointestinal tract (Zoetendal *et al.,* 2002); wastewater treatment bioreactors (Gray *et al.,* 2002; Stamper *et al.,* 2003); insects (Reeson *et al.,* 2003); and clinical samples (Burton *et al.,* 2003; Donskey *et al.,* 2003; Mc Bain *et al.,* 2003).

The composition of microbial communities, in any environment, differ both qualitatively and quantitatively (Wintzingerode *et al.,* 1997). Thus, the real composition and dynamics of bacterial communities in surface waters are far from being assessed and understood in detail (Hahn, 2006). Also, few studies have determined how these communities are affected by the seasonal fluctuations in physical and chemical parameters that typically occur in rivers and beaches. This chapter thus focused on the use of the PCR-DGGE method to evaluate seasonal changes in the bacterial community of the Umgeni and Umdloti Rivers and six beaches in Durban, South Africa.

4.2 Materials and methods

4.2.1 Sample collection

Water samples were collected as described in Section 2.2.2 of Chapter two.

4.2.2 Extraction of total DNA from water samples

Water samples (1 L to 1.5 L) were filtered through 0.2 µm pore size Supor membrane disc filters (Pall, 47 mm) within 24 hrs of sample collection and filters were stored at –70°C until DNA isolation was required. Bacterial cells were recovered from the filters as a cell suspension by placing the filters in 20 ml of phosphate buffered saline (PBS) (Appendix A) and vigorously shaken. The filters were also incubated overnight in the PBS solution at 4°C. Following incubation, the filters were aseptically removed and the cell-PBS suspension was centrifuged at 10 000 rpm for 15 – 20 min at 4°C to harvest the bacterial cells. The bacterial cell pellet was washed and resuspended in 200 µl of PBS and thereafter used for total DNA extraction using the ZR Fungal/Bacterial DNA kit (ZymoResearch), according to the manufacturer's instructions. DNA quality and quantity was measured using the NanoDrop 1000 Spectrophotometer (Thermo Scientific) and DNA extracts stored at –70°C. In addition, the extracted DNA was visualized on a 0.8% (w/v) agarose (SeaKem) gel in 1 × TAE running buffer (Appendix A) with an applied voltage of 90 V for 90 min (BioRad electrophoresis system). After electrophoresis, the gel was stained using ethidium bromide (1 µg/ml) (Sigma) and visualized by UV transillumination (Chemi-Genius^2 BioImaging System, Syngene).

4.2.3 PCR amplification of 16S rDNA region

The total DNA extracted from the water samples was subsequently used as template for the amplification of the 16S rDNA region (1324 bp) using the 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-3') universal bacterial primer set (Marchesi *et al.*, 1998). Primer stocks (100 µM) (Inqaba Biotech) were prepared by adding an appropriate amount of sterile double distilled water to the lyophilized oligo pellets while working primer solutions were prepared to a final concentration of 10 µM and stored at –20°C. Each amplification reaction mixture (25 µl) consisted of 10 × PCR buffer, a mixture containing 40 µM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 0.4 µM of each primer, 1 mM $MgCl_2$, 0.5 U of SuperTherm *Taq* DNA polymerase (Southern Cross Biotech), template DNA (~10 ng) and sterile double distilled water brought up to volume. PCR was performed using the PE Applied Biosystems GeneAmp PCR System 9700 (Perkin-Elmer) and was programmed to implement an initial denaturation at 95°C for 5 min followed by 30 cycles of annealing and extension at 95°C for 1 min, 55°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 5 min. The amplicons were analyzed (2 µl gel loading buffer:8 µl PCR amplicons) by electrophoresis on 1% (w/v) agarose gels in 1 × TAE running buffer with an applied voltage of 90 V for 90 min. After electrophoresis, the gel was stained using ethidium bromide (1 µg/ml) and visualized by UV

transillumination. Molecular weight marker, O'GeneRuler 100bp DNA Ladder mix (Fermentas) was used to determine amplicon size. The correct product size (1324 bp) of the PCR amplification of the 16S rDNA region was obtained for all water samples.

4.2.4 PCR amplification of V3 to V5 region

The V3 to V5 region of bacterial 16S rDNA fragments (585 bp) were amplified using the conventional eubacterial primer pair 341F-GC (5'-CCTACGGGAGGCAGCAG-3') consisting of a 40 bp GC clamp (CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG) attached to its 5' end and 907R (5'-CCGTCAATTCMTTTGAGTTT-3') (Muyzer *et al.,* 1997). The GC-clamp prevents complete separation of the strands during DGGE (Muyzer *et al*., 1993). Primer stocks (Inqaba Biotech) and working primer solutions were prepared as described in Section 4.2.3. Each amplification reaction mixture (50 µl) consisted of 10 × PCR buffer, a mixture containing 200 µM of each deoxynucleoside triphosphate (dNTP) (Fermentas), 0.5 µM of each primer, 1 mM $MgCl_2$, 0.5 U of SuperTherm *Taq* DNA polymerase, template DNA (10 – 50 ng) and sterile double distilled water brought up to volume. A touchdown thermal profile was implemented as follows: initial denaturation at 94°C for 5 min, 10 cycles of annealing at 94°C for 1 min, 65°C for 1 min (decreasing by 1°C every cycle), 72°C for 3 min, 20 cycles of extension at 94°C for 1 min, 55°C for 1 min, 72°C for 3 min and a final extension at 72°C for 5 min. The amplicons were analyzed (2 µl gel loading buffer:10 µl PCR amplicons) by electrophoresis on 2% (w/v) agarose gels in 1 × TAE running buffer with an applied voltage of 90 V for 120 min. After electrophoresis, the gel was stained using ethidium bromide (1 µg/ml) and visualized by UV transillumination. Molecular weight marker, O'GeneRuler 100bp DNA Ladder mix (Fermentas) was used to determine amplicon size.

4.2.5 Denaturing Gradient Gel Electrophoresis (DGGE)

PCR amplicons were separated by DGGE using the D-Code Universal Mutation Detection System (BioRad) (Muyzer *et al.,* 1997). Firstly, 0% and 100% denaturing solutions for 6% acrylamide gels were prepared in calibrated 100 ml volumetric flasks, filtered through 0.45 µm pore size GN-6 Metricel membrane filters (Pall, 47 mm) and stored in brown bottles at 4°C. The 0% denaturing solution was prepared as follows: 15 ml of 40% acrylamide/bisacrylamide (BioRad), 2 ml of 50 × TAE buffer (20 mM Tris-acetate, pH 7.4, 10 mM sodium acetate, 0.5 mM Na-EDTA) (BioRad) and 83 ml of sterile double distilled water. The 100% denaturing solution was prepared in the same manner as the 0% denaturing solution; however 40 ml of 40% (v/v) deionized formamide (BioRad) and 42 g of 7 M urea crystals (BioRad) was added and the solution was brought up to the 100 ml mark with sterile double distilled water. DGGE gels were cast by preparing 20 ml each of low and high density solutions containing 20 µl TEMED (N,N,N',N' -tetramethylenediamine) (BioRad) and 200 µl of 10% ammonium persulphate (APS) (Promega), for gradient formation. The different gradients used in this study are as

follows: 30% to 60% for the Umgeni River and beach water samples and 40% to 60% for the Umdloti River water samples. The gel sandwich was assembled and the density solutions were then applied to the gradient delivery system to cast the perpendicular 6% acrylamide DGGE gels (dimensions: 200 mm by 200 mm by 1 mm). Once the gels were cast and allowed to polymerize for 2 – 3 hrs, the gel comb was removed and the wells were washed with 1 × TAE buffer to remove residual acrylamide that may prevent sample migration out of the wells. Thereafter, 7 L of running buffer was preheated to 60°C in the buffer chamber after which the core gel assembly was carefully placed in the buffer chamber. Prior to sample loading, a pre-run was performed at a constant voltage of 150 V at 60°C for 30 min to facilitate sample migration out of the wells during the electrophoretic run. Following the pre-run, samples were loaded into the gels (5 µl gel loading buffer:10 – 15 µl PCR-DGGE amplicons) and DGGE was conducted at a constant voltage of 60 V in 1 × TAE buffer at 60°C for 16 – 17 hrs. After electrophoresis, gels were stained with ethidium bromide (0.5 µg/ml) (BioRad) for 30 min, destained in 1 × TAE buffer for a further 20 min and thereafter visualized by UV transillumination.

4.2.6 Analysis of DGGE patterns

Each sample analyzed in this study was run in duplicate, and the data presented in this study are consensus data compiled from all gels of the same sample to further minimize any error variation. The DGGE band profile was analyzed with an image-analyzing system UV transilluminator (Chemi-Genius^2 BioImaging System, Syngene) for fragment detection and migration of the bands.

4.3 Results

4.3.1 Bacterial community profiles of Umgeni River samples

The DGGE profiles of the bacterial communities of the five sampling stations along the Umgeni River (A1 – A5), during a seasonal cycle, are illustrated in Fig. 4.1. Few bands were observed in the profiles with slight variations in the patterns occurring between the seasons. The bacterial community of the Umgeni River gradually and continuously changed from upstream to downstream and between the seasons. Generally, the bands were dispersed across the entire gradient. With regard to bacterial richness, defined as the number of DGGE bands, a total of 87 different bands were detected in the twenty water samples collected from the Umgeni River over the four seasons, with the number of bands varying from two to nine for the individual samples. Two bands (designated as 1 and 2) were obvious in all five samples collected from the river during summer and winter, though the intensity of the bands differed among the samples. Other bands showed different distribution patterns as some were present in one or several sampling positions while others were present in one or several seasons. Dominant bands observed during the summer and winter seasons were distributed near the bottom of the gel while a widespread diversity pattern was observed during the spring and autumn seasons. One bright band (designated as 3) observed in the Umgeni River mouth sample (A1) during spring disappeared during the summer season. This was accompanied by the appearance of two other bands (red arrows) in the same sample during summer.

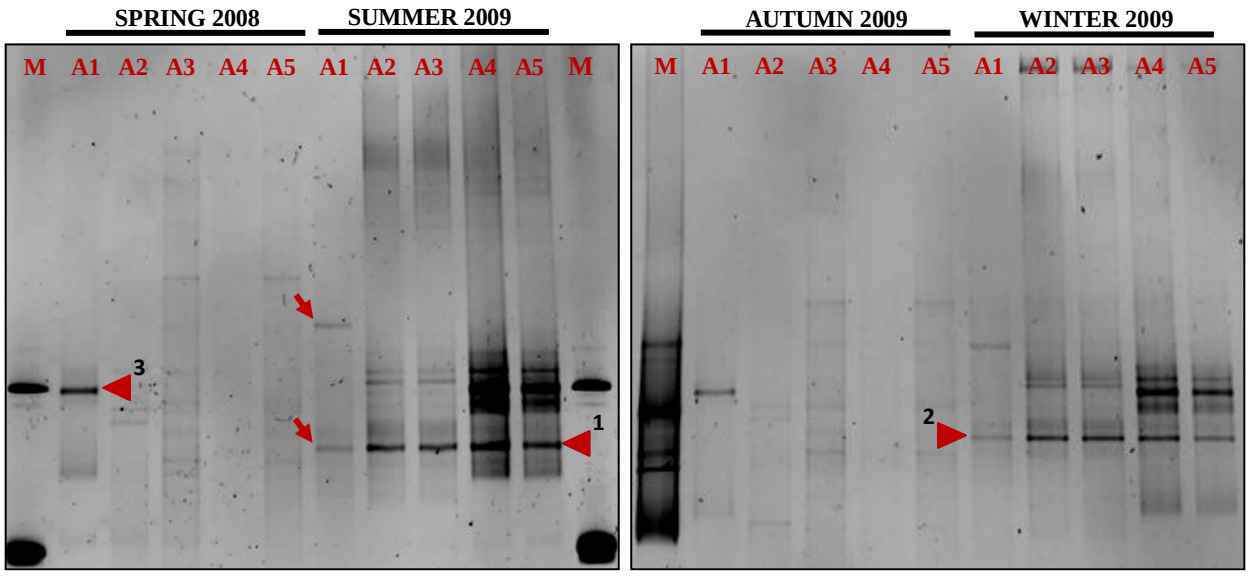


Fig. 4.1: Inverted DGGE band profiles of PCR amplified 16S rDNA gene fragments from samples obtained along the Umgeni River during the different seasons. The DGGE gels were loaded according to sampling time while lane numbers correspond to sampling position along the river. M, marker; A1, Umgeni River mouth; A2, Reservoir Hills; A3, New Germany; A4, KrantzKloof Nature Reserve; A5, Inanda dam. The position(s) of the dominant bands are indicated by red arrows (◄) while individual bands are designated by numbers. Bands of interest are indicated by red arrows (➔).

This banding pattern was once again seen over the autumn and winter seasons. The water sample collected from sampling point A4, during spring and autumn, revealed very low diversity evidenced by the few and decreased intensity of bands compared to the other sampling stations along the river. In contrast, the diversity observed in the water sample collected from sampling point A3 (New Germany) was indicated by eight bands during spring, six bands during summer and autumn and nine bands during winter. This suggests that there was a greater seasonal variation of band patterns in the A3 sample than that observed in the other samples. In contrast to the other positions, a little seasonal variation was observed at sampling points A1 and A2.

4.3.2 Bacterial community profiles of beach water samples

The DGGE profiles of the bacterial communities of six sampling stations representing six different beaches (B1 – B6), during a seasonal cycle, are illustrated in Figs. 4.2 and 4.3. The bacterial diversity of the six beaches was indicated by a few bands that were distributed near the bottom end of the gels. A total of 58 different bands were detected in the twelve water samples collected from Virginia Aerodome beach (B1), Beachwood beach (B2) and Umgeni South beach (B3) over the seasonal periods. A further 69 different bands were detected in the twelve water samples collected from Battery beach (B4), Sunkist beach (B5) and Addington beach (B6) over the four seasons. For the individual samples, the number of bands varied from two to ten. Four prominent bands (designated as 4 to 7) with varying intensities were detected in the beach water samples, B1 to B3 during the different seasons (Fig. 4.2). Two bands (designated as 8 and 9) observed in the lanes belonging to the sampling stations, B2 and B3 were located at the top end of the DGGE gel and were not detected in the water samples collected from the other four beaches. Both bands were detected during spring but disappeared during summer and autumn and thereafter reappeared during winter. Furthermore, pronounced seasonal variations was observed in the banding patterns of the beaches (B1 – B3) where three dominant bands (designated as 4) detected during spring had disappeared during summer and autumn and reappeared during winter (Fig. 4.2). However, the intensity of the band (designated as 4) detected in the beach water sample, B1 was very weak in comparison to the same band detected in the water samples, B2 and B3.

Five bands (designated as 10 to 14) were detected in the water samples collected from the beaches, B4 to B6, during summer and autumn (Fig. 4.3). The other bands that were detected were dispersed differently, illustrating seasonal variations in the banding patterns. Two light bands (designated as 15) was initially detected during spring in water sample, B4 and was found to be absent during the summer and autumn seasons and was again detected during winter.

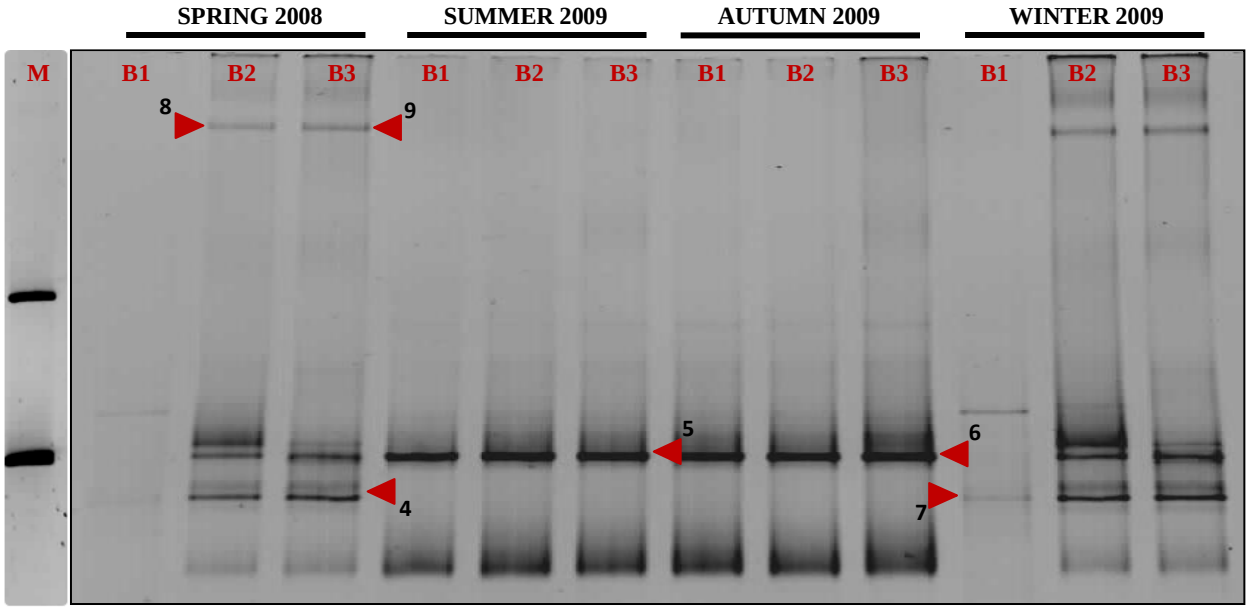


Fig. 4.2: Inverted DGGE band profiles of PCR amplified 16S rDNA gene fragments from three beaches during the different seasons. The DGGE gel was loaded according to sampling time while lane numbers correspond to sampling position or a particular beach. M, marker; B1, Virginia Aerodome beach; B2, Beachwood beach; B3, Umgeni South beach. The position(s) of the dominant bands are indicated by red arrows (◄) while individual bands are designated by numbers.

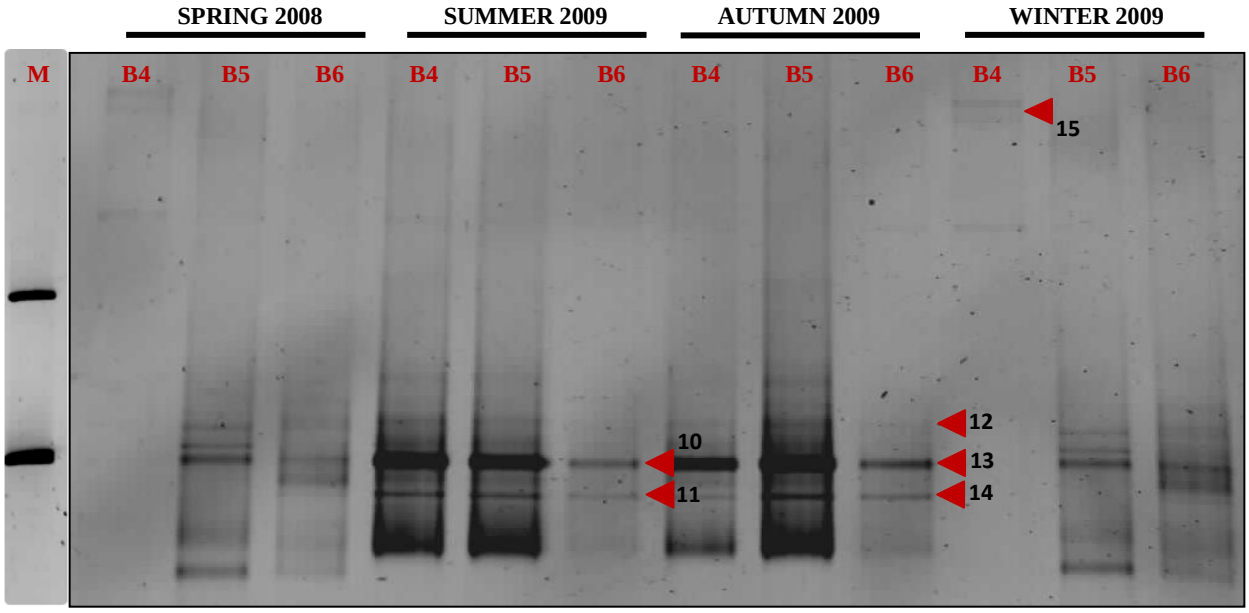


Fig. 4.3: Inverted DGGE band profiles of PCR amplified 16S rDNA gene fragments from three beaches during the different seasons. The DGGE gel was loaded according to sampling time while lane numbers correspond to sampling position or a particular beach. M, marker; B4, Battery beach; B5, Sunkist beach; B6, Addington beach. The position(s) of the dominant bands are indicated by red arrows (◄) while individual bands are designated by numbers.

4.3.3 Bacterial community profiles of Umdloti River samples

The DGGE profiles of the bacterial communities of the four sampling stations along the Umdloti River (C1 – C4), during a seasonal cycle, are illustrated in Fig. 4.4. The banding patterns exhibited pronounced differences between the sampling stations along the Umdloti River, as well as, from season to season. Many DGGE bands were observed in the profiles with a total of 107 bands been detected in the sixteen water samples collected over the seasonal cycle. Furthermore, ten bands (designated as 16 to 25) common to the four water samples (C1 – C4) were detected during particular seasons. Five (designated as 21 to 25) of these ten bands were detected in the water samples collected during winter which suggests stable bacterial community composition from upstream to downstream during the season. Surprisingly, diversity of the bacterial microflora was higher during spring and winter compared to summer and autumn as the number of bands decreased from spring to summer and autumn followed by an increase in the number of bands from autumn to the winter season. With respect to season, the lowest diversities occurred during summer for sampling points, C1 and C2 and during autumn for sampling points, C3 and C4. The highest diversities for point C1 occurred in winter (14 DGGE bands), C2 in spring (8 DGGE bands), C3 and C4 in summer (11 DGGE bands for each position). Interestingly, the dominant band (designated as 16) detected among the spring samples had the highest intensity in the water sample collected from the Hazelmere dam. This observation was also evident among the winter samples (designated as 22). Majority of bands were common in the spring samples (C1 to C3) whereas the summer samples (C3 and C4) exhibited similar band profiles.

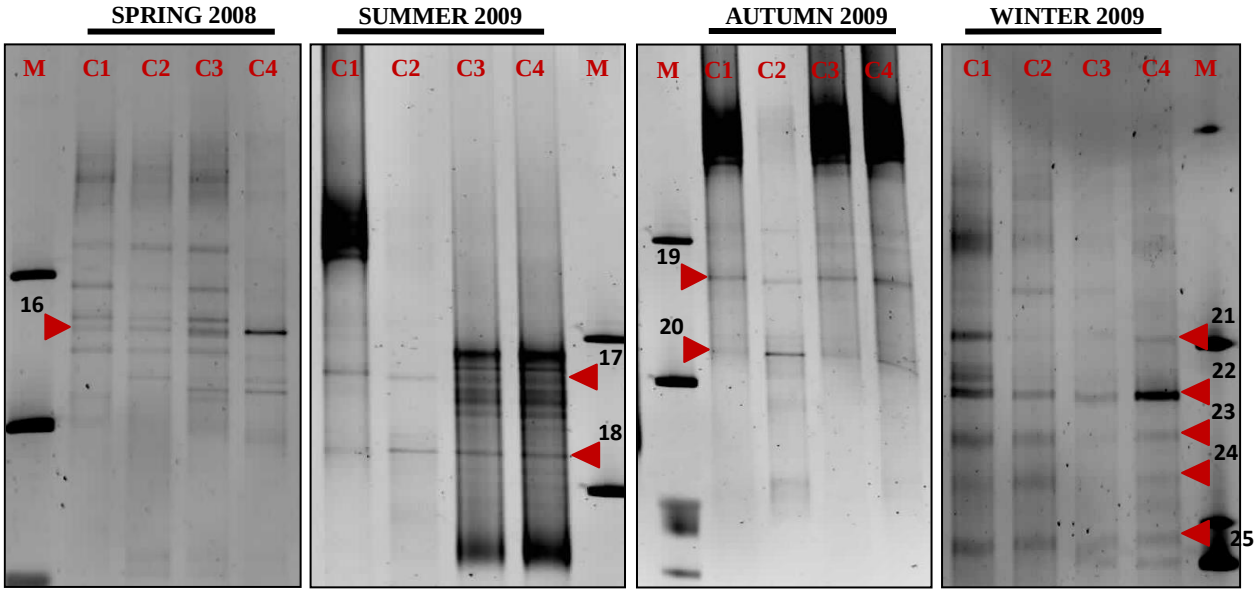


Fig. 4.4: Inverted DGGE band profiles of PCR amplified 16S rDNA gene fragments from samples obtained along the Umdloti River during the different seasons. The DGGE gels were loaded according to sampling time while lane numbers correspond to sampling position along the river. M, marker; C1, Umdloti River mouth; C2, Verulam; C3, New Glasgow; C4, Hazelmere dam. The position(s) of the dominant bands are indicated by red arrows (◄) while individual bands are designated by numbers.

4.4 Discussion

The use of 16S rRNA gene as a molecular marker is now an established method for determining phylogenetic relationships and for analyzing ecosystems mainly based on cloning of 16S rDNA fragments amplified directly from extracted nucleic acids (Eichler *et al.,* 2006; Lui and Stahl, 2002; Schwieger and Tebbe, 1998; Servais *et al.,* 1995; Zwart *et al.,* 2002). While the cloning and sequencing strategies are rather labour and cost intensive, the development of fingerprinting techniques, such as Denaturing Gradient Gel Electrophoresis (DGGE), Single Strand Conformation Polymorphism (SSCP) or T-RFLP of PCR-amplified 16S rDNA have the potential needed to analyze large numbers of samples. The fundamental principle of these fingerprinting methods is that differences in banding patterns result from differences in microbial species that comprise the community. The amplicons may be separated based on sequence-specific melting behaviour of amplicons by DGGE (Madigan *et al.,* 2003) or Temperature Gradient Gel Electrophoresis (TGGE) (Muyzer and Smalla, 1998). DGGE has been applied to characterize bacterial communities from many habitats such as soil (Stephen *et al.,* 1998), marine (Diéz *et al.,* 2001), polluted river (Cébron *et al.,* 2004), hot spring (Aminin *et al.,* 2008) and microbes in gas industry pipelines (Zhu *et al.,* 2003), thus revealing the adequacy of the DGGE technique to follow temporal and spatial dynamics of various environmental microbial assemblages. It is important to note that heteroduplex formation and the co-migration of bands containing DNA of different sequences but similar melting behavior can lead to misinterpretations of community structure (Esseili *et al.,* 2008). Therefore, interpretation of DGGE fingerprints should be approached with caution.

The study of bacterial diversity in aquatic habitats has strongly advanced with the introduction of molecular techniques with the choice of method dependent on the number of samples to be processed within a reasonable period of time. To the best of our knowledge, this is the first study to examine the bacterial diversity of two large rivers and six beaches in Durban, South Africa, over a seasonal cycle using the DGGE technique. A seasonal cycle provides a template for the temporal dynamics of the community, since samples from the same season are expected to have a more similar fingerprint than samples that are distant in time (Sánchez *et al.,* 2007). The present study suggests that the bacterial assemblages detected in the water samples collected from the rivers and beaches in Durban, followed seasonal dynamics as previous studies have indicated that shifts in bacterial community compositions are, to a great extent, the consequence of fluctuations in certain environmental factors, such as pH, water temperature, water chemistry, water retention time, metazooplankton and protistan predation, phytoplankton composition, intensity of ultraviolet radiation, nutrient conditions, and regional differences (Hahn, 2006; Lindström *et al.,* 2005; Lindström and Leskinen, 2002; Muylaert *et al.,* 2002; Tian *et al.,* 2009). The differences in banding patterns, either to a greater or lesser degree, between samples from a common resource or between seasons was evident and this could have been a result of varying physico-chemical parameters recorded at each sampling point and season during the study period. It is typically expected that changes in bacterial communities are related to changes in water quality, such as changes in

nutrient concentrations, pH and temperature (Sekiguchi *et al.*, 2002). These water quality parameters depend on the inflow from surrounding tributaries, surface runoff and precipitation. However, changes in the physico-chemical parameters along the rivers were not appreciable to result in drastic changes in bacterial community composition. Furthermore, the buffering action of the large amount of water flowing into these rivers, as well as the rapid flow, is not conducive to allowing changes in bacterial composition. This may also explain the simple bacterial community profiles produced from the samples collected from the rivers.

The detection of some dominant bands in all samples suggests that these bacterial taxa might survive at all the different positions in their different physico-chemical environments, in all four seasons. The bright band (designated as 3) (Fig. 4.1) detected in the Umgeni River mouth sample (A1) during spring was not detected in the other water samples during the same season. Furthermore, the banding patterns detected in all five samples collected from the Umgeni River during summer and winter indicates that the communities in the different water samples showed seasonal variations and may have formed stable and somewhat predictable summer and winter communities. This observation correlates with the findings of Crump and Hobbie (2005). Crump and Hobbie (2005) demonstrated the similarity between bacterial communities in two neighbouring, temperate rivers and also observed a shift in community composition due to changes in temperature and river flow rate, suggesting that diversity is directly or indirectly influenced by complex seasonal shifts in environmental conditions. An increase in diversity was observed among the beaches, from spring into summer; however little change in the fingerprints was detected in the samples during summer and autumn, indicating stability of the bacterial community composition over several months (~6 months). Minimal bacterial microflora was detected in water samples collected from the Virginia Aerodome and Battery beaches as evidenced by the low number and low intensity of bands that are present. Two very bright bands (designated as 10 and 13) (Fig. 4.3) were found to be common in beach water samples (B4 – B6) during summer and autumn.

The number of bands constituting a DGGE fingerprint provides a rough estimate of the number of dominant phylotypes in the sample. Twenty one dominant bands were found among all sampling sites of the Umdloti River, indicating widespread phylotypes, whereas 14 bands were exclusively detected at only one sampling site (C1), potentially indicating indigenous phylotypes. Some bands appeared year-round, whereas some other bands were specific to a particular season. Very diverse bacterial assemblages, such as soils, produce so many bands that, in some cases, can not be discriminated by DGGE (Muyzer *et al.*, 1997). On the other hand, simpler bacterial communities, such as microbial mats, are represented by a few bands (Ferris *et al.*, 1996), while aquatic systems generally present an intermediate number of bacterial bands. In freshwater systems, 15 to 20 bands were detected in a meromictic lake (Øvreås *et al.*, 1997), 10 to 15 bands in a mesotrophic lake (Zwisler *et al.*, 2003), 7 to 22 bands in the Chaohu Lake, China (Wei *et al.*, 2008) and 6 to 15 bands in a boreal forest lake (Lindström, 1998). According to DGGE fingerprints, marine bacterial assemblages are generally dominated by few taxa, although many more are likely to be

present at low abundances, and this agrees with the findings obtained with other techniques (Schauer *et al.,* 2000). In marine systems, 15 to 30 bands were found in two Californian estuaries (Murray *et al.,* 1996), 17 to 35 bands in Catalan coastal waters, Mediterranean Sea (Schauer *et al.,* 2000), 20 to 30 bands in the Weser estuary, Germany (Selje and Simon, 2003), 23 to 32 bands in Antarctic coastal waters (Murray *et al.,* 1998) and around 15 bands in coastal and offshore samples in the Arabian Sea (Riemann *et al.,* 1999). The samples analyzed in this study, presented 2 to 14 bands, which is consistent with previous results for fresh and marine bacterial composition and further confirming that DGGE is a powerful tool to investigate natural microbial assemblages. It also allows many samples to be processed in a less labour-intensive way than by cloning and sequencing.

Molecular biology approaches have been applied to various natural environments to assess spatio-temporal variations in diversity and succession in bacterial communities. In freshwater systems, usually β-*Proteobacteria* dominate, and cells of the *Cytophaga/Flavobacteria* (CF) cluster also comprise high proportions, whereas in marine environments members of the CF cluster, followed by α- and γ-*Proteobacteria*, constitute largest proportions of the detectable bacterial cells. β-*Proteobacteria* appear to be absent in marine environments except in estuarine and coastal areas (Zwisler *et al.,* 2003). In the Ter River, Ŝimek *et al.* (2001) analyzed α- and β-*Proteobacteria*, the *Cytophaga-Flexibacter-Bacteroides* (CFB) group, and high-G+C-content gram-positive bacteria by fluorescence *in situ* hybridization (FISH) and reported that these groups accounted for the major proportion of the community. Dominance of the same bacterial groups has been found in most studies involving bacterial communities in lakes (Glöckner *et al.,* 2000; Hiorns *et al.,* 1997; Urbach *et al.,* 2001). The bacterial community structure in the Changjiang River revealed that the dominant bacterial groups changed from β-*Proteobacteria* and the CFB group upstream to high-G+C- content gram-positive bacteria downstream (Sekiguchi *et al.,* 2002). Zwart and colleagues (2002) identified patterns in bacterial community composition from Parker River (Massachusetts, USA); Lake Soyang (South Korea) and Lake IJssel (The Netherlands) by analyzing the available database of 16S rDNA sequences from freshwater plankton. The study concluded that rivers and lakes have a specific planktonic bacterial community distinct from bacteria in neighboring environments such as soil and sediments and points out that these planktonic bacteria are distributed in diverse freshwater ecosystems around the world.

Therefore, identification of the organisms represented by the dominant bands obtained in this study is required to reveal the dominant bacterial groups in the water samples during the different seasons. Also, the DGGE approach used in this study does not allow for a complete characterization of the bacterial community composition of the investigated water samples. For instance, bands found at the same positions on a gel do not always represent the same taxon. Therefore, differences and similarities in gel patterns may not completely correspond to differences and similarities in nature. However, the technique is capable of assessing changes in bacterial community composition. The observed resultant

seasonal fluctuation in the bacterial diversity along the investigated rivers and the beaches makes it difficult to predict accurately the future structure of the bacterial communities of these water resources.

5.1 The research in perspective

Microbial contamination of inland surface waters and coastal waters, in Southern Africa, is one of the leading challenges facing the water industry and regulatory authorities (DEAT, 2006). Deteriorating water quality due to both anthropogenic and natural influences has restricted the country's capability to provide sufficient water to meet its needs and to ensure environmental sustainability (DWAF, 2007). Despite increasing stresses on water resources in both developed and developing countries, understanding of microbial pollutants in these aquatic environments is fairly scattered. In addition, chemical pollutants that enter surface waters through various pathways may give rise to serious health hazards, especially persistent chemicals as they can pose a significant health hazard even at extremely low concentrations (McMichael *et al.,* 2001; Yassi *et al.,* 2001). Apart from the microbial pollution crisis our surface water resources are facing, the voluminous use of antimicrobial substances has resulted in extensive contamination of these waters (McEwen and Fedorka-Cray 2002). Consequently, the pathogens disseminated into these environments are exposed to large quantities of the antibiotics resulting in accelerated development of antibiotic resistance and proliferation of these resistant pathogens (Begum *et al.,* 2005; Furuya and Lowy, 2006; Hamelin *et al.,* 2006; Hu *et al.,* 2008). The quality of surface waters (both fresh and marine) in Durban has not been adequately investigated, despite the fact that the microbiological qualities of water sources in other South African provinces have been widely reported. Therefore, this study assessed the microbial and physico-chemical quality of two major rivers and six recreational beaches in Durban, KwaZulu-Natal, South Africa.

Firstly, baseline information regarding the microbial and physico-chemical quality of the investigated water resources was generated and the effects of seasonal variability on the water quality indices were assessed and results presented in Chapter two. The physico-chemical characteristics of a surface water resource are influenced by various factors that dominate that water ecosystem. In summary, spatial and seasonal fluctuations of the physical and chemical environmental variables differed significantly ($p < 0.05$) among the water samples. This dynamism of surface water resources may present challenges in monitoring and managing these water bodies. Water temperature, pH, turbidity, biological oxygen demand (BOD_5), chemical oxygen demand (COD) and conductivity were higher during spring and summer with the inorganic water quality parameters reaching peak values during the autumn and winter seasons. The observed trend was especially noted in the river water samples which could be attributed to the evaporation and decreased flow of water from rivers during the dry seasons and subsequent dilution due to heavy precipitation and run-off from the catchment areas during the wet season (Radhika and Gangaderr, 2004). Gross heavy metal contamination of the water resources was evidenced by the repeated non-compliance of the water samples with the South African Water Quality guidelines (DWAF, 1996) across the seasonal cycle. Presumptive total coliform (TC), faecal coliform (FC) and total heterotrophic bacterial (THB) counts varied significantly ($p < 0.05$) in all river and beach water samples during the four seasons. All points along both rivers and the six beaches grossly violated the guidelines

for these indicators, with peak indicator concentrations being recorded during the summer months and lowest during winter. Furthermore, *Vibrio cholerae*, *Salmonella spp.* and *Shigella spp.* were detected in all water samples on all collection occasions. Sampling points A1 (Umgeni River mouth), A2 (Reservoir Hills), B2 (Beachwood beach), B4 (Battery beach), B6 (Addington beach), C1 (Umdloti River mouth) and C2 (Verulam) were the most contaminated with regard to microbial pollution. These indicator levels can have serious consequences for disease treatment while potential for outbreaks of water-borne diseases within these local communities is also imminent. The information generated in this study can assist local authorities to gain further insight into the state of rivers and beaches located within these municipalities. Furthermore, the information can be utilized to revise the established microbiological and physico-chemical water quality assessment procedures thereby assisting water resource managers to restore these impaired water resources.

The antibiotic resistance patterns and multi-drug resistant patterns of *Escherichia coli* and *Vibrio cholerae* strains recovered from the river and beach water samples were established. Rivers and ultimately beaches have become the main receptacles for antibiotic-resistant bacteria and antibiotics being discharged in various amounts into these environments (Begum *et al.,* 2007; Ram *et al.,* 2009). Consequently, pressure is placed on the success of medical treatment, which is further compounded by the emergence of multi-drug resistant bacterial strains (Heuer *et al.,* 2002; Okesola and Oni, 2009). As presented in Chapter three, all 96 *E. coli* isolates were resistant to rifampicin, penicillin G, erythromycin, trimethoprim, fusidic acid and clindamycin. High prevalence of resistance to ampicillin was encountered among the *E. coli* isolates from the Umgeni River, followed by tetracycline, streptomycin, amikacin, cotrimoxazole and augmentin while the majority of isolates were susceptible to cefoxitin, cefotaxime, ceftriaxone, gentamicin and tobramycin. In contrast, low prevalence of resistance against chloramphenicol, nalidixic acid and cefuroxime was observed among these isolates. The *E. coli* isolates obtained from the different beaches demonstrated high resistance frequencies against tetracycline, ampicillin, streptomycin and amikacin. In addition, these strains were susceptible to ciprofloxacin, cefoxitin, cefotaxime and ceftriaxone while majority were sensitive to chloramphenicol, gentamicin and tobramycin. Similar susceptibility patterns were observed among the *E. coli* isolates obtained from the Umdloti River, with all isolates being sensitive to chloramphenicol, ciprofloxacin, cefoxitin, cefotaxime, ceftriaxone, gentamicin and tobramycin with predominant resistance demonstrated against augmentin, ampicillin, amikacin and cotrimoxazole. Multi-drug resistance among the *E. coli* isolates was clearly indicated by the twenty nine- (Umgeni River), twenty six- (beaches) and fourteen (Umdloti River) different resistance patterns ranging from six-drug patterns to eleven-drug patterns. Additionally, the antibiotic resistance patterns revealed that majority of the Umgeni River *E. coli* strains were resistant to six antibiotic classes while majority of the isolates recovered from the beach water samples were resistant to eight antibiotic classes and almost half of the Umdloti River *E. coli* isolates were resistant to seven antibiotic classes. Interestingly, one *E. coli* isolate, recovered from the Umgeni River, was resistant to

eleven antibiotic classes. In general, all *V. cholerae* isolates retrieved from the Umgeni River were consistently sensitive to ciprofloxacin and ceftriaxone while all isolates were resistant to penicillin G. The most frequently encountered form of resistance in all Umgeni River samples was resistance to cotrimoxazole, followed by resistance to erythromycin, trimethoprim, streptomycin, augmentin, rifampicin, tetracycline, cefoxitin, ampicillin, cefuroxime and mupirocin. Moreover, low prevalence of resistance to tobramycin, amikacin, nalidixic acid, cefotaxime and gentamicin was observed among these isolates. High sensitivity levels against ciprofloxacin and ceftriaxone was observed among the *V. cholerae* isolates obtained from the beach water samples while extensive antibiotic resistance was exhibited by these strains against trimethoprim, streptomycin, cotrimoxazole, chloramphenicol, erythromycin, rifampicin, cefoxitin and amikacin. In contrast, lower antibiotic resistance levels were detected against gentamicin, augmentin, tetracycline, ampicillin, cefuroxime, cefotaxime and nalidixic acid. Overall, the most frequently encountered form of resistance among the *V. cholerae* isolates obtained from the Umdloti River samples, was resistance to cotrimoxazole, followed by streptomycin, trimethoprim, chloramphenicol, mupirocin, erythromycin, ampicillin, cefoxitin, rifampicin, cefuroxime, amikacin and cefotaxime. Multi-drug resistance was also evident among the *V. cholerae* isolates as eighteen- (Umgeni River), thirty five- (beaches) and twenty nine (Umdloti River) different resistance patterns were produced. Proportional resistance of the total *V. cholerae* isolates to the different classes of antibiotics ranged from four to eleven different antibiotic classes. The antibiotic resistance frequencies of the *E. coli* and *V. cholerae* isolates documented in this study show good corroboration with previous studies (Alhaj *et al.,* 2007; Chander *et al.,* 2009; Chandran *et al.,* 2008; Khan *et al.,* 2002; Nyberg *et al.,* 2007; Okesola and Oni, 2009; Ozgumus *et al.,* 2007; Ram *et al.,* 2009; Sáenz *et al.,* 2001; Sayah *et al.,* 2005) while numerous reports on the detection of multi-antimicrobial resistant *E. coli* in surface water samples (Edge and Hill, 2005; Hamelin *et al.,* 2006; Hu *et al.,* 2008; Kaneene *et al.,* 2007; Qadri *et al.,* 2005; Ram and Shanker, 2005), have been published. The high level of multi-antibiotic resistances demonstrated by these environmental isolates is evidenced by the number of antibiotics and antibiotic classes these isolates were resistant to. This study reiterates the need to continuously monitor the changing trends in antimicrobial resistance patterns of diarrhoeal-related bacterial pathogens such as *E. coli* and *V. cholerae*, to fast track antimicrobial drug discovery and develop strategies to overcome bacterial resistance which can greatly influence control strategies for future outbreaks.

Finally, Chapter four concentrated on analyzing the seasonal changes in the bacterial community composition of the water samples, over the collection period, using denaturing gradient gel electrophoresis (DGGE). This study suggested that the bacterial composition detected in the river and beach water samples, were influenced by seasonal variations. This finding corroborated with previous studies that have indicated that shifts in bacterial community compositions are, to a great extent, the consequence of fluctuations in environmental factors (Lindström *et al.,* 2005; Muylaert *et al.,* 2002). The various dominant bands that were detected in all water samples suggest that these bacterial taxa might

have survived in their different physico-chemical environments, in all four seasons. The bacterial diversity fingerprint produced from the water samples collected from the Umgeni River gradually changed from upstream to downstream and showed no abrupt changes in banding patterns between the seasons. A total of 127 different DGGE bands were detected in the twenty four water samples collected from the six beaches over the seasonal period. An increase in diversity was observed among the beaches, from spring into summer; however little change in the fingerprints was detected in the samples during summer and autumn, indicating stability of the bacterial community composition over several months (~6 months). Bacterial diversity increased, among the water samples collected from the Umdloti River, from upstream to downstream during spring and winter; however during summer and autumn greater diversity was observed in the water samples collected from sampling points located upstream of the river. The work presented in this study has shown that the fingerprinting technique, DGGE is a powerful tool to investigate natural microbial assemblages; however the current knowledge of the diversity and function of microorganisms in fresh- and marine water habitats is insufficient for the sustainable management of freshwater resources.

Overall, the present study successfully demonstrated the poor microbiological quality of the investigated surface water resources which raise concerns over the management of these water resources and the subsequent deleterious effects these environments have on the end users. In addition, the high level of resistance to antimicrobial agents and multi-drug resistance was extremely common. Therefore, continued surveillance of these surface waters used for recreational or domestic purposes and the development of adequate prevention strategies to diminish the spread of multi-resistant bacteria are needed for public health reasons.

5.2 Potential for future development of the work

The current study reinforced the contention that no one bacterial indicator organism or simple physical/chemical characteristic can be used to monitor water quality or predict contamination. Therefore, the development of alternative faecal indicators to replace or combine with the current conventional ones requires additional investigation. It has become more evident that combining the use of conventional faecal indicators with molecular-based techniques can successfully lead to more conclusive results of microbial and, in particular, faecal contamination of water.

As mentioned earlier, surface water resources that harbour pathogenic *E. coli* and *V. cholerae* strains pose a serious risk to public health, as users are exposed to water-borne pathogens. These pathogens are able to cause disease as they carry virulence genes that encode specific factors (adhesins, invasins, haemolysins, toxins, effacement factors, cytotoxic necrotic factors and capsules) necessary for causing disease. It is therefore important to comprehensively determine the virulence gene signatures of the environmental isolates obtained in this study, for their proper classification into pathogenic groups. Future studies should also be geared toward the complete characterization of the *E. coli* and *V. cholerae*

strains isolated during the study, in order to establish the individual phenotypic profiles (biotypes) among the strains. Quantitative assessment of the strains to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of antibiotics currently used to treat diseases caused by these pathogens should also be established. Also, the multi-drug resistant strains collected during this study should be screened for antibiotic resistance genes and elucidate the antibiotic resistance mechanisms in these isolates. In addition, the expression of these antibiotic resistance genes should also be investigated to ascertain the potential danger inherent with the use of water contaminated with these bacterial pathogens. In addition, molecular characterization of the strains to determine the genetic diversity among the strains should be established. Lastly, it will be important to identify the organisms represented by the dominant bands on the DGGE gels, in order to properly understand the different bacterial phylotypes present. This would enhance the understanding of the microbial diversity in these surface water resources over the seasonal period.

Composition of media, test reagents and buffers used in this study

1. Media

• *Bile aesculin agar* (Biolab)	62.5	g/L
• *Eosin methylene blue agar* (Fluka BioChemika)	37.5	g/L
• *Kligler iron agar* (Oxoid)	55	g/L
• *mEndo agar* (Biolab)	51	g/L
• *m-FC agar* (Biolab)	50	g/L
• *Motility test medium* (Difco)	22	g/L
• *MRVP test medium* (Biolab)	17	g/L
• *Müeller Hinton agar* (Biolab)	38	g/L
• *Nutrient agar* (Biolab)	31	g/L
• *Nutrient broth* (Biolab)	16	g/L
• *Nutrient gelatinase test medium* (Oxoid)	128	g/L
• *Simmon's citrate agar* (Oxoid)	23	g/L
• *S-S agar* (Oxoid)	63	g/L
• *Thiosulphate citrate bile salts sucrose agar* (Biolab)	90	g/L
• *Triple sugar iron agar* (Biolab)	63	g/L
• *Trypticase soy broth* (Biolab)	20	g/L
• *Tryptone broth* (Biolab)	10	g/L

- *Alkaline peptone water*

Peptone powder (Biolab)	1	g
Sodium chloride (NaCl) (Saarchem)	1	g
Water (pH 8.6)	100	ml

- *Arginine dihydrolase test medium*

Yeast Extract (Biolab)	5	g
Bacto-Tryptone powder (Biolab)	10	g
Sodium chloride	10	g
L-Arginine (Merck)	10	g
Phenol red (Merck)	0.01	g
Water	1000	ml

- *Autoagglutination test medium*

Yeast Extract	5	g
Bacto-Tryptone powder	10	g
Sodium chloride	20	g
Water	1000	ml

- *Carbohydrate utilization test medium*

Purple broth base (Difco)	16	g
Mannitol (Saarchem)	10	g
Arabinose (Saarchem)	10	g
Water	1000	ml

- *Esculin hydrolysis test medium*

Heart infusion agar (Difco)	40	g
Esculin hydrate (Sigma)	1	g
Ferric chloride ($FeCl_3$) (Saarchem)	0.5	g
Water	1000	ml

- *Luria-Bertani agar*

Yeast Extract	5	g
Bacto-Tryptone powder	10	g
Sodium chloride	10	g
Bacteriological agar (Biolab)	12	g
Water	1000	ml

- *Membrane enterococcus agar*

Tryptose (Biolab)	20	g
Yeast Extract	5	g
Glucose (Saarchem)	2	g
Dipotassium hydrogen phosphate (K_2HPO_4) (Merck)	4	g
Sodium azide (NaN_3) (Sigma-Aldrich)	0.4	g
2,3,5 Triphenyl tetrazolium chloride (TTC) (Merck)	0.1	g
Bacteriological agar	12	g
Water	1000	ml

- *Oxolinic acid aesculin azide agar*

Kanamycin aesculin azide agar base (Oxoid)	42.6	g
Sodium azide (Sigma)	0.25	g
Water	1000	ml
Oxolinic acid solution	0.5	ml
Oxolinic acid (Sigma-Aldrich)	1	g
0.1 N Sodium hydroxide solution (NaOH)	50	ml
Distilled water	50	ml
Filter sterilize solution before addition to test medium		

- *Sodium ion requirement test medium*

Nutrient broth (Biolab)	16	g
Sodium chloride (0%)	0	g
Sodium chloride (6%)	60	g
Sodium chloride (8%)	80	g
Water	1000	ml

- *Urease production test medium*

Christensen's urea agar (Fluka)	21	g
Water	950	ml
Urea solution	50	ml
Urea crystals (Saarchem)	20	g
Distilled water	50	ml
Filter sterilize solution before addition to test medium		

2. Test reagents

- *IMViC test reagents*

Kovac's reagent

Isoamyl alcohol (Merck)	150	ml
ρ-dimethylaminobenzaldehyde (Merck)	10	g
Hydrochloric acid (HCl)	50	ml

Methyl red indicator

Methyl red (Merck)	0.05	g
95% Ethanol	150	ml

Barritt's A solution

Potassium hydroxide (KOH) (Saarchem)	40	g
Water	100	ml

Barritt's B solution

Alpha-naphthol (Merck)	5	g
Absolute ethanol	100	ml

3. Buffers

- *0.5 M Disodium ethylenediaminetetraacetate (EDTA)*

EDTA (Saarchem)	186.12	g
Double distilled water (bring up)	1000	ml
pH adjustment (sodium hydroxide pellets ~20 g)	pH 8	

- *1 M Tris-hydroxymethyl-aminomethane (Tris)*

Tris base (Roche)	121.14	g
Double distilled water (bring up)	1000	ml
pH adjustment (hydrochloric acid ~42 ml)	pH 8	

- *Tris-EDTA buffer (TE)*

0.5 M EDTA stock solution	2	ml
1 M Tris stock solution	10	ml
Double distilled water (bring up)	1000	ml

- *50 X Tris-acetate EDTA buffer (TAE)*

Tris base	242	g
Glacial acetic acid (Merck)	57.1	ml
0.5 M EDTA (pH 8)	100	ml
Double distilled water (bring up)	1000	ml
pH adjustment (sodium hydroxide pellets/glacial acetic acid)	pH 8	

- *Phosphate buffered saline (PBS)*

Sodium chloride	8	g
Potassium chloride (KCl) (Saarchem)	0.2	g
Disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) (Saarchem)	3.58	g
Potassium dihydrogen phosphate (KH_2PO_4) (Saarchem)	0.24	g
Double distilled water (bring up)	1000	ml
pH adjustment (hydrochloric acid)	pH 7.4	

- *Ethidium bromide stain (EtBr)*

Ethidium bromide (Sigma)	50	µl
Double distilled water	500	ml

- *Primer stocks (16S rDNA region) (Inqaba Biotec)*

Double distilled water added to 63F primer	408.73	µl
Double distilled water added to 1387R primer	250.81	µl
Final concentration	100	µM

- *Primer stocks (V3 – V5 region) (Inqaba Biotec)*

Double distilled water added to 341F-GC primer	280.36	µl
Double distilled water added to 907R primer	343.99	µl
Final concentration	100	µM

- *Denaturing solution (0%)*

40% Acrylamide/bisacrylamide (BioRad)	15	ml
50 × TAE buffer (pH 8) (BioRad)	2	ml
Double distilled water	83	ml

- *Denaturing solution (100%)*

40% Acrylamide/bisacrylamide	15	ml
50 × TAE buffer (pH 8)	2	ml
40% (v/v) Deionized formamide (BioRad)	40	ml
7 M Urea (BioRad)	42	g
Double distilled water (bring up)	100	ml

- *Ammonium persulphate (APS)*

APS (10%) (Promega)	0.05	g
Double distilled water	500	µl

Numerical data and statistical analysis

Table B1: Presumptive bacterial indicator counts for water samples along the Umgeni River during the spring season.

Indicator	A1		A2		A3		A4		A5	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count										
10^{-4}	367	7.34×10^{4}	164	3.28×10^{4}	136	2.72×10^{4}	102	2.04×10^{4}	75	1.50×10^{4}
	341	6.82×10^{4}	161	3.22×10^{4}	128	2.56×10^{4}	95	1.90×10^{4}	70	1.40×10^{4}
10^{-5}	125	2.50×10^{5}	28	5.60×10^{4}	36	7.20×10^{4}	8	1.60×10^{4}	8	1.60×10^{4}
	139	2.78×10^{5}	15	3.00×10^{4}	21	4.20×10^{4}	8	1.60×10^{4}	9	1.80×10^{4}
10^{-6}	89	1.78×10^{6}	1	2.00×10^{4}	1	2.00×10^{4}	1	2.00×10^{4}	1	2.00×10^{4}
	91	1.82×10^{6}	1	2.00×10^{4}	2	4.00×10^{4}	0	0	0	0
10^{-7}	28	5.60×10^{6}	0	0	0	0	0	0	0	0
	25	5.00×10^{6}	0	0	0	0	0	0	0	0
Average (x 10^{4})		**7.08**		**3.775**		**2.82**		**1.785**		**1.575**
SD		**0.367696**		**1.222607**		**0.845064**		**0.221133**		**0.170783**
Total Coliforms										
10^{-1}	150	3.00×10^{1}	282	5.64×10^{1}	183	3.66×10^{1}	151	3.02×10^{1}	192	3.84×10^{1}
	125	2.50×10^{1}	285	5.70×10^{1}	162	3.24×10^{1}	127	2.54×10^{1}	175	3.50×10^{1}
10^{-2}	26	5.20×10^{1}	43	8.60×10^{1}	9	1.80×10^{1}	31	6.20×10^{1}	10	2.00×10^{1}
	13	2.60×10^{1}	21	4.20×10^{1}	9	1.80×10^{1}	17	3.40×10^{1}	8	1.60×10^{1}
10^{-3}	4	8.00×10^{1}	2	4.00×10^{1}	0	0	1	2.00×10^{1}	1	2.00×10^{1}
	3	6.00×10^{1}	1	2.00×10^{1}	0	0	1	2.00×10^{1}	0	0
Average (x 10^{1})		**3.325**		**4.885**		**2.625**		**2.74**		**2.835**
SD		**1.268529**		**0.910439**		**0.967936**		**0.60597**		**0.974115**
Faecal Coliforms										
10^{-1}	135	2.70×10^{1}	122	2.44×10^{1}	95	1.90×10^{1}	51	1.02×10^{1}	77	1.54×10^{1}
	130	2.60×10^{1}	146	2.92×10^{1}	116	2.32×10^{1}	46	9.20×10^{0}	65	1.30×10^{1}
10^{-2}	3	6.00×10^{0}	23	4.60×10^{1}	5	1.00×10^{1}	6	1.20×10^{1}	12	2.40×10^{1}
	9	1.80×10^{1}	21	4.20×10^{1}	9	1.80×10^{1}	7	1.40×10^{1}	8	1.60×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	0	0	0	0	0	0
	0	0	1	2.00×10^{1}	0	0	0	0	1	2.00×10^{1}
Average (x 10^{1})		**2.275**		**2.84**		**1.755**		**1.135**		**1.71**
SD		**0.442531**		**0.859767**		**0.551453**		**0.211266**		**0.477912**
Enterococci										
10^{-1}	2	4.00×10^{-1}	10	2.00×10^{0}	0	0	21	4.20×10^{0}	9	1.80×10^{0}
	3	6.00×10^{-1}	11	2.20×10^{0}	0	0	17	3.40×10^{0}	9	1.80×10^{0}
10^{-2}	0	0	1	2.00×10^{0}	0	0	2	4.00×10^{0}	0	0
	0	0	1	2.00×10^{0}	0	0	2	4.00×10^{0}	1	2.00×10^{0}
Average		**0.5**		**2.05**		**-**		**3.9**		**1.867**
SD		**0.141421**		**0.1**		**-**		**0.34641**		**0.11547**

Table B1/cont.

Faecal Streptococci										
10^{-1}	12	2.40×10^{0}	33	6.60×10^{0}	11	2.20×10^{0}	21	4.20×10^{0}	14	2.80×10^{0}
	10	2.00×10^{0}	48	9.60×10^{0}	11	2.20×10^{0}	18	3.60×10^{0}	15	3.00×10^{0}
10^{-2}	1	2.00×10^{0}	3	6.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	0	0	5	1.00×10^{1}	1	2.00×10^{0}	0	0	0	0
Average		**2.133**		**7.4**		**2.133333**		**3.267**		**2.6**
SD		**0.23094**		**1.92873**		**0.11547**		**1.137248**		**0.52915**
Vibrio cholerae										
10^{-1}	163	3.26×10^{1}	102	2.04×10^{1}	*	*	177	3.54×10^{1}	*	*
	139	2.78×10^{1}	100	2.00×10^{1}	282	5.64×10^{1}	153	3.06×10^{1}	200	4.00×10^{1}
10^{-2}	32	6.40×10^{1}	15	3.00×10^{1}	34	6.80×10^{1}	30	6.00×10^{1}	27	5.40×10^{1}
	54	1.08×10^{2}	29	5.80×10^{1}	29	5.80×10^{1}	25	5.00×10^{1}	44	8.80×10^{1}
10^{-3}	2	4.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}	5	1.00×10^{2}	3	6.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	4	8.00×10^{1}	3	6.00×10^{1}	3	6.00×10^{1}
Average (x 10^{1})		**3.51**		**2.26**		**5.56**		**4.4**		**5.35**
SD		**0.598777**		**0.493694**		**1.15977**		**1.348481**		**0.943398**
Salmonella spp.										
10^{-1}	61	1.22×10^{1}	47	9.40×10^{0}	16	3.20×10^{0}	29	5.80×10^{0}	33	6.60×10^{0}
	54	1.08×10^{1}	36	7.20×10^{0}	13	2.60×10^{0}	31	6.20×10^{0}	35	7.00×10^{0}
10^{-2}	5	1.00×10^{1}	6	1.20×10^{1}	1	2.00×10^{0}	7	1.40×10^{1}	3	6.00×10^{0}
	9	1.80×10^{1}	3	6.00×10^{0}	1	2.00×10^{0}	4	8.00×10^{0}	3	6.00×10^{0}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	0	0	1	2.00×10^{1}	0	0
	1	2.00×10^{1}	0	0	0	0	0	0	0	0
Average (x 10^{1})		**1.275**		**0.865**		**0.245**		**0.85**		**0.64**
SD		**0.361617**		**0.264008**		**0.057446**		**0.378946**		**0.04899**
Shigella spp.										
10^{-1}	75	1.50×10^{1}	56	1.12×10^{1}	44	8.80×10^{0}	47	9.40×10^{0}	73	1.46×10^{1}
	67	1.34×10^{1}	70	1.40×10^{1}	49	9.80×10^{0}	50	1.00×10^{1}	81	1.62×10^{1}
10^{-2}	8	1.60×10^{1}	9	1.80×10^{1}	4	8.00×10^{0}	6	1.20×10^{1}	8	1.60×10^{1}
	8	1.60×10^{1}	6	1.20×10^{1}	3	6.00×10^{0}	5	1.00×10^{1}	10	2.00×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	0	0	2	4.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	0	0	0	0	0	0
Average (x 10^{1})		**1.51**		**1.38**		**0.815**		**1.035**		**1.67**
SD		**0.122746**		**0.303754**		**0.161142**		**0.113578**		**0.231229**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B2: Presumptive bacterial indicator counts for water samples along the Umgeni River during the summer season.

Indicator	A1		A2		A3		A4		A5	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count										
10^{-4}	247	4.94×10^{4}	179	3.58×10^{4}	206	4.12×10^{4}	146	2.92×10^{4}	111	2.22×10^{4}
	243	4.86×10^{4}	192	3.84×10^{4}	181	3.60×10^{4}	173	3.46×10^{4}	94	1.88×10^{4}
10^{-5}	85	1.70×10^{5}	33	6.60×10^{4}	55	1.10×10^{5}	25	5.00×10^{4}	31	6.20×10^{4}
	66	1.32×10^{5}	45	9.90×10^{4}	56	1.12×10^{5}	21	4.20×10^{4}	17	3.40×10^{4}
10^{-6}	11	2.20×10^{5}	21	4.20×10^{5}	16	3.20×10^{5}	6	1.20×10^{5}	8	1.60×10^{5}
	17	3.40×10^{5}	11	2.20×10^{5}	9	1.80×10^{5}	4	8.00×10^{4}	5	1.00×10^{5}
10^{-7}	2	4.00×10^{5}	8	1.60×10^{6}	1	2.00×10^{5}	0	0	1	2.00×10^{5}
	7	1.40×10^{6}	5	1.00×10^{6}	2	4.00×10^{5}	1	2.00×10^{5}	1	2.00×10^{5}
Average (x 10^{4})		**21.55**		**5.98**		**15.05**		**11.25**		**16.5**
SD		**9.048573**		**2.949034**		**4.634292**		**6.5**		**4.725816**
Total Coliforms										
10^{-1}	222	4.44×10^{1}	*	*	163	3.26×10^{1}	*	*	*	*
	246	4.92×10^{1}	*	*	144	2.88×10^{1}	*	*	*	*
10^{-2}	15	3.00×10^{1}	72	1.44×10^{2}	12	2.40×10^{1}	53	1.06×10^{2}	25	5.00×10^{1}
	26	5.20×10^{1}	59	1.18×10^{2}	18	3.60×10^{1}	31	6.20×10^{1}	35	7.00×10^{1}
10^{-3}	2	4.00×10^{1}	4	8.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}
	2	4.00×10^{1}	3	6.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**4.64**		**8.6**		**3.055**		**6.93333**		**3**
SD		**0.529654**		**2.946184**		**0.515461**		**3.360556**		**1.732051**
Faecal Coliforms										
10^{-1}	165	3.30×10^{1}	170	3.40×10^{1}	156	3.12×10^{1}	135	2.70×10^{1}	121	2.42×10^{1}
	153	3.06×10^{1}	160	3.20×10^{1}	172	3.44×10^{1}	138	2.76×10^{1}	137	2.74×10^{1}
10^{-2}	21	4.20×10^{1}	22	4.40×10^{1}	16	3.20×10^{1}	15	3.00×10^{1}	10	2.00×10^{1}
	18	3.60×10^{1}	19	3.80×10^{1}	19	3.80×10^{1}	15	3.00×10^{1}	11	2.20×10^{1}
10^{3}	2	4.00×10^{1}	3	6.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	4	8.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**3.54**		**3.7**		**3.39**		**2.865**		**2.34**
SD		**0.492341**		**0.52915**		**0.305287**		**0.157797**		**0.31707**
Enterococci										
10^{-1}	25	5.00×10^{0}	27	5.40×10^{0}	8	1.60×10^{0}	13	2.60×10^{0}	6	1.20×10^{0}
	16	3.20×10^{0}	19	3.80×10^{0}	8	1.60×10^{0}	9	1.80×10^{0}	10	2.00×10^{0}
10^{-2}	3	6.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
Average		**4.05**		**4.8**		**1.8**		**2.1**		**1.8**
SD		**1.791647**		**1.070825**		**0.23094**		**0.34641**		**0.4**
Faecal Streptococci										
10^{-1}	30	6.00×10^{0}	13	2.60×10^{0}	22	4.40×10^{0}	12	2.40×10^{0}	8	1.60×10^{0}

Table B2/cont.

	19	3.80×10^{0}	10	2.00×10^{0}	31	6.20×10^{0}	12	2.40×10^{0}	11	2.20×10^{0}
10^{-2}	4	8.00×10^{0}	2	4.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
	7	1.40×10^{1}	1	2.00×10^{0}	5	1.00×10^{1}	1	2.00×10^{0}	1	2.00×10^{0}
Average		**5.933333**		**2.2**		**5.533333**		**2.266667**		**1.933333**
SD		**2.100794**		**0.34641**		**0.986577**		**0.23094**		**0.305505**
Vibrio cholerae										
10^{-1}	240	4.80×10^{1}	177	3.54×10^{1}	236	4.72×10^{1}	*	*	142	2.84×10^{1}
	269	5.38×10^{1}	193	3.86×10^{1}	218	4.36×10^{1}	218	4.36×10^{1}	186	3.72×10^{1}
10^{-2}	59	1.18×10^{2}	53	1.06×10^{2}	44	8.80×10^{1}	60	1.20×10^{2}	27	5.40×10^{1}
	31	6.20×10^{1}	49	9.80×10^{1}	39	7.80×10^{1}	43	8.60×10^{1}	36	7.20×10^{1}
10^{-3}	7	1.40×10^{2}	5	1.00×10^{2}	8	1.60×10^{2}	4	8.00×10^{1}	5	1.00×10^{2}
	4	8.00×10^{1}	6	1.20×10^{2}	10	2.00×10^{2}	4	8.00×10^{1}	2	4.00×10^{1}
Average (x 10^{1})		**6.095**		**10.6**		**6.42**		**7.24**		**5.08**
SD		**1.393832**		**0.993311**		**2.213775**		**1.940722**		**1.592985**
Salmonella spp.										
10^{-1}	109	2.18×10^{1}	37	7.40×10^{0}	93	1.86×10^{1}	52	1.04×10^{1}	115	2.30×10^{1}
	120	2.40×10^{1}	43	8.60×10^{0}	71	1.42×10^{1}	59	1.18×10^{1}	127	2.54×10^{1}
10^{-2}	9	1.80×10^{1}	12	2.40×10^{1}	13	2.60×10^{1}	11	2.20×10^{1}	10	2.00×10^{1}
	8	1.60×10^{1}	7	1.40×10^{1}	9	1.80×10^{1}	7	1.40×10^{1}	2	4.00×10^{0}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	0	0	1	2.00×10^{1}
	0	0	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**1.895**		**2.45**		**1.92**		**1.455**		**2.21**
SD		**0.250533**		**1.112055**		**0.493423**		**0.518298**		**0.261534**
Shigella spp.										
10^{-1}	81	1.62×10^{1}	48	9.60×10^{0}	103	2.06×10^{1}	35	7.00×10^{0}	62	1.24×10^{1}
	90	1.80×10^{1}	44	8.80×10^{0}	87	1.74×10^{1}	41	8.20×10^{0}	50	1.00×10^{1}
10^{-2}	7	1.40×10^{1}	3	6.00×10^{0}	6	1.20×10^{1}	5	1.00×10^{1}	3	6.00×10^{0}
	7	1.40×10^{1}	5	1.00×10^{1}	7	1.40×10^{1}	5	1.00×10^{1}	2	4.00×10^{0}
10^{-3}	1	2.00×10^{1}	0	0	0	0	1	2.00×10^{1}	0	0
	0	0	0	0	1	2.00×10^{1}	0	0	0	0
Average (x 10^{1})		**1.555**		**0.86**		**1.6**		**0.88**		**0.81**
SD		**0.193477**		**0.18037**		**0.379122**		**0.146969**		**0.38**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B3: Presumptive bacterial indicator counts for water samples along the Umgeni River during the autumn season.

Indicator	A1		A2		A3		A4		A5	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count										
10^{-4}	158	3.16×10^{4}	127	2.54×10^{4}	173	3.46×10^{4}	105	2.10×10^{4}	132	2.64×10^{4}
	164	3.28×10^{4}	151	3.02×10^{4}	139	2.78×10^{4}	118	2.36×10^{4}	146	2.92×10^{4}
10^{-5}	29	5.80×10^{4}	33	6.60×10^{4}	15	3.00×10^{4}	17	3.40×10^{4}	15	3.00×10^{4}
	36	7.20×10^{4}	25	5.00×10^{4}	15	3.00×10^{4}	24	4.80×10^{4}	19	3.80×10^{4}
10^{-6}	11	2.20×10^{5}	6	1.20×10^{5}	3	6.00×10^{4}	4	8.00×10^{4}	2	4.00×10^{4}
	9	1.80×10^{5}	5	1.00×10^{5}	2	4.00×10^{4}	3	6.00×10^{4}	7	1.40×10^{5}
10^{-7}	2	4.00×10^{4}	1	2.00×10^{4}	0	0	1	2.00×10^{4}	1	2.00×10^{4}
	0	0	1	2.00×10^{4}	0	0	0	0	1	2.00×10^{4}
Average (x 10^{4})		**4.06**		**2.39**		**3.06**		**2.465**		**3.09**
SD		**1.217867**		**0.491121**		**0.286124**		**0.641535**		**0.497862**
Total Coliforms										
10^{-1}	135	2.70×10^{1}	169	3.38×10^{1}	185	3.70×10^{1}	144	2.88×10^{1}	121	2.42×10^{1}
	117	2.34×10^{1}	182	3.64×10^{1}	173	3.46×10^{1}	128	2.56×10^{1}	130	2.60×10^{1}
10^{-2}	24	4.80×10^{1}	23	4.60×10^{1}	29	5.80×10^{1}	32	6.40×10^{1}	14	2.80×10^{1}
	21	4.20×10^{1}	28	5.60×10^{1}	23	4.60×10^{1}	27	5.40×10^{1}	16	3.20×10^{1}
10^{-3}	5	1.00×10^{2}	10	2.00×10^{2}	9	1.80×10^{2}	2	4.00×10^{1}	3	6.00×10^{1}
	8	1.60×10^{2}	7	1.40×10^{2}	9	1.80×10^{2}	3	6.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**3.51**		**4.305**		**4.39**		**3.71**		**2.755**
SD		**1.178304**		**1.010264**		**1.060377**		**1.284731**		**0.334813**
Faecal Coliforms										
10^{-1}	149	2.98×10^{1}	130	2.60×10^{1}	80	1.60×10^{1}	61	1.22×10^{1}	106	2.12×10^{1}
	135	2.70×10^{1}	129	2.58×10^{1}	98	1.96×10^{1}	72	1.44×10^{1}	127	2.54×10^{1}
10^{-2}	26	5.20×10^{1}	10	2.00×10^{1}	11	2.20×10^{1}	6	1.20×10^{1}	16	3.20×10^{1}
	15	3.00×10^{1}	9	1.80×10^{1}	11	2.20×10^{1}	6	1.20×10^{1}	9	1.80×10^{1}
10^{3}	3	6.00×10^{1}	1	2.00×10^{1}	0	0	2	4.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**2.67**		**1.95**		**1.99**		**1.265**		**2.115**
SD		**0.46719**		**0.1**		**0.283549**		**0.117047**		**0.31257**
Enterococci										
10^{-1}	14	2.80×10^{0}	7	1.40×10^{0}	23	4.60×10^{0}	11	2.20×10^{0}	26	5.20×10^{0}
	12	2.40×10^{0}	10	2.00×10^{0}	17	3.40×10^{0}	10	2.00×10^{0}	22	4.40×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
Average		**2.3**		**1.85**		**3**		**2.05**		**3.9**
SD		**0.382971**		**0.3**		**1.254326**		**0.1**		**1.361372**
Faecal Streptococci										
10^{-1}	24	4.80×10^{0}	9	1.80×10^{0}	18	3.60×10^{0}	8	1.60×10^{0}	14	2.80×10^{0}

Table B3/cont.

	24	4.80×10^0	10	2.00×10^0	23	4.60×10^0	7	1.40×10^0	18	3.60×10^0
10^{-2}	2	4.00×10^0	1	2.00×10^0	3	6.00×10^0	1	2.00×10^0	2	4.00×10^0
	2	4.00×10^0	1	2.00×10^0	2	4.00×10^0	0	0	1	2.00×10^0
Average		**4.4**		**1.95**		**4.55**		**1.6667**		**3.1**
SD		**0.46188**		**0.1**		**1.050397**		**0.305505**		**0.886942**
Vibrio cholerae										
10^{-1}	207	4.14×10^1	183	3.66×10^1	160	3.20×10^1	195	3.90×10^1	110	2.20×10^1
	186	3.72×10^1	158	3.16×10^1	164	3.28×10^1	177	3.54×10^1	129	2.58×10^1
10^{-2}	32	6.40×10^1	24	4.80×10^1	20	4.00×10^1	33	6.60×10^1	16	3.20×10^1
	34	6.80×10^1	30	6.00×10^1	15	3.00×10^1	41	8.20×10^1	25	5.00×10^1
10^{-3}	10	2.00×10^2	5	1.00×10^2	3	6.00×10^1	2	4.00×10^1	2	4.00×10^1
	7	1.40×10^2	2	4.00×10^1	1	2.00×10^1	2	4.00×10^1	1	2.00×10^1
Average (x 10^1)		**5.265**		**3.905**		**3.37**		**3.86**		**2.495**
SD		**1.559605**		**0.689227**		**0.436196**		**0.21848**		**0.527984**
Salmonella spp.										
10^{-1}	75	1.50×10^1	60	1.20×10^1	53	1.06×10^1	57	1.14×10^1	89	1.78×10^1
	68	1.36×10^1	44	8.80×10^0	51	1.02×10^1	70	1.40×10^1	95	1.90×10^1
10^{-2}	18	3.60×10^1	9	1.80×10^1	6	1.20×10^1	13	2.60×10^1	7	1.40×10^1
	12	2.40×10^1	11	2.20×10^1	8	1.60×10^1	15	3.00×10^1	7	1.40×10^1
10^{-3}	1	2.00×10^1	1	2.00×10^1	1	2.00×10^1	2	4.00×10^1	1	2.00×10^1
	2	4.00×10^1	1	2.00×10^1	0	0	2	4.00×10^1	0	0
Average (x 10^1)		**2.172**		**1.616**		**1.376**		**2.428**		**1.696**
SD		**0.898844**		**0.556129**		**0.417469**		**1.177251**		**0.281212**
Shigella spp.										
10^{-1}	38	7.60×10^0	81	1.62×10^1	99	1.98×10^1	51	1.02×10^1	25	5.00×10^0
	40	8.00×10^0	67	1.34×10^1	106	2.12×10^1	48	9.60×10^0	32	6.40×10^0
10^{-2}	3	6.00×10^0	12	2.40×10^1	15	3.00×10^1	13	2.60×10^1	5	1.00×10^1
	2	4.00×10^0	10	2.00×10^1	19	3.80×10^1	9	1.80×10^1	4	8.00×10^0
10^{-3}	0	0	4	8.00×10^1	3	6.00×10^1	1	2.00×10^1	1	2.00×10^1
	0	0	2	4.00×10^1	3	6.00×10^1	2	4.00×10^1	0	0
Average (x 10^1)		**0.64**		**1.84**		**2.725**		**1.595**		**0.735**
SD		**0.181842**		**0.461013**		**0.847014**		**0.771557**		**0.215019**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B4: Presumptive bacterial indicator counts for water samples along the Umgeni River during the winter season.

Indicator	A1		A2		A3		A4		A5	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count										
10^{-4}	113	2.26×10^{4}	121	2.42×10^{4}	134	2.68×10^{4}	91	1.82×10^{4}	103	2.06×10^{4}
	107	2.14×10^{4}	114	2.28×10^{4}	128	2.56×10^{4}	90	1.80×10^{4}	111	2.22×10^{4}
10^{-5}	15	3.00×10^{4}	18	3.60×10^{4}	20	4.00×10^{4}	11	2.20×10^{4}	12	2.40×10^{4}
	10	2.00×10^{4}	16	3.20×10^{4}	17	3.40×10^{4}	8	1.60×10^{4}	16	3.20×10^{4}
10^{-6}	1	2.00×10^{4}	5	1.00×10^{5}	3	6.00×10^{4}	1	2.00×10^{4}	2	4.00×10^{4}
	2	4.00×10^{4}	2	4.00×10^{4}	3	6.00×10^{4}	2	4.00×10^{4}	2	4.00×10^{4}
10^{-7}	0	0	1	2.00×10^{5}	1	2.00×10^{5}	0	0	0	0
	0	0	0	0	1	2.00×10^{5}	0	0	0	0
Average (x 10^{4})		**2.1**		**2.875**		**3.16**		**1.855**		**2.47**
SD		**0.125433**		**0.630423**		**0.671714**		**0.250533**		**0.506096**
Total Coliforms										
10^{-1}	109	2.18×10^{1}	120	2.40×10^{1}	98	1.96×10^{1}	73	1.46×10^{1}	129	2.58×10^{1}
	112	2.24×10^{1}	114	2.28×10^{1}	83	1.66×10^{1}	91	1.82×10^{1}	136	2.72×10^{1}
10^{-2}	7	1.40×10^{1}	9	1.80×10^{1}	10	2.00×10^{1}	12	2.40×10^{1}	10	2.00×10^{1}
	6	1.20×10^{1}	7	1.40×10^{1}	8	1.60×10^{1}	8	1.60×10^{1}	10	2.00×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}
	0	0	1	2.00×10^{1}	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**1.955**		**2.12**		**1.805**		**1.82**		**2.145**
SD		**0.383797**		**0.271293**		**0.204206**		**0.414085**		**0.29**
Faecal Coliforms										
10^{-1}	67	1.34×10^{1}	88	1.76×10^{1}	59	1.18×10^{1}	70	1.40×10^{1}	82	1.64×10^{1}
	81	1.62×10^{1}	81	1.62×10^{1}	55	1.10×10^{1}	54	1.08×10^{1}	75	1.50×10^{1}
10^{-2}	5	1.00×10^{1}	10	2.00×10^{1}	3	6.00×10^{0}	2	4.00×10^{0}	9	1.80×10^{1}
	3	6.00×10^{0}	9	1.80×10^{1}	2	4.00×10^{0}	2	4.00×10^{0}	8	1.60×10^{1}
10^{3}	0	0	1	2.00×10^{1}	0	0	0	0	1	2.00×10^{1}
	0	0	0	0	0	0	0	0	0	0
Average (x 10^{1})		**1.141**		**1.795**		**0.82**		**0.82**		**1.635**
SD		**0.440303**		**0.15695**		**0.379825**		**0.502262**		**0.124766**
Enterococci										
10^{-1}	8	1.40×10^{0}	12	2.40×10^{0}	14	2.80×10^{0}	21	4.20×10^{0}	20	4.00×10^{0}
	10	2.00×10^{0}	13	2.60×10^{0}	11	2.20×10^{0}	16	3.20×10^{0}	17	3.40×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	3	6.00×10^{0}	2	4.00×10^{0}
	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}
Average		**1.80**		**2.333**		**2.333**		**3.80**		**3.80**
SD		**0.34641**		**0.305505**		**0.416333**		**0.52915**		**0.34641**
Faecal Streptococci										
10^{-1}	23	4.60×10^{0}	15	3.00×10^{0}	27	5.40×10^{0}	24	4.80×10^{0}	19	3.80×10^{0}

Table B4/cont.

	20	4.00×10^0	15	3.00×10^0	21	4.20×10^0	20	4.00×10^0	16	3.20×10^0
10^{-2}	2	4.00×10^0	1	2.00×10^0	3	6.00×10^0	1	2.00×10^0	1	2.00×10^0
	1	2.00×10^0	2	4.00×10^0	4	8.00×10^0	2	4.00×10^0	2	4.00×10^0
Average		**4.20**		**2.6667**		**5.20**		**4.26667**		**3.6667**
SD		**0.34641**		**0.57735**		**0.916515**		**0.46188**		**0.416333**
Vibrio cholerae										
10^{-1}	155	3.10×10^1	160	3.20×10^1	117	2.34×10^1	82	1.64×10^1	93	1.86×10^1
	138	2.76×10^1	149	2.98×10^1	110	2.20×10^1	100	2.00×10^1	116	2.32×10^1
10^{-2}	20	4.00×10^1	10	2.00×10^1	12	2.40×10^1	15	3.00×10^1	14	2.80×10^1
	26	5.20×10^1	13	2.60×10^1	18	3.60×10^1	11	2.20×10^1	12	2.40×10^1
10^{-3}	5	1.00×10^2	2	4.00×10^1	2	4.00×10^1	1	2.00×10^1	1	2.00×10^1
	3	6.00×10^1	1	2.00×10^1	2	4.00×10^1	2	4.00×10^1	1	2.00×10^1
Average (x 10^1)		**4.212**		**2.556**		**2.908**		**2.168**		**2.304**
SD		**1.375035**		**0.551072**		**0.829651**		**0.50707**		**0.331783**
Salmonella spp.										
10^{-1}	40	8.00×10^0	29	5.80×10^0	74	1.48×10^1	70	1.40×10^1	49	9.80×10^0
	53	1.06×10^1	38	7.60×10^0	66	1.32×10^1	52	1.04×10^1	42	8.40×10^0
10^{-2}	7	1.40×10^1	4	8.00×10^0	10	2.00×10^1	13	2.60×10^1	8	1.60×10^1
	5	1.00×10^1	2	4.00×10^0	10	2.00×10^1	9	1.80×10^1	6	1.20×10^1
10^{-3}	1	2.00×10^1	1	2.00×10^1	3	6.00×10^1	1	2.00×10^1	1	2.00×10^1
	0	0	0	0	2	4.00×10^1	3	6.00×10^1	1	2.00×10^1
Average (x 10^1)		**1.065**		**0.635**		**1.7**		**1.56**		**1.155**
SD		**0.249466**		**0.183576**		**0.352515**		**0.427083**		**0.331612**
Shigella spp.										
10^{-1}	62	1.24×10^1	46	9.20×10^0	87	1.74×10^1	25	5.00×10^0	51	1.02×10^1
	69	1.38×10^1	60	1.20×10^1	74	1.48×10^1	26	5.20×10^0	53	1.06×10^1
10^{-2}	8	1.60×10^1	7	1.40×10^1	21	4.20×10^1	3	6.00×10^0	7	1.40×10^1
	8	1.60×10^1	10	2.00×10^1	14	2.80×10^1	4	8.00×10^0	6	1.20×10^1
10^{-3}	0	0	1	2.00×10^1	2	4.00×10^1	0	0	0	0
	0	0	1	2.00×10^1	1	2.00×10^1	0	0	1	2.00×10^1
Average (x 10^1)		**1.455**		**1.38**		**2.005**		**0.605**		**1.17**
SD		**0.176918**		**0.457821**		**0.570935**		**0.136991**		**0.171659**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B5: Presumptive bacterial indicator counts for water samples from the six beaches during the spring season.

Indicator	B1		B2		B3		B4		B5		B6	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count												
10^{-2}	111	2.22×10^{2}	98	1.96×10^{2}	95	1.90×10^{2}	59	1.18×10^{2}	141	2.82×10^{2}	167	3.34×10^{2}
	106	2.12×10^{2}	87	1.74×10^{2}	117	2.34×10^{2}	45	9.00×10^{1}	124	2.48×10^{2}	133	2.66×10^{2}
10^{-3}	11	2.20×10^{2}	8	1.60×10^{2}	7	1.40×10^{2}	5	1.00×10^{2}	11	2.20×10^{2}	10	2.00×10^{2}
	15	3.00×10^{2}	5	1.00×10^{2}	9	1.80×10^{2}	7	1.40×10^{2}	12	2.40×10^{2}	14	2.80×10^{2}
10^{-4}	2	4.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	0	0
	3	6.00×10^{2}	0	0	1	2.00×10^{2}	0	0	1	2.00×10^{2}	1	2.00×10^{2}
Average (x 10^{2})		**2.708**		**1.66**		**1.888**		**1.296**		**2.38**		**2.56**
SD		**0.80568**		**0.403485**		**0.340176**		**0.437127**		**0.308545**		**0.570789**
Total Coliforms												
10^{-0}	130	2.60×10^{0}	144	2.88×10^{0}	172	3.44×10^{0}	163	3.26×10^{0}	139	2.78×10^{0}	161	3.22×10^{0}
	118	2.36×10^{0}	120	2.40×10^{0}	155	3.10×10^{0}	180	3.60×10^{0}	143	2.86×10^{0}	158	3.16×10^{0}
10^{-1}	9	1.80×10^{0}	10	2.00×10^{0}	24	4.80×10^{0}	20	4.00×10^{0}	12	2.40×10^{0}	21	4.20×10^{0}
	12	2.40×10^{0}	16	3.20×10^{0}	19	3.80×10^{0}	22	4.40×10^{0}	16	3.20×10^{0}	18	3.60×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	3	6.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	0	0	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	0	0
Average		**2.272**		**2.496**		**2.868**		**3.852**		**2.648**		**3.236**
SD		**0.264424**		**0.534864**		**0.830133**		**0.435339**		**0.460565**		**0.805531**
Faecal Coliforms												
10^{-0}	43	8.60×10^{-1}	119	2.38×10^{0}	88	1.76×10^{0}	59	1.18×10^{0}	50	1.00×10^{0}	85	1.70×10^{0}
	45	9.00×10^{-1}	94	1.88×10^{0}	70	1.40×10^{0}	66	1.32×10^{0}	38	7.60×10^{-1}	101	2.02×10^{0}
10^{-1}	4	8.00×10^{-1}	13	2.60×10^{0}	11	2.20×10^{0}	6	1.20×10^{0}	5	1.00×10^{0}	10	2.00×10^{0}
	5	1.00×10^{0}	7	1.40×10^{0}	8	1.60×10^{0}	9	1.80×10^{0}	5	1.00×10^{0}	9	1.80×10^{0}
Average		**0.89**		**2.065**		**1.74**		**1.375**		**0.94**		**1.88**
SD		**0.084063**		**0.536004**		**0.340196**		**0.29**		**0.12**		**0.155778**
Enterococci												
10^{-0}	23	4.60×10^{-1}	13	2.60×10^{-1}	49	9.80×10^{-1}	8	1.60×10^{-1}	39	7.80×10^{-1}	52	1.04×10^{0}
	20	4.00×10^{-1}	17	3.40×10^{-1}	66	1.32×10^{0}	8	1.60×10^{-1}	45	9.00×10^{-1}	48	9.60×10^{-1}
10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	9	1.80×10^{0}	1	2.00×10^{-1}	3	6.00×10^{-1}	6	1.20×10^{0}
	0	0	2	4.00×10^{-1}	5	1.00×10^{0}	0	0	3	6.00×10^{-1}	6	1.20×10^{0}
Average		**0.353333**		**0.266667**		**1.373333**		**0.173333**		**0.66**		**1.066667**
SD		**0.136137**		**0.070238**		**0.402658**		**0.023094**		**0.103923**		**0.122202**
Faecal Streptococci												
10^{-0}	13	2.60×10^{-1}	26	5.20×10^{-1}	20	4.00×10^{-1}	9	1.80×10^{-1}	17	3.40×10^{-1}	12	2.40×10^{-1}
	16	3.20×10^{-1}	35	7.00×10^{-1}	21	4.20×10^{-1}	10	2.00×10^{-1}	22	4.40×10^{-1}	13	2.60×10^{-1}
10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	0	0	2	4.00×10^{-1}	1	2.00×10^{-1}
	1	2.00×10^{-1}	3	6.00×10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}
Average		**0.22**		**0.606667**		**0.406667**		**0.193333**		**0.393333**		**0.233333**

Table B5/cont.

SD		**0.034641**		**0.090185**		**0.011547**		**0.011547**		**0.050332**		**0.030551**
Vibrio cholerae												
10^{-0}	160	3.20×10^{0}	73	1.46×10^{0}	180	3.60×10^{0}	158	3.16×10^{0}	88	1.76×10^{0}	61	1.22×10^{0}
	160	3.20×10^{0}	69	1.38×10^{0}	192	3.84×10^{0}	172	3.44×10^{0}	95	1.90×10^{0}	84	1.68×10^{0}
10^{-1}	12	2.40×10^{0}	7	1.40×10^{0}	7	1.40×10^{0}	24	4.80×10^{0}	10	2.00×10^{0}	7	1.40×10^{0}
	15	3.00×10^{0}	9	1.80×10^{0}	11	2.20×10^{0}	40	8.00×10^{0}	10	2.00×10^{0}	11	2.20×10^{0}
10^{-2}	1	2.00×10^{0}	0	0	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
Average		**2.76**		**1.608**		**3.128**		**3.08**		**1.932**		**1.7**
SD		**0.536656**		**0.277345**		**0.951798**		**1.164818**		**0.105451**		**0.406448**
Salmonella spp.												
10^{-0}	59	1.18×10^{0}	56	1.12×10^{0}	13	2.60×10^{-1}	49	9.80×10^{-1}	40	8.00×10^{-1}	90	1.80×10^{0}
	57	1.14×10^{0}	54	1.08×10^{0}	17	3.40×10^{-1}	63	1.26×10^{0}	45	9.00×10^{-1}	100	2.00×10^{0}
10^{-1}	8	1.60×10^{0}	10	2.00×10^{0}	4	8.00×10^{-1}	9	1.80×10^{0}	2	4.00×10^{-1}	11	2.20×10^{0}
	7	1.40×10^{0}	8	1.60×10^{0}	5	1.00×10^{0}	9	1.80×10^{0}	6	1.20×10^{0}	9	1.80×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	0	0	1	2.00×10^{0}	0	0	2	4.00×10^{0}
	0	0	1	2.00×10^{0}	0	0	0	0	0	0	1	2.00×10^{0}
Average		**1.33**		**1.45**		**0.6**		**1.715**		**0.825**		**1.9**
SD		**0.213229**		**0.436196**		**0.357398**		**0.317648**		**0.330404**		**0.11547**
Shigella spp.												
10^{-0}	102	2.04×10^{0}	92	1.84×10^{0}	50	1.00×10^{0}	88	1.76×10^{0}	69	1.38×10^{0}	55	1.10×10^{0}
	87	1.74×10^{0}	71	1.42×10^{0}	69	1.38×10^{0}	115	2.30×10^{0}	97	1.94×10^{0}	44	8.80×10^{-1}
10^{-1}	15	3.00×10^{0}	21	4.20×10^{0}	9	1.80×10^{0}	23	4.60×10^{0}	11	2.20×10^{0}	6	1.20×10^{0}
	13	2.60×10^{0}	16	3.20×10^{0}	10	2.00×10^{0}	21	4.20×10^{0}	19	3.80×10^{0}	6	1.20×10^{0}
10^{-2}	1	2.00×10^{0}	2	2.00×10^{0}	1	2.00×10^{0}	4	8.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	2	4.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	3	6.00×10^{0}
Average		**2.095**		**2.115**		**1.795**		**3.775**		**2.33**		**1.375**
SD		**0.361985**		**0.763566**		**0.29229**		**1.014479**		**1.038011**		**0.419325**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B6: Presumptive bacterial indicator counts for water samples from the six beaches during the summer season.

Indicator	B1		B2		B3		B4		B5		B6	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count												
10^{-2}	144	2.88×10^{2}	129	2.58×10^{2}	151	3.02×10^{2}	233	4.66×10^{2}	182	3.64×10^{2}	227	4.54×10^{2}
	131	2.62×10^{2}	95	1.90×10^{2}	139	2.78×10^{2}	241	4.82×10^{2}	200	4.00×10^{2}	250	5.00×10^{2}
10^{-3}	17	3.40×10^{2}	15	3.00×10^{2}	21	4.20×10^{2}	16	3.20×10^{2}	30	6.00×10^{2}	33	6.60×10^{2}
	16	3.20×10^{2}	11	2.20×10^{2}	18	3.60×10^{2}	29	5.80×10^{2}	24	4.80×10^{2}	27	5.40×10^{2}
10^{-4}	5	1.00×10^{3}	3	6.00×10^{2}	6	1.20×10^{3}	6	1.20×10^{3}	5	1.00×10^{3}	1	2.00×10^{2}
	3	6.00×10^{2}	4	8.00×10^{2}	4	8.00×10^{2}	1	2.00×10^{2}	3	6.00×10^{2}	4	8.00×10^{2}
Average (x 10^{2})		**3.025**		**2.42**		**3.4**		**4.62**		**4.61**		**5.385**
SD		**0.344625**		**0.476375**		**0.63477**		**1.072443**		**1.045817**		**0.882931**
Total Coliforms												
10^{-0}	278	5.56×10^{0}	306	6.12×10^{0}	193	3.86×10^{0}	154	3.08×10^{0}	182	3.64×10^{0}	337	6.74×10^{0}
	293	5.86×10^{0}	284	5.68×10^{0}	216	4.32×10^{0}	166	3.32×10^{0}	219	4.38×10^{0}	309	6.18×10^{0}
10^{-1}	65	1.30×10^{1}	31	6.20×10^{0}	25	5.00×10^{0}	23	4.60×10^{0}	12	2.40×10^{0}	82	1.64×10^{1}
	70	1.40×10^{1}	38	7.60×10^{0}	29	5.80×10^{0}	33	6.60×10^{0}	16	3.20×10^{0}	66	1.32×10^{1}
10^{-2}	2	4.00×10^{0}	6	1.20×10^{1}	3	6.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}	3	6.00×10^{0}
	2	4.00×10^{0}	4	8.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
Average		**4.855**		**6.4**		**4.295**		**3.75**		**2.4**		**5.73**
SD		**0.994837**		**0.832026**		**0.507904**		**0.687701**		**0.565685**		**1.195603**
Faecal Coliforms												
10^{-0}	155	3.10×10^{0}	116	2.32×10^{0}	123	2.46×10^{0}	132	2.64×10^{0}	92	1.84×10^{0}	150	3.00×10^{0}
	149	2.98×10^{0}	137	2.74×10^{0}	125	2.50×10^{0}	95	1.90×10^{0}	81	1.62×10^{0}	137	2.74×10^{0}
10^{-1}	10	2.00×10^{0}	20	4.00×10^{0}	8	1.60×10^{0}	16	3.20×10^{0}	14	2.80×10^{0}	10	2.00×10^{0}
	13	2.60×10^{0}	19	3.80×10^{0}	10	2.00×10^{0}	11	2.20×10^{0}	12	2.40×10^{0}	11	2.20×10^{0}
Average		**2.67**		**3.215**		**2.14**		**2.485**		**2.165**		**2.485**
SD		**0.494907**		**0.813449**		**0.425519**		**0.565302**		**0.535755**		**0.464292**
Enterococci												
10^{-0}	20	4.00×10^{-1}	9	1.80×10^{-1}	26	5.20×10^{-1}	42	8.40×10^{-1}	14	2.80×10^{-1}	35	7.00×10^{-1}
	15	3.00×10^{-1}	11	2.20×10^{-1}	34	6.80×10^{-1}	30	6.00×10^{-1}	14	2.80×10^{-1}	31	6.20×10^{-1}
10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	6	1.20×10^{0}
	3	6.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	4	8.00×10^{-1}	1	2.00×10^{-1}	7	1.40×10^{0}
Average		**0.366667**		**0.206667**		**0.533333**		**0.746667**		**0.253333**		**0.84**
SD		**0.057735**		**0.011547**		**0.140475**		**0.128582**		**0.046188**		**0.314325**
Faecal Streptococci												
10^{-0}	15	3.00×10^{-1}	5	1.00×10^{-1}	8	1.60×10^{-1}	7	1.40×10^{-1}	19	3.80×10^{-1}	10	2.00×10^{-1}
	18	3.60×10^{-1}	9	1.80×10^{-1}	12	2.40×10^{-1}	4	8.00×10^{-1}	11	2.20×10^{-1}	11	2.20×10^{-1}
10^{-1}	7	1.40×10^{0}	1	2.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	4	8.00×10^{-1}	1	2.00×10^{-1}
	2	4.00×10^{-1}	1	2.00×10^{-1}	3	6.00×10^{-1}	1	2.00×10^{-1}	6	1.20×10^{0}	1	2.00×10^{-1}
Average		**0.353333**		**0.16**		**0.2**		**0.18**		**0.466667**		**0.2066667**

Table B6/cont.

SD		**0.050332**		**0.052915**		**0.04**		**0.034641**		**0.299555**		**0.011547**
Vibrio cholerae												
10^{-0}	192	3.84×10^{0}	149	2.98×10^{0}	178	3.56×10^{0}	89	1.78×10^{0}	105	2.10×10^{0}	164	3.28×10^{0}
	222	4.44×10^{0}	126	2.52×10^{0}	152	3.04×10^{0}	71	1.42×10^{0}	110	2.20×10^{0}	206	4.12×10^{0}
10^{-1}	27	5.40×10^{0}	12	2.40×10^{0}	23	4.60×10^{0}	10	2.00×10^{0}	19	3.80×10^{0}	25	5.00×10^{0}
	19	3.80×10^{0}	9	1.80×10^{0}	16	3.20×10^{0}	10	2.00×10^{0}	21	4.20×10^{0}	29	5.80×10^{0}
10^{-2}	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	3	6.00×10^{0}	3	6.00×10^{0}
	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}
Average		**4.016**		**2.34**		**3.68**		**1.84**		**3.26**		**4.44**
SD		**0.253929**		**0.461736**		**0.633088**		**0.253377**		**1.023719**		**0.975295**
Salmonella spp.												
10^{-0}	70	1.40×10^{0}	47	9.40×10^{-1}	62	1.24×10^{0}	111	2.22×10^{0}	111	2.22×10^{0}	103	2.06×10^{0}
	82	1.64×10^{0}	60	1.20×10^{0}	57	1.14×10^{0}	88	1.76×10^{0}	124	2.48×10^{0}	79	1.58×10^{0}
10^{-1}	12	2.40×10^{0}	13	2.60×10^{0}	7	1.40×10^{0}	9	1.80×10^{0}	18	3.60×10^{0}	15	3.00×10^{0}
	9	1.80×10^{0}	18	3.60×10^{0}	8	1.60×10^{0}	14	2.80×10^{0}	25	5.00×10^{0}	12	2.40×10^{0}
10^{-2}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	5	1.00×10^{1}	2	4.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
Average		**1.71**		**1.685**		**1.345**		**1.89**		**3.075**		**2.01**
SD		**0.253772**		**0.758661**		**0.200915**		**0.128062**		**0.859515**		**0.336452**
Shigella spp.												
10^{-0}	134	2.68×10^{0}	83	1.66×10^{0}	74	1.48×10^{0}	95	1.90×10^{0}	118	2.36×10^{0}	54	1.08×10^{0}
	109	2.18×10^{0}	115	2.30×10^{0}	66	1.32×10^{0}	81	1.62×10^{0}	129	2.58×10^{0}	70	1.40×10^{0}
10^{-1}	16	3.20×10^{0}	12	2.40×10^{0}	11	2.20×10^{0}	14	2.80×10^{0}	7	1.40×10^{0}	9	1.80×10^{0}
	19	3.80×10^{0}	12	2.40×10^{0}	13	2.60×10^{0}	10	2.00×10^{0}	10	2.00×10^{0}	8	1.60×10^{0}
10^{-2}	3	6.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	3	6.00×10^{0}	3	6.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	0	0×10^{0}
Average		**2.965**		**2.19**		**1.9**		**2.08**		**2.085**		**1.47**
SD		**0.695198**		**0.356464**		**0.603545**		**0.506228**		**0.515461**		**0.307029**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B7: Presumptive bacterial indicator counts for water samples from the six beaches during the autumn season.

Indicator	B1		B2		B3		B4		B5		B6	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count												
10^{-2}	79	1.58×10^{2}	161	3.22×10^{2}	133	2.60×10^{2}	160	3.20×10^{2}	146	2.92×10^{2}	192	3.84×10^{2}
	101	2.02×10^{2}	132	2.64×10^{2}	117	2.34×10^{2}	170	3.40×10^{2}	150	3.00×10^{2}	175	3.50×10^{2}
10^{-3}	13	2.60×10^{2}	5	1.00×10^{2}	15	3.00×10^{2}	34	6.80×10^{2}	38	7.60×10^{2}	25	5.00×10^{2}
	13	2.60×10^{2}	9	1.80×10^{2}	15	3.00×10^{2}	27	5.40×10^{2}	18	3.60×10^{2}	29	5.80×10^{2}
10^{-4}	1	2.00×10^{2}	1	2.00×10^{2}	2	4.00×10^{2}	2	4.00×10^{2}	1	2.00×10^{2}	9	1.80×10^{3}
	1	2.00×10^{2}	0	0	3	6.00×10^{2}	4	8.00×10^{2}	2	4.00×10^{2}	10	2.00×10^{3}
Average (x 10^{2})		**2.305**		**1.86**		**2.735**		**4**		**2.88**		**4.535**
SD		**0.340735**		**0.676067**		**0.323883**		**0.993311**		**0.660505**		**1.059984**
Total Coliforms												
10^{-0}	125	2.50×10^{0}	146	2.92×10^{0}	130	2.60×10^{0}	76	1.52×10^{0}	150	3.00×10^{0}	99	1.98×10^{0}
	129	2.58×10^{0}	152	3.04×10^{0}	105	2.10×10^{0}	80	1.60×10^{0}	159	3.18×10^{0}	135	2.70×10^{0}
10^{-1}	11	2.20×10^{0}	15	3.00×10^{0}	12	2.40×10^{0}	7	1.40×10^{0}	18	3.60×10^{0}	14	2.80×10^{0}
	13	2.60×10^{0}	20	4.00×10^{0}	9	1.80×10^{0}	10	2.00×10^{0}	20	4.00×10^{0}	19	3.80×10^{0}
10^{-2}	0	0	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	0	0
Average		**2.376**		**3.608**		**2.18**		**1.704**		**3.556**		**2.656**
SD		**0.264348**		**0.536954**		**0.319374**		**0.279428**		**0.460087**		**0.744768**
Faecal Coliforms												
10^{-0}	75	1.50×10^{0}	111	2.22×10^{0}	54	1.08×10^{0}	73	1.46×10^{0}	81	1.62×10^{0}	155	3.10×10^{0}
	73	1.46×10^{0}	119	2.38×10^{0}	60	1.20×10^{0}	77	1.54×10^{0}	96	1.92×10^{0}	169	3.38×10^{0}
10^{-1}	7	1.40×10^{0}	8	1.60×10^{0}	3	6.00×10^{-1}	8	1.60×10^{0}	5	1.00×10^{0}	22	4.40×10^{0}
	10	2.00×10^{0}	6	1.20×10^{0}	2	4.00×10^{-1}	7	1.40×10^{0}	9	1.80×10^{0}	13	2.60×10^{0}
Average		**1.59**		**1.85**		**0.82**		**1.5**		**1.585**		**3.37**
SD		**0.276405**		**0.548574**		**0.381576**		**0.087939**		**0.409023**		**0.758683**
Enterococci												
10^{-0}	13	2.60×10^{-1}	25	5.00×10^{-1}	8	1.60×10^{-1}	21	4.20×10^{-1}	5	1.00×10^{-1}	22	4.40×10^{-1}
	16	3.20×10^{-1}	16	3.20×10^{-1}	12	2.40×10^{-1}	15	3.00×10^{-1}	9	1.80×10^{-1}	13	2.60×10^{-1}
10^{-1}	2	4.00×10^{-1}	4	8.00×10^{-1}	1	2.00×10^{-1}	3	6.00×10^{-1}	2	4.00×10^{-1}	2	4.00×10^{-1}
	1	2.00×10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}
Average		**0.26**		**0.406667**		**0.213333**		**0.373333**		**0.16**		**0.413333**
SD		**0.06**		**0.090185**		**0.023094**		**0.064291**		**0.052915**		**0.023094**
Faecal Streptococci												
10^{-0}	11	2.20×10^{-1}	3	6.00×10^{-2}	20	4.00×10^{-1}	14	2.80×10^{-1}	12	2.40×10^{-1}	6	1.20×10^{-1}
	8	1.60×10^{-1}	4	8.00×10^{-2}	17	3.40×10^{-1}	10	2.00×10^{-1}	7	1.40×10^{-1}	8	1.60×10^{-1}
10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}
	1	2.00×10^{-1}	0	0	1	2.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}	0	0
Average		**0.186667**		**0.113333**		**0.38**		**0.226667**		**0.193333**		**0.16**

Table B7/cont.

SD		**0.023094**		**0.075719**		**0.034641**		**0.046188**		**0.050332**		**0.04**
Vibrio cholerae												
10^{-0}	153	3.06×10^{0}	111	2.22×10^{0}	136	2.72×10^{0}	102	2.04×10^{0}	72	1.44×10^{0}	171	3.42×10^{0}
	162	3.24×10^{0}	85	1.70×10^{0}	105	2.10×10^{0}	83	1.66×10^{0}	80	1.60×10^{0}	150	3.00×10^{0}
10^{-1}	25	5.00×10^{0}	13	2.60×10^{0}	16	3.20×10^{0}	9	1.80×10^{0}	9	1.80×10^{0}	21	4.20×10^{0}
	18	3.60×10^{0}	14	2.80×10^{0}	13	2.60×10^{0}	12	2.40×10^{0}	10	2.00×10^{0}	16	3.20×10^{0}
10^{-2}	2	4.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
	2	4.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	3	6.00×10^{0}
Average		**3.58**		**2.264**		**2.924**		**1.98**		**1.768**		**3.564**
SD		**0.429884**		**0.444387**		**0.717551**		**0.280713**		**0.247225**		**0.516217**
Salmonella spp.												
10^{-0}	44	8.80×10^{-1}	37	7.40×10^{-1}	23	4.60×10^{-1}	63	1.26×10^{0}	34	6.80×10^{-1}	94	1.88×10^{0}
	30	6.00×10^{-1}	52	1.04×10^{0}	41	8.20×10^{-1}	54	1.08×10^{0}	27	5.40×10^{-1}	72	1.44×10^{0}
10^{-1}	7	1.40×10^{0}	6	1.20×10^{0}	10	2.00×10^{0}	6	1.20×10^{0}	11	2.20×10^{0}	12	2.40×10^{0}
	7	1.40×10^{0}	7	1.40×10^{0}	8	1.60×10^{0}	9	1.80×10^{0}	7	1.40×10^{0}	9	1.80×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
Average		**1.256**		**1.276**		**1.684**		**1.468**		**1.364**		**1.904**
SD		**0.540074**		**0.471254**		**0.513108**		**0.405857**		**0.750253**		**0.347102**
Shigella spp.												
10^{-0}	60	1.20×10^{0}	102	2.04×10^{0}	87	1.74×10^{0}	50	1.00×10^{0}	77	1.54×10^{0}	40	8.00×10^{-1}
	69	1.38×10^{0}	96	1.92×10^{0}	65	1.30×10^{0}	42	8.40×10^{-1}	84	1.68×10^{0}	51	1.02×10^{0}
10^{-1}	5	1.00×10^{0}	11	2.20×10^{0}	10	2.00×10^{0}	9	1.80×10^{0}	12	2.40×10^{0}	8	1.60×10^{0}
	8	1.60×10^{0}	12	2.40×10^{0}	13	2.60×10^{0}	9	1.80×10^{0}	10	2.00×10^{0}	8	1.60×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	2	4.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
Average		**1.436**		**2.112**		**2.068**		**1.72**		**1.924**		**1.644**
SD		**0.385331**		**0.190578**		**0.317994**		**0.414729**		**0.333587**		**0.402095**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B8: Presumptive bacterial indicator counts for water samples from the six beaches during the winter season.

Indicator	B1		B2		B3		B4		B5		B6	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count												
10^{-2}	125	2.50×10^{2}	100	2.00×10^{2}	67	1.34×10^{2}	75	1.50×10^{2}	104	2.08×10^{2}	81	1.62×10^{2}
	113	2.26×10^{2}	91	1.82×10^{2}	52	1.04×10^{2}	96	1.92×10^{2}	80	1.60×10^{2}	73	1.46×10^{2}
10^{-3}	23	4.60×10^{2}	13	2.60×10^{2}	10	2.00×10^{2}	10	2.00×10^{2}	22	4.40×10^{2}	16	3.20×10^{2}
	18	3.60×10^{2}	11	2.20×10^{2}	8	1.60×10^{2}	10	2.00×10^{2}	15	3.00×10^{2}	12	2.40×10^{2}
10^{-4}	3	6.00×10^{2}	2	4.00×10^{2}	1	2.00×10^{2}	0	0	3	6.00×10^{2}	2	4.00×10^{2}
	2	4.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	1	2.00×10^{2}	2	4.00×10^{2}
Average (x 10^{2})		**3.392**		**2.124**		**1.596**		**1.884**		**2.616**		**2.536**
SD		**0.99364**		**0.298127**		**0.418665**		**0.21744**		**1.121285**		**1.072325**
Total Coliforms												
10^{-0}	53	1.06×10^{0}	104	2.08×10^{0}	70	1.40×10^{0}	62	1.24×10^{0}	132	2.64×10^{0}	81	1.62×10^{0}
	60	1.20×10^{0}	88	1.76×10^{0}	100	2.00×10^{0}	65	1.30×10^{0}	118	2.36×10^{0}	69	1.38×10^{0}
10^{-1}	8	1.60×10^{0}	12	2.40×10^{0}	14	2.80×10^{0}	7	1.40×10^{0}	7	1.40×10^{0}	10	2.00×10^{0}
	9	1.80×10^{0}	12	2.40×10^{0}	11	2.20×10^{0}	7	1.40×10^{0}	9	1.80×10^{0}	9	1.80×10^{0}
10^{-2}	0	0	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	3	6.00×10^{0}	2	4.00×10^{0}	0	0	1	2.00×10^{0}	1	2.00×10^{0}
Average		**1.532**		**2.128**		**2.08**		**1.468**		**2.04**		**1.76**
SD		**0.396384**		**0.274809**		**0.501996**		**0.305156**		**0.482494**		**0.264953**
Faecal Coliforms												
10^{-0}	52	1.04×10^{0}	68	1.36×10^{0}	36	7.20×10^{-1}	40	8.00×10^{-1}	58	1.16×10^{0}	70	1.40×10^{0}
	46	9.20×10^{-1}	74	1.48×10^{0}	50	1.00×10^{0}	49	9.80×10^{-1}	34	6.80×10^{-1}	78	1.56×10^{0}
10^{-1}	5	1.00×10^{0}	9	1.80×10^{0}	5	1.00×10^{0}	4	8.00×10^{-1}	2	4.00×10^{-1}	7	1.40×10^{0}
	5	1.00×10^{0}	6	1.20×10^{0}	6	1.20×10^{0}	3	6.00×10^{-1}	1	2.00×10^{-1}	4	8.00×10^{-1}
Average		**0.99**		**1.46**		**0.98**		**0.795**		**0.61**		**1.29**
SD		**0.050332**		**0.254034**		**0.197315**		**0.155242**		**0.416173**		**0.335261**
Enterococci												
10^{-0}	20	4.00×10^{-1}	19	3.80×10^{-1}	0	0	7	1.40×10^{-1}	0	0	23	4.60×10^{-1}
	16	3.20×10^{-1}	13	2.60×10^{-1}	0	0	11	2.20×10^{-1}	0	0	20	4.00×10^{-1}
10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	0	0	1	2.00×10^{-1}	0	0	2	4.00×10^{-1}
	1	2.00×10^{-1}	1	2.00×10^{-1}	0	0	1	2.00×10^{-1}	0	0	1	2.00×10^{-1}
Average		**0.33**		**0.26**		**-**		**1.9**		**-**		**0.365**
SD		**0.094516**		**0.084853**		**-**		**0.34641**		**-**		**0.113578**
Faecal Streptococci												
10^{-0}	13	2.60×10^{-1}	10	2.00×10^{-1}	6	1.20×10^{-1}	3	6.00×10^{-2}	9	1.80×10^{-1}	4	8.00×10^{-2}
	8	1.60×10^{-1}	15	3.00×10^{-1}	11	2.20×10^{-1}	7	1.40×10^{-1}	9	1.80×10^{-1}	10	2.00×10^{-1}
10^{-1}	2	4.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	0	0	1	2.00×10^{-1}	1	2.00×10^{-1}
	1	2.00×10^{-1}	3	6.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	1	2.00×10^{-1}	2	4.00×10^{-1}
Average		**0.206667**		**0.233333**		**0.206667**		**0.133333**		**0.186667**		**0.16**

Table B8/cont.

SD		**0.050332**		**0.057735**		**0.011547**		**0.070238**		**0.011547**		**0.069282**
Vibrio cholerae												
10^{-0}	115	2.30×10^{0}	92	1.84×10^{0}	76	1.52×10^{0}	59	1.18×10^{0}	46	9.20×10^{-1}	113	2.26×10^{0}
	132	2.64×10^{0}	68	1.36×10^{0}	100	2.00×10^{0}	67	1.34×10^{0}	55	1.10×10^{0}	80	1.60×10^{0}
10^{-1}	23	4.60×10^{0}	11	2.20×10^{0}	12	2.40×10^{0}	9	1.80×10^{0}	7	1.40×10^{0}	17	3.40×10^{0}
	20	4.00×10^{0}	8	1.60×10^{0}	15	3.00×10^{0}	10	2.00×10^{0}	7	1.40×10^{0}	11	2.20×10^{0}
10^{-2}	3	6.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	0	0	2	4.00×10^{0}
Average		**3.508**		**1.928**		**2.184**		**1.828**		**1.364**		**3.172**
SD		**0.986063**		**0.223428**		**0.552521**		**0.286217**		**0.410463**		**0.894382**
Salmonella spp												
10^{-0}	32	6.40×10^{-1}	29	5.80×10^{-1}	60	1.20×10^{0}	77	1.54×10^{0}	18	3.60×10^{-1}	44	8.80×10^{-1}
	28	5.60×10^{-1}	21	4.20×10^{-1}	38	7.60×10^{-1}	50	1.00×10^{0}	31	6.20×10^{-1}	25	5.00×10^{-1}
10^{-1}	5	1.00×10^{0}	8	1.60×10^{0}	9	1.80×10^{0}	10	2.00×10^{0}	4	8.00×10^{-1}	8	1.60×10^{0}
	3	6.00×10^{-1}	7	1.40×10^{0}	9	1.80×10^{0}	6	1.20×10^{0}	4	8.00×10^{-1}	11	2.20×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	0	0	1	2.00×10^{0}
Average		**0.96**		**1.2**		**1.512**		**1.548**		**0.916**		**1.436**
SD		**0.607289**		**0.676905**		**0.516449**		**0.455544**		**0.632202**		**0.726966**
Shigella spp.												
10^{-0}	46	9.20×10^{-1}	52	1.04×10^{0}	26	5.20×10^{-1}	80	1.60×10^{0}	15	3.00×10^{-1}	50	1.00×10^{0}
	70	1.40×10^{0}	39	7.80×10^{-1}	25	5.00×10^{-1}	66	1.32×10^{0}	29	5.80×10^{-1}	53	1.06×10^{0}
10^{-1}	4	8.00×10^{-1}	9	1.80×10^{0}	3	6.00×10^{-1}	12	2.40×10^{0}	5	1.00×10^{0}	6	1.20×10^{0}
	3	6.00×10^{-1}	8	1.60×10^{0}	3	6.00×10^{-1}	6	1.20×10^{0}	7	1.40×10^{0}	6	1.20×10^{0}
10^{-2}	0	0	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	0	0	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
Average		**1.144**		**1.444**		**0.844**		**1.704**		**1.056**		**1.292**
SD		**0.561854**		**0.515829**		**0.647827**		**0.496065**		**0.672815**		**0.405364**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B9: Presumptive bacterial indicator counts for water samples along the Umdloti River during the spring season.

Indicator	C1		C2		C3		C4	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count								
10^{-4}	93	1.86×10^{4}	116	2.32×10^{4}	156	3.12×10^{4}	72	1.44×10^{4}
	106	2.12×10^{4}	125	2.50×10^{4}	147	2.94×10^{4}	75	1.50×10^{4}
10^{-5}	32	6.40×10^{4}	24	4.80×10^{4}	28	5.60×10^{4}	10	2.00×10^{4}
	19	3.80×10^{4}	13	2.60×10^{4}	33	6.60×10^{4}	6	1.20×10^{4}
10^{-6}	2	4.00×10^{4}	2	4.00×10^{4}	2	4.00×10^{4}	3	6.00×10^{4}
	3	6.00×10^{4}	4	8.00×10^{4}	6	1.20×10^{5}	2	4.00×10^{4}
10^{-7}	1	2.00×10^{5}	2	4.00×10^{5}	1	2.00×10^{5}	1	2.00×10^{5}
	0	0	0	0	1	2.00×10^{5}	0	0
Average (x 10^{4})		**2.945**		**2.855**		**3.915**		**1.535**
SD		**1.110841**		**0.772075**		**1.215058**		**0.336006**
Total Coliforms								
10^{-1}	99	1.98×10^{1}	173	3.46×10^{1}	139	2.78×10^{1}	105	2.10×10^{1}
	87	1.74×10^{1}	162	3.24×10^{1}	150	3.00×10^{1}	121	2.42×10^{1}
10^{-2}	9	1.80×10^{1}	20	4.00×10^{1}	12	2.40×10^{1}	6	1.20×10^{1}
	18	3.60×10^{1}	7	1.40×10^{1}	25	5.00×10^{1}	9	1.80×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	0	0	2	4.00×10^{1}
	0	0	4	8.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**1.88**		**3.675**		**2.545**		**1.88**
SD		**0.129615**		**0.385876**		**0.439811**		**0.51923**
Faecal Coliforms								
10^{-1}	52	1.04×10^{1}	66	1.32×10^{1}	52	1.04×10^{1}	39	7.80×10^{0}
	77	1.54×10^{1}	49	9.80×10^{0}	56	1.12×10^{1}	50	1.00×10^{1}
10^{-2}	9	1.80×10^{1}	5	1.00×10^{1}	3	6.00×10^{0}	4	8.00×10^{0}
	8	1.60×10^{1}	4	8.00×10^{0}	1	2.00×10^{0}	4	8.00×10^{0}
10^{-3}	1	2.00×10^{1}	0	0	0	0	1	2.00×10^{1}
	0	0	0	0	0	0	0	0
Average (x 10^{1})		**1.495**		**1.025**		**0.74**		**0.845**
SD		**0.323058**		**0.216256**		**0.426458**		**0.103763**
Enterococci								
10^{-1}	42	8.40×10^{0}	17	3.40×10^{0}	10	2.00×10^{0}	27	5.40×10^{0}
	31	6.20×10^{0}	23	4.60×10^{0}	9	1.80×10^{0}	11	2.20×10^{0}
10^{-2}	5	1.00×10^{1}	2	4.00×10^{0}	2	4.00×10^{0}	6	1.20×10^{1}
	5	1.00×10^{1}	2	4.00×10^{0}	3	6.00×10^{0}	1	2.00×10^{0}
Average (x 10^{1})		**0.82**		**0.4**		**0.26**		**0.32**
SD		**0.190788**		**0.06**		**0.121655**		**0.190788**
Faecal Streptococci								
10^{-1}	62	1.24×10^{1}	19	3.80×10^{0}	29	5.80×10^{0}	33	6.60×10^{0}

Table B9/cont.

	59	1.18×10^1	25	5.00×10^0	22	4.40×10^0	40	8.00×10^0
10^{-2}	14	2.80×10^1	5	1.00×10^1	4	8.00×10^0	10	2.00×10^1
	9	1.80×10^1	3	6.00×10^0	7	1.40×10^1	6	1.20×10^1
Average (x 10^1)		**1.755**		**0.62**		**0.805**		**1.165**
SD		**0.750533**		**0.268825**		**0.423438**		**0.601858**
Vibrio cholerae								
10^{-1}	142	2.84×10^1	73	1.46×10^1	101	2.02×10^1	35	7.00×10^0
	161	3.22×10^1	69	1.38×10^1	128	2.56×10^1	40	8.00×10^0
10^{-2}	49	9.80×10^1	10	2.00×10^1	16	3.20×10^1	7	1.40×10^1
	23	4.60×10^1	8	1.60×10^1	22	4.40×10^1	13	2.60×10^1
10^{-3}	3	6.00×10^1	2	4.00×10^1	5	1.00×10^2	1	2.00×10^1
	1	2.00×10^1	0	0	3	6.00×10^1	1	2.00×10^1
Average (x 10^1)		**3.165**		**1.61**		**3.045**		**2**
SD		**1.08399**		**0.275439**		**1.024028**		**0.489898**
Salmonella spp.								
10^{-1}	57	1.14×10^1	83	1.66×10^1	*	*	33	6.60×10^0
	80	1.60×10^1	81	1.62×10^1	*	*	40	8.00×10^0
10^{-2}	14	2.80×10^1	6	1.20×10^1	18	3.60×10^1	3	6.00×10^0
	12	2.40×10^1	9	1.80×10^1	18	3.60×10^1	3	6.00×10^0
10^{-3}	5	1.00×10^2	0	0	1	2.00×10^1	1	2.00×10^1
	2	4.00×10^1	1	2.00×10^1	1	2.00×10^1	0	0
Average (x 10^1)		**1.985**		**1.57**		**2.8**		**0.65**
SD		**0.752485**		**0.258457**		**0.92376**		**0.1**
Shigella spp.								
10^{-1}	46	9.20×10^0	61	1.22×10^1	14	2.80×10^0	54	1.08×10^1
	37	7.40×10^0	40	8.00×10^0	27	5.40×10^0	31	6.20×10^0
10^{-2}	12	2.40×10^1	5	1.00×10^1	9	1.80×10^1	11	2.20×10^1
	7	1.40×10^1	10	2.00×10^1	9	1.80×10^1	8	1.60×10^1
10^{-3}	1	2.00×10^1	1	2.00×10^1	2	4.00×10^1	3	6.00×10^1
	1	2.00×10^1	0	0	1	2.00×10^1	1	2.00×10^1
Average (x 10^1)		**1.492**		**1.404**		**1.284**		**1.5**
SD		**0.704216**		**0.563986**		**0.807267**		**0.652074**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B10: Presumptive bacterial indicator counts for water samples along the Umdloti River during the summer season.

Indicator	C1		C2		C3		C4	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count								
10^{-4}	177	3.54×10^{4}	213	4.26×10^{4}	139	2.78×10^{4}	111	2.22×10^{4}
	190	3.80×10^{4}	172	3.44×10^{4}	112	2.24×10^{4}	101	2.02×10^{4}
10^{-5}	30	6.00×10^{4}	23	4.60×10^{4}	7	1.40×10^{4}	13	2.60×10^{4}
	25	5.00×10^{4}	19	3.80×10^{4}	11	2.20×10^{4}	18	3.60×10^{4}
10^{-6}	15	3.00×10^{5}	6	1.20×10^{5}	3	6.00×10^{4}	5	1.00×10^{5}
	8	1.60×10^{5}	3	6.00×10^{4}	2	4.00×10^{4}	9	1.80×10^{5}
10^{-7}	2	4.00×10^{5}	1	2.00×10^{5}	0	0	1	2.00×10^{5}
	1	2.00×10^{5}	1	2.00×10^{5}	0	0	1	2.00×10^{5}
Average (x 10^{4})		**4.585**		**4.025**		**2.155**		**2.61**
SD		**1.137644**		**0.509477**		**0.568595**		**0.702472**
Total Coliforms								
10^{-1}	172	3.44×10^{1}	265	5.30×10^{1}	193	3.86×10^{1}	149	2.98×10^{1}
	196	3.92×10^{1}	221	4.42×10^{1}	187	3.74×10^{1}	160	3.20×10^{1}
10^{-2}	25	5.00×10^{1}	23	4.60×10^{1}	31	6.20×10^{1}	19	3.80×10^{1}
	21	4.20×10^{1}	18	3.60×10^{1}	18	3.60×10^{1}	17	3.40×10^{1}
10^{-3}	2	4.00×10^{1}	3	6.00×10^{1}	2	4.00×10^{1}	8	1.60×10^{2}
	0	0	2	4.00×10^{1}	3	6.00×10^{1}	11	2.20×10^{2}
Average (x 10^{1})		**3.89**		**4.155**		**3.8**		**3.345**
SD		**0.322284**		**0.447325**		**0.170489**		**0.348473**
Faecal Coliforms								
10^{-1}	103	2.06×10^{1}	112	2.24×10^{1}	73	1.46×10^{1}	58	1.16×10^{1}
	89	1.78×10^{1}	80	1.60×10^{1}	90	1.80×10^{1}	62	1.24×10^{1}
10^{-2}	14	2.80×10^{1}	13	2.60×10^{1}	11	2.20×10^{1}	8	1.60×10^{1}
	15	3.00×10^{1}	10	2.00×10^{1}	8	1.60×10^{1}	7	1.40×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**2.328**		**2.088**		**1.812**		**1.48**
SD		**0.537141**		**0.367042**		**0.297859**		**0.335857**
Enterococci								
10^{-1}	22	4.40×10^{0}	8	1.60×10^{0}	27	5.40×10^{0}	16	3.20×10^{0}
	13	2.60×10^{0}	10	2.00×10^{0}	24	4.80×10^{0}	18	3.60×10^{0}
10^{-2}	4	8.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	3	6.00×10^{0}
	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
Average (x 10^{1})		**0.475**		**0.19**		**0.355**		**0.37**
SD		**0.23**		**0.02**		**0.180647**		**0.16773**
Faecal Streptococci								
10^{-1}	9	1.80×10^{0}	23	4.60×10^{0}	15	3.00×10^{0}	14	2.80×10^{0}

Table B10/cont.

	9	1.80×10^{0}	30	6.00×10^{0}	14	2.80×10^{0}	17	3.40×10^{0}
10^{-2}	1	2.00×10^{0}	4	8.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	2	4.00×10^{0}	5	1.00×10^{1}	2	4.00×10^{0}	1	2.00×10^{0}
Average (x 10^{1})		**0.24**		**0.715**		**0.345**		**0.255**
SD		**0.107083**		**0.235726**		**0.064031**		**0.068069**
Vibrio cholerae								
10^{-1}	77	1.54×10^{1}	141	2.82×10^{1}	95	1.90×10^{1}	91	1.82×10^{1}
	58	1.16×10^{1}	126	2.52×10^{1}	117	2.34×10^{1}	79	1.58×10^{1}
10^{-2}	9	1.80×10^{1}	10	2.00×10^{1}	9	1.80×10^{1}	13	2.60×10^{1}
	6	1.20×10^{1}	10	2.00×10^{1}	10	2.00×10^{1}	12	2.40×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	2	4.00×10^{1}
	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**1.616667**		**2.556667**		**2.006667**		**2.4**
SD		**0.378294**		**0.785307**		**0.181842**		**0.868194**
Salmonella spp.								
10^{-1}	125	2.50×10^{1}	60	1.20×10^{1}	101	2.02×10^{1}	63	1.26×10^{1}
	103	2.06×10^{1}	69	1.38×10^{1}	110	2.20×10^{1}	81	1.62×10^{1}
10^{-2}	32	6.40×10^{1}	19	3.80×10^{1}	22	4.40×10^{1}	12	2.40×10^{1}
	18	3.60×10^{1}	15	3.00×10^{1}	18	3.60×10^{1}	15	3.00×10^{1}
10^{-3}	6	1.20×10^{2}	1	2.00×10^{1}	3	6.00×10^{1}	3	6.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	3	6.00×10^{1}
Average (x 10^{1})		**3.04**		**2.7**		**2.455**		**2.07**
SD		**0.910531**		**0.87178**		**0.768613**		**0.781537**
Shigella spp.								
10^{-1}	66	1.32×10^{1}	108	2.16×10^{1}	34	6.80×10^{0}	59	1.18×10^{1}
	72	1.44×10^{1}	75	1.50×10^{1}	43	8.60×10^{0}	54	1.08×10^{1}
10^{-2}	10	2.00×10^{1}	16	3.20×10^{1}	12	2.40×10^{1}	6	1.20×10^{1}
	7	1.40×10^{1}	9	1.80×10^{1}	11	2.20×10^{1}	8	1.60×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	3	6.00×10^{1}	1	2.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**1.632**		**2.132**		**1.628**		**1.412**
SD		**0.338703**		**0.645848**		**0.798448**		**0.384083**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B11: Presumptive bacterial indicator counts for water samples along the Umdloti River during the autumn season.

Indicator	C1		C2		C3		C4	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count								
10^{-4}	153	3.06×10^{4}	142	2.84×10^{4}	98	1.96×10^{4}	85	1.70×10^{4}
	125	2.50×10^{4}	137	2.74×10^{4}	119	2.38×10^{4}	102	2.04×10^{4}
10^{-5}	21	4.20×10^{4}	18	3.60×10^{4}	13	2.60×10^{4}	9	1.80×10^{4}
	16	3.20×10^{4}	15	3.00×10^{4}	17	3.40×10^{4}	13	2.60×10^{4}
10^{-6}	7	1.40×10^{4}	6	1.20×10^{5}	4	8.00×10^{4}	5	1.00×10^{5}
	5	1.00×10^{5}	3	6.00×10^{4}	4	8.00×10^{4}	5	1.00×10^{5}
10^{-7}	1	2.00×10^{5}	1	2.00×10^{5}	1	2.00×10^{5}	1	2.00×10^{5}
	2	4.00×10^{5}	1	2.00×10^{5}	0	0	1	2.00×10^{5}
Average (x 10^{4})		**3.24**		**3.045**		**2.585**		**2.035**
SD		**0.707861**		**0.385184**		**0.604731**		**0.402782**
Total Coliforms								
10^{-1}	127	2.54×10^{1}	158	3.16×10^{1}	139	2.78×10^{1}	124	2.48×10^{1}
	114	2.28×10^{1}	170	3.40×10^{1}	147	2.94×10^{1}	107	2.14×10^{1}
10^{-2}	10	2.00×10^{1}	13	2.60×10^{1}	19	3.80×10^{1}	7	1.40×10^{1}
	8	1.60×10^{1}	11	2.20×10^{1}	22	4.40×10^{1}	8	1.60×10^{1}
10^{-3}	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**1.976**		**2.672**		**2.704**		**1.924**
SD		**0.242652**		**0.601598**		**0.750653**		**0.430442**
Faecal Coliforms								
10^{-1}	93	1.86×10^{1}	140	2.80×10^{1}	71	1.42×10^{1}	27	5.40×10^{0}
	85	1.70×10^{1}	117	2.34×10^{1}	120	2.40×10^{1}	32	6.40×10^{0}
10^{-2}	10	2.00×10^{1}	9	1.80×10^{1}	3	6.00×10^{0}	1	2.00×10^{1}
	9	1.80×10^{1}	9	1.80×10^{1}	8	1.60×10^{1}	1	2.00×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	0	0	0	0
	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**1.84**		**1.9**		**1.405**		**1.295**
SD		**0.125433**		**0.11547**		**0.588869**		**0.815087**
Enterococci								
10^{-1}	28	5.60×10^{0}	17	3.40×10^{0}	13	2.60×10^{0}	8	1.60×10^{0}
	21	4.20×10^{0}	14	2.80×10^{0}	15	3.00×10^{0}	11	2.20×10^{0}
10^{-2}	3	6.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}	1	2.00×10^{0}
	2	4.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
Average (x 10^{1})		**0.495**		**0.255**		**0.29**		**0.195**
SD		**0.099833**		**0.068069**		**0.084063**		**0.025166**
Faecal Streptococci								
10^{-1}	12	2.40×10^{0}	13	2.60×10^{0}	7	1.40×10^{0}	22	4.40×10^{0}

Table B11/cont.

	10	2.00×10^{0}	9	1.80×10^{0}	7	1.40×10^{0}	16	3.20×10^{0}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	2	4.00×10^{0}
Average (x 10^{1})		**0.21**		**0.21**		**0.17**		**0.34**
SD		**0.02**		**0.034641**		**0.034641**		**0.10583**
Vibrio cholerae								
10^{-1}	45	9.00×10^{0}	117	2.34×10^{1}	68	1.36×10^{1}	71	1.42×10^{1}
	51	1.02×10^{1}	108	2.16×10^{1}	57	1.14×10^{1}	101	2.02×10^{1}
10^{-2}	13	2.60×10^{1}	15	3.00×10^{1}	9	1.80×10^{1}	14	2.80×10^{1}
	8	1.60×10^{1}	9	1.80×10^{1}	6	1.20×10^{1}	14	2.80×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	1	2.00×10^{1}	0	0	1	2.00×10^{1}
Average (x 10^{1})		**1.504**		**2.06**		**1.5**		**2.324**
SD		**0.524481**		**0.20199**		**0.380526**		**0.434603**
Salmonella spp.								
10^{-1}	105	2.10×10^{1}	52	1.04×10^{1}	34	6.80×10^{0}	95	1.90×10^{1}
	71	1.42×10^{1}	76	1.52×10^{1}	50	1.00×10^{1}	64	1.28×10^{1}
10^{-2}	24	4.80×10^{1}	15	3.00×10^{1}	16	3.20×10^{1}	5	1.00×10^{1}
	13	2.60×10^{1}	18	3.60×10^{1}	9	1.80×10^{1}	3	6.00×10^{0}
10^{-3}	2	4.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	2	4.00×10^{1}	1	2.00×10^{1}	0	0
Average (x 10^{1})		**2.03**		**1.89**		**1.37**		**1.545**
SD		**0.484011**		**0.837377**		**0.631084**		**0.483149**
Shigella spp.								
10^{-1}	37	7.40×10^{0}	49	9.80×10^{0}	57	1.14×10^{1}	22	4.40×10^{0}
	18	3.60×10^{0}	24	4.80×10^{0}	62	1.24×10^{1}	28	5.60×10^{0}
10^{-2}	7	1.40×10^{1}	6	1.20×10^{1}	10	2.00×10^{1}	3	6.00×10^{0}
	5	1.00×10^{1}	4	8.00×10^{0}	6	1.20×10^{1}	2	4.00×10^{0}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	0	0	0	0	1	2.00×10^{1}	0	0
Average (x 10^{1})		**0.875**		**0.865**		**1.81**		**0.5**
SD		**0.437683**		**0.304357**		**0.38**		**0.095219**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B12: Presumptive bacterial indicator counts for water samples along the Umdloti River during the winter season.

Indicator	C1		C2		C3		C4	
	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml	COUNT	cfu/ml
Total Heterotrophic Bacterial Count								
10^{-4}	130	2.60×10^{4}	87	1.74×10^{4}	103	2.06×10^{4}	76	1.52×10^{4}
	134	2.68×10^{4}	63	1.26×10^{4}	112	2.24×10^{4}	69	1.38×10^{4}
10^{-5}	11	2.20×10^{4}	23	4.60×10^{4}	19	3.80×10^{4}	21	4.20×10^{4}
	14	2.80×10^{4}	16	3.20×10^{4}	12	2.40×10^{4}	11	2.20×10^{4}
10^{-6}	3	6.00×10^{4}	2	4.00×10^{4}	3	6.00×10^{4}	4	8.00×10^{4}
	4	8.00×10^{4}	2	4.00×10^{4}	2	4.00×10^{4}	2	4.00×10^{4}
10^{-7}	1	2.00×10^{5}	0	0	1	2.00×10^{5}	1	2.00×10^{5}
	0	0	0	0	1	2.00×10^{5}	1	2.00×10^{5}
Average ($\times 10^{4}$)		**2.57**		**3.95**		**2.625**		**2.275**
SD		**0.26**		**0.574456**		**0.79555**		**1.204478**
Total Coliforms								
10^{-1}	103	2.06×10^{1}	134	2.68×10^{1}	111	2.22×10^{1}	70	1.40×10^{1}
	82	1.64×10^{1}	139	2.78×10^{1}	120	2.40×10^{1}	61	1.22×10^{1}
10^{-2}	12	2.40×10^{1}	12	2.40×10^{1}	18	3.60×10^{1}	9	1.80×10^{1}
	7	1.40×10^{1}	10	2.00×10^{1}	16	3.20×10^{1}	8	1.60×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	1	2.00×10^{1}	2	4.00×10^{1}	2	4.00×10^{1}	0	0
Average ($\times 10^{1}$)		**1.82**		**2.372**		**2.684**		**1.604**
SD		**0.28775**		**0.367042**		**0.683579**		**0.309968**
Faecal Coliforms								
10^{-1}	48	9.60×10^{0}	59	1.18×10^{1}	88	1.76×10^{1}	37	7.40×10^{0}
	52	1.04×10^{1}	74	1.48×10^{1}	60	1.20×10^{1}	45	9.00×10^{0}
10^{-2}	6	1.20×10^{1}	8	1.60×10^{1}	10	2.00×10^{1}	7	1.40×10^{1}
	6	1.20×10^{1}	5	1.00×10^{1}	7	1.40×10^{1}	6	1.20×10^{1}
10^{-3}	1	2.00×10^{1}	0	0	1	2.00×10^{1}	1	2.00×10^{1}
	0	0	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
Average ($\times 10^{1}$)		**1.28**		**1.452**		**1.832**		**1.5**
SD		**0.415692**		**0.387711**		**0.262907**		**0.489898**
Enterococci								
10^{-1}	5	1.00×10^{0}	9	1.80×10^{0}	23	4.60×10^{0}	6	1.20×10^{0}
	3	6.00×10^{-1}	12	2.40×10^{0}	14	2.80×10^{0}	4	8.00×10^{-1}
10^{-2}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}	1	2.00×10^{0}
	0	0	1	2.00×10^{0}	1	2.00×10^{0}	0	0
Average ($\times 10^{1}$)		**0.12**		**0.213333**		**0.226667**		**0.133333**
SD		**0.072111**		**0.023094**		**0.046188**		**0.061101**
Faecal Streptococci								
10^{-1}	5	1.00×10^{0}	9	1.80×10^{0}	2	4.00×10^{-1}	4	8.00×10^{-1}

Table B12/cont.

	3	6.00×10^{-1}	6	1.20×10^{0}	3	6.00×10^{-1}	5	1.00×10^{0}
10^{-2}	0	0	1	2.00×10^{0}	0	0	0	0
	0	0	0	0	0	0	0	0
Average (x 10^{1})		**0.08**		**0.15**		**0.05**		**0.09**
SD		**0.028284**		**0.042426**		**0.014142**		**0.014142**
Vibrio cholerae								
10^{-1}	73	1.46×10^{1}	64	1.28×10^{1}	31	6.20×10^{0}	25	5.00×10^{0}
	70	1.40×10^{1}	89	1.78×10^{1}	29	5.80×10^{0}	48	9.60×10^{0}
10^{-2}	10	2.00×10^{1}	11	2.20×10^{1}	9	1.80×10^{1}	6	1.20×10^{1}
	12	2.40×10^{1}	11	2.20×10^{1}	7	1.40×10^{1}	10	2.00×10^{1}
10^{-3}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}	1	2.00×10^{1}
	2	4.00×10^{1}	1	2.00×10^{1}	0	0	1	2.00×10^{1}
Average (x 10^{1})		**1.852**		**2.036**		**1.28**		**1.632**
SD		**0.418951**		**0.174585**		**0.657419**		**0.510999**
Salmonella spp.								
10^{-1}	65	1.30×10^{1}	20	4.00×10^{0}	33	6.60×10^{0}	72	1.44×10^{1}
	41	8.20×10^{0}	15	3.00×10^{0}	37	7.40×10^{0}	46	9.20×10^{0}
10^{-2}	7	1.40×10^{1}	3	6.00×10^{0}	6	1.20×10^{1}	10	2.00×10^{1}
	8	1.60×10^{1}	5	1.00×10^{1}	8	1.60×10^{1}	7	1.40×10^{1}
10^{-3}	1	2.00×10^{1}	0	0	0	0	1	2.00×10^{1}
	0	0	0	0	1	2.00×10^{1}	1	2.00×10^{1}
Average (x 10^{1})		**1.575**		**0.575**		**1.05**		**1.85**
SD		**0.30957**		**0.30957**		**0.437112**		**0.3**
Shigella spp.								
10^{-1}	26	5.20×10^{0}	14	2.80×10^{0}	39	7.80×10^{0}	29	5.80×10^{0}
	24	4.80×10^{0}	21	4.20×10^{0}	46	9.20×10^{0}	48	9.60×10^{0}
10^{-2}	3	6.00×10^{0}	4	8.00×10^{0}	5	1.00×10^{1}	3	6.00×10^{0}
	2	4.00×10^{0}	4	8.00×10^{0}	4	8.00×10^{0}	2	4.00×10^{0}
10^{-3}	0	0	0	0	1	2.00×10^{1}	1	2.00×10^{1}
	0	0	1	2.00×10^{1}	0	0	1	2.00×10^{1}
Average (x 10^{1})		**0.5**		**0.575**		**0.875**		**0.635**
SD		**0.083267**		**0.26602**		**0.103763**		**0.234592**

* – colonies were too numerous to count for a definitive colony count

SD – standard deviation

cfu/ml – colony forming units per milliliter

Shaded areas highlight the values used to calculate average

Table B13: Statistical analysis: Post Hoc tests

Dependent Variable: Temperature (Umgeni River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	4.0000*	1.26095	.006	1.4269	6.5731
	Autumn	1.4000	1.26095	.269	-1.1731	3.9731
	Winter	5.1000*	1.26095	.001	2.5269	7.6731
Summer	Springs	-4.0000*	1.26095	.006	-6.5731	-1.4269
	Autumn	-2.6000*	1.26095	.051	-5.1731	.0269
	Winter	1.1000	1.26095	.389	-1.4731	3.6731
Autumn	Springs	-1.4000	1.26095	.269	-3.9731	1.1731
	Summer	2.6000*	1.26095	.051	-.0269	5.1731
	Winter	3.7000*	1.26095	.010	1.1269	6.2731
Winter	Springs	-5.1000*	1.26095	.001	-7.6731	-2.5269
	Summer	-1.1000	1.26095	.389	-3.6731	1.4731
	Autumn	-3.7000*	1.26095	.010	-6.2731	-1.1269

* The mean difference is significant at the .05 level.

Dependent Variable: Temperature (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-4.7500*	.77560	.000	-6.4399	-3.0601
	Autumn	1.1250	.77560	.173	-.5649	2.8149
	Winter	5.5000*	.77560	.000	3.8101	7.1899
Summer	Springs	4.7500*	.77560	.000	3.0601	6.4399
	Autumn	5.8750*	.77560	.000	4.1851	7.5649
	Winter	12.2500*	.77560	.000	10.5601	13.9399
Autumn	Springs	-1.1250	.77560	.173	-2.8149	.5649
	Summer	-5.8750*	.77560	.000	-7.5649	-4.1851
	Winter	6.3750*	.77560	.000	4.6851	8.0649
Winter	Springs	-5.5000*	.77560	.000	-7.1899	-3.8101
	Summer	-12.2500*	.77560	.000	-13.9399	-10.5601
	Autumn	-6.3750*	.77560	.000	-8.0649	-4.6851

*. The mean difference is significant at the .05 level.

Dependent Variable: Temperature (Umhlanga)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-6.4000*	.66020	.000	-7.8610	-4.9390
	Autumn	-3.5000*	.66020	.000	-4.9610	-2.0390
	Winter	4.1000*	.66020	.000	2.6390	5.5610
Summer	Springs	6.4000*	.66020	.000	4.9390	7.8610
	Autumn	2.9000*	.66020	.001	1.4390	4.3610
	Winter	10.5000*	.66020	.000	9.0390	11.9610
Autumn	Springs	3.5000*	.66020	.000	2.0390	4.9610
	Summer	2.9000*	.66020	.001	1.4390	4.3610
	Winter	7.6000*	.66020	.000	6.1390	9.0610
Winter	Springs	-4.1000*	.66020	.000	-5.5610	-2.6390
	Summer	-10.5000*	.66020	.000	-11.9610	-9.0390
	Autumn	-7.6000*	.66020	.000	-9.0610	-6.1390

*. The mean difference is significant at the .05 level.

Dependent Variable: pH (Umgeni River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-.7460*	.33659	.042	-1.4595	-.0325
	Autumn	.2020	.33659	.460	-.5115	.9155
	Winter	-.7360*	.33659	.043	-1.4515	-.0205
Summer	Springs	.7460*	.33659	.042	.0325	1.4595
	Autumn	.9480	.33659	.102	-.1655	2.0615
	Winter	.0100	.33659	.981	-.7055	.7215
Autumn	Springs	-.2020	.33659	.460	-.9155	.5115
	Summer	-.9480	.33659	.102	-2.0615	.1655
	Winter	-.9380	.33659	.168	-1.6535	.2275
Winter	Springs	.7360*	.33659	.043	.0205	1.4515
	Summer	-.0080	.33659	.981	-.7215	.7055
	Autumn	.9480	.33659	.168	-.2275	2.1235

*. The mean difference is significant at the .05 level.

Dependent Variable: pH (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.1340	.14781	.378	-.1573	.4473
	Autumn	.7500*	.14781	.000	1.0633	.4367
	Winter	.5320*	.14781	.000	1.1453	.5187
Summer	Springs	-.1340	.14781	.370	-.4473	.1760
	Autumn	-.6840*	.14781	.000	-1.1973	-.5707
	Winter	-.9960*	.14781	.000	-1.2793	-.6527
Autumn	Springs	.7500*	.14781	.000	.4367	1.0633
	Summer	.6840*	.14781	.000	.5707	1.1973
	Winter	.0820	.14781	.587	.3653	.2313
Winter	Springs	.5320*	.14781	.000	.5187	1.1453
	Summer	.9960*	.14781	.000	.6527	1.2793
	Autumn	.0820	.14781	.587	.2313	.3953

* The mean difference is significant at the .05 level.

Dependent Variable: pH (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-.5225*	.24606	.035	-1.0186	-.0264
	Autumn	-.5525*	.24606	.030	-1.1186	-.0464
	Winter	-.8375*	.24606	.000	-1.3736	-.3014
Summer	Springs	.5225*	.24606	.035	.0264	1.0186
	Autumn	.2400	.24606	.340	-.2561	.7361
	Winter	-.0150	.24606	.952	-.5111	.5211
Autumn	Springs	.5525*	.24606	.030	.0464	1.1186
	Summer	-.2400	.24606	.340	-.7361	.2561
	Winter	-.2550	.24606	.320	-.7911	.2811
Winter	Springs	.8375*	.24606	.000	.3014	1.3736
	Summer	.0150	.24606	.952	-.5211	.5511
	Autumn	.2550	.24606	.320	-.2811	.7911

* The mean difference is significant at the .05 level.

Dependent Variable: Turbidity (Umgeni River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-4.4940*	2.06690	.045	-8.8756	-.1124
	Autumn	.3020	2.06690	.056	4.7836	3.9796
	Winter	1.4180	2.06690	.503	5.7996	2.9636
Summer	Springs	4.4940*	2.06690	.045	.1124	8.8756
	Autumn	4.1120	2.06690	.064	-.2696	8.4936
	Winter	5.9760	2.06690	.156	1.3056	7.4576
Autumn	Springs	-.3020	2.06690	.056	-3.9796	4.7636
	Summer	-4.1120	2.06690	.064	-8.4936	.2696
	Winter	-1.0360	2.06690	.623	-5.4176	3.3456
Winter	Springs	-1.4180	2.06690	.503	-2.9636	5.7996
	Summer	-5.9760	2.06690	.156	-7.4576	1.3056
	Autumn	-1.0360	2.06690	.623	-3.3456	5.4176

* The mean difference is significant at the .05 level.

Dependent Variable: Turbidity (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-.3400	.32191	.298	-1.0284	.3364
	Autumn	.0360	.32191	.912	-.6484	.7104
	Winter	.4060	.32191	.149	-.1944	1.1704
Summer	Springs	.3400	.32191	.298	-.3364	1.0284
	Autumn	.3820	.32191	.253	-.3001	1.0641
	Winter	.8540*	.32191	.020	.1518	1.5164
Autumn	Springs	-.0360	.32191	.912	-.7104	.6484
	Summer	-.3820	.32191	.253	-1.0641	.3001
	Winter	.4520	.32191	.179	-.2304	1.1344
Winter	Springs	-.4060	.32191	.149	-1.1704	.1944
	Summer	-.8540*	.32191	.020	-1.5164	-.1518
	Autumn	-.4520	.32191	.179	-1.1344	.2304

* The mean difference is significant at the .05 level.

Dependent Variable: BOD (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-.1000	.86094	.907	-1.9079	1.6079
	Autumn	2.7360*	.86094	.005	.9241	4.5479
	Winter	2.8080*	.86094	.004	1.0041	4.7019
Summer	Springs	.1000	.86094	.907	-1.6079	1.9079
	Autumn	2.8440*	.86094	.004	1.0401	4.6479
	Winter	3.0040*	.86094	.003	1.2001	4.8079
Autumn	Springs	-2.7360*	.86094	.005	-4.5479	-.9241
	Summer	-2.8440*	.86094	.004	-4.6479	-1.0401
	Winter	.1600	.86094	.853	-1.6439	1.9639
Winter	Springs	-2.8080*	.86094	.004	-4.7019	-1.0041
	Summer	-3.0040*	.86094	.003	-4.8079	-1.2001
	Autumn	-.1600	.86094	.853	-1.9639	1.6439

* The mean difference is significant at the .05 level.

Dependent Variable: Conductivity (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-2.0000	46.64762	.966	-100.8885	96.8885
	Autumn	292.0000*	46.64762	.000	193.1115	390.8885
	Winter	282.0000*	46.64762	.000	183.1115	380.8885
Summer	Springs	2.0000	46.64762	.966	-96.8885	100.8885
	Autumn	294.0000*	46.64762	.000	195.1115	392.8885
	Winter	284.0000*	46.64762	.000	185.1115	382.8885
Autumn	Springs	-292.0000*	46.64762	.000	-390.8885	-193.1115
	Summer	-294.0000*	46.64762	.000	-392.8885	-195.1115
	Winter	-10.0000	46.64762	.833	-108.8885	88.8885
Winter	Springs	-282.0000*	46.64762	.000	-380.8885	-183.1115
	Summer	-284.0000*	46.64762	.000	-382.8885	-185.1115
	Autumn	10.0000	46.64762	.833	-88.8885	108.8885

* The mean difference is significant at the .05 level.

Dependent Variable: Phosphate (Umgeni River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0000	322.41123	1.000	-683.4813	683.4813
	Autumn	852.0000*	322.41123	.018	168.5187	1535.4813
	Winter	-918.0000*	322.41123	.012	-1601.4813	-234.5187
Summer	Springs	.0000	322.41123	1.000	-683.4813	683.4813
	Autumn	-852.0000*	322.41123	.018	-1535.4813	-168.5187
	Winter	-918.0000*	322.41123	.012	-1601.4813	-234.5187
Autumn	Springs	852.0000*	322.41123	.018	168.5187	1535.4813
	Summer	852.0000*	322.41123	.018	168.5187	1535.4813
	Winter	-66.0000	322.41123	.840	-749.4813	617.4813
Winter	Springs	918.0000*	322.41123	.012	234.5187	1601.4813
	Summer	918.0000*	322.41123	.012	234.5187	1601.4813
	Autumn	66.0000	322.41123	.840	-617.4813	749.4813

*. The mean difference is significant at the .05 level.

Dependent Variable: Phosphate (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0000	102.12576	1.000	-396.0179	396.0179
	Autumn	-1697.5000*	182.12576	.000	-1991.3179	-1200.6821
	Winter	-1650.0000*	102.12576	.000	-2046.0179	-1253.1821
Summer	Springs	.0000	102.12576	1.000	-396.0179	396.0179
	Autumn	-1697.5000*	182.12576	.000	-1991.3179	-1200.6821
	Winter	-1650.0000*	102.12576	.000	-2046.0179	-1253.1821
Autumn	Springs	1697.5000*	102.12576	.000	1200.6821	1994.3179
	Summer	1697.5000*	182.12576	.000	1200.6821	1994.3179
	Winter	47.5000	182.12576	.778	-149.3179	344.3179
Winter	Springs	1650.0000*	102.12576	.000	1253.1821	2046.8179
	Summer	1650.0000*	182.12576	.000	1253.1821	2046.8179
	Autumn	-47.5000	182.12576	.778	-344.3179	149.3179

*. The mean difference is significant at the .05 level.

Dependent Variable: Nitrate (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0000	.00000	1.000	.0000	.0000
	Autumn	-2.4500*	.00000	.000	-2.4500	-2.4500
	Winter	2.4500*	.00000	.000	2.4500	2.4500
Summer	Springs	.0000	.00000	1.000	.0000	.0000
	Autumn	-2.4500*	.00000	.000	-2.4500	-2.4500
	Winter	2.4500*	.00000	.000	2.4500	2.4500
Autumn	Springs	2.4500*	.00000	.000	2.4500	2.4500
	Summer	2.4500*	.00000	.000	2.4500	2.4500
	Winter	.0000	.00000	1.000	.0000	.0000
Winter	Springs	-2.4500*	.00000	.000	-2.4500	-2.4500
	Summer	-2.4500*	.00000	.000	-2.4500	-2.4500
	Autumn	.0000	.00000	1.000	.0000	.0000

*. The mean difference is significant at the .05 level.

Dependent Variable: Nitrate (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0000	.10596	1.000	-.2309	.2309
	Autumn	-.1400	.10596	.211	-.3709	.0909
	Winter	-.3025*	.10596	.014	-.5334	-.0716
Summer	Springs	.0000	.10596	1.000	-.2309	.2309
	Autumn	-.1400	.10596	.211	-.3709	.0909
	Winter	-.3025*	.10596	.014	-.5334	-.0716
Autumn	Springs	.1400	.10596	.211	-.0909	.3709
	Summer	.1400	.10596	.211	-.0909	.3709
	Winter	-.1625	.10596	.151	-.3934	.0684
Winter	Springs	.3025*	.10596	.014	.0716	.5334
	Summer	.3025*	.10596	.014	.0716	.5334
	Autumn	.1625	.10596	.151	-.0684	.3934

*. The mean difference is significant at the .05 level.

Dependent Variable: Sulphate (Beaches)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	93.2000	78.14858	.250	-72.4676	258.8676
	Autumn	-133.2000	78.14858	.106	-298.8676	32.4676
	Winter	-146.2000	78.14858	.080	-311.8676	19.4676
Summer	Springs	-93.2000	78.14858	.250	-258.8676	72.4676
	Autumn	-226.4000*	78.14858	.011	-392.0676	-60.7324
	Winter	-239.4000*	78.14858	.007	-405.0676	-73.7324
Autumn	Springs	133.2000	78.14858	.106	-32.4676	298.8676
	Summer	226.4000*	78.14858	.011	60.7324	392.0676
	Winter	-13.0000	78.14858	.870	-178.6676	152.6676
Winter	Springs	146.2000	78.14858	.080	-19.4676	311.8676
	Summer	239.4000*	78.14858	.007	73.7324	405.0676
	Autumn	13.0000	78.14858	.870	-152.6676	178.6676

*. The mean difference is significant at the .05 level.

Dependent Variable: Lead (Umgeni River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0014	.00739	.852	-.0131	.0143
	Autumn	.0166*	.00739	.039	.0009	.0323
	Winter	.0260*	.00739	.001	.0103	.0417
Summer	Springs	-.0014	.00739	.852	-.0171	.0131
	Autumn	.0152*	.00739	.047	.0003	.0309
	Winter	.0246*	.00739	.004	.0097	.0403
Autumn	Springs	-.0166*	.00739	.039	-.0323	-.0009
	Summer	-.0152*	.00739	.047	-.0309	-.0003
	Winter	.0094	.00739	.221	-.0063	.0251
Winter	Springs	-.0260*	.00739	.003	-.0417	-.0103
	Summer	-.0246*	.00739	.004	-.0403	-.0097
	Autumn	-.0094	.00739	.221	-.0251	.0063

*. The mean difference is significant at the .05 level.

Dependent Variable: Cadmium (Ortigan River)
LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	-.1056*	.04790	.042	-.2071	-.0041
	Autumn	.0650	.04790	.180	-.0357	.1671
	Winter	.0031	.04790	.944	-.0981	.1019
Summer	Springs	.1056*	.04790	.042	.0041	.2071
	Autumn	.1714*	.04790	.003	.0699	.2729
	Winter	.1090*	.04790	.037	.0076	.2105
Autumn	Springs	-.0650	.04790	.180	-.1671	.0357
	Summer	-.1714*	.04790	.003	-.2729	-.0699
	Winter	-.0624	.04790	.211	-.1639	.0391
Winter	Springs	.0034	.04790	.944	-.1049	.0981
	Summer	-.1090*	.04790	.037	-.2105	-.0075
	Autumn	.0624	.04790	.211	-.0391	.1639

*. The mean difference is significant at the .05 level.

Dependent Variable: Copper (Ortigan River)
LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0066	.00849	.449	-.0117	.0247
	Autumn	.0076	.00849	.380	-.0107	.0257
	Winter	-.0609*	.00901	.000	-.0801	-.0506
Summer	Springs	-.0066	.00849	.449	-.0247	.0117
	Autumn	.0010	.00849	.909	-.0191	.0171
	Winter	-.0633*	.00901	.000	-.0825	-.0440
Autumn	Springs	-.0076	.00849	.380	-.0257	.0107
	Summer	-.0010	.00849	.909	-.0171	.0191
	Winter	-.0623*	.00901	.000	-.0815	-.0430
Winter	Springs	.0609*	.00901	.000	.0506	.0801
	Summer	.0633*	.00901	.000	.0440	.0825
	Autumn	.0623*	.00901	.000	.0430	.0815

*. The mean difference is significant at the .05 level.

Dependent Variable: Aluminium (Beaches)
LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0076	.02830	.789	-.0701	.0549
	Autumn	-.1086*	.02830	.001	-.1911	-.0661
	Winter	.1786*	.02830	.000	.2423	.1173
Summer	Springs	-.0076	.02830	.789	-.0549	.0701
	Autumn	.1210*	.02785	.001	.1789	.0621
	Winter	.1722*	.02785	.000	.2311	.1133
Autumn	Springs	.1086*	.02830	.001	.0661	.1911
	Summer	-.1210*	.02785	.001	-.0621	-.1789
	Winter	.0512	.02785	.084	-.0101	.0077
Winter	Springs	-.1786*	.02830	.000	-.1173	-.2423
	Summer	-.1722*	.02785	.000	-.1133	-.2311
	Autumn	-.0512	.02785	.084	-.0077	-.1101

*. The mean difference is significant at the .05 level.

Dependent Variable: Copper (Beaches)
LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0020	.01646	.903	-.0371	.0330
	Autumn	-.0114	.01646	.497	-.0465	.0236
	Winter	.0619*	.01646	.002	.0969	.0268
Summer	Springs	-.0020	.01646	.903	-.0330	.0371
	Autumn	-.0094	.01652	.584	-.0426	.0237
	Winter	.0599*	.01652	.002	.0929	.0267
Autumn	Springs	.0114	.01646	.497	-.0236	.0465
	Summer	.0094	.01652	.584	-.0237	.0426
	Winter	.0504*	.01552	.005	.0835	.0173
Winter	Springs	-.0619*	.01646	.002	-.0268	-.0969
	Summer	-.0599*	.01552	.002	-.0267	-.0929
	Autumn	-.0504*	.01552	.005	-.0173	-.0835

*. The mean difference is significant at the .05 level.

Dependent Variable: Aluminium (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0005	.23579	.998	-.5132	.5142
	Autumn	-.8518*	.23579	.001	-1.3660	-.3376
	Winter	.0507	.23579	.833	-.5045	.4020
Summer	Springs	.0005	.23579	.998	-.5142	.5132
	Autumn	-.8523*	.23579	.001	-1.3660	-.3386
	Winter	.0512	.23579	.832	-.5650	.4625
Autumn	Springs	.8518*	.23579	.004	.3376	1.3660
	Summer	.8523*	.23579	.001	.3386	1.3660
	Winter	.9010*	.23579	.005	.2873	1.3147
Winter	Springs	-.0507	.23579	.833	-.4020	.5045
	Summer	-.0512	.23579	.832	-.4625	.5650
	Autumn	-.9010*	.23579	.005	-1.3147	-.2873

*. The mean difference is significant at the .05 level.

Dependent Variable: Copper (Umdloti River)

LSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Springs	Summer	.0000	.01656	1.000	-.0404	.0404
	Autumn	.0040*	.01656	.004	.0243	.1052
	Winter	-.0125	.01656	.515	-.0529	.0279
Summer	Springs	.0000	.01656	1.000	-.0404	.0404
	Autumn	.0040*	.01656	.004	.0243	.1052
	Winter	-.0125	.01656	.515	-.0529	.0279
Autumn	Springs	.0646*	.01656	.004	.1052	.0243
	Summer	-.0646*	.01656	.001	-.1052	-.0243
	Winter	-.0771*	.01656	.001	-.1177	-.0366
Winter	Springs	.0125	.01656	.515	-.0279	.0529
	Summer	.0125	.01656	.515	-.0279	.0529
	Autumn	.0771*	.01656	.001	.0366	.1177

*. The mean difference is significant at the .05 level.

Table B14: Diameter(s) of inhibition zones (mm) and classification of *E. coli* isolates as susceptible, intermediate susceptibility and resistant.

Isolate	Antibiotics																					
	CIP (5µg)		C (30µg)		TS (25µg)		RP (5µg)		NA (30µg)		CXM (30µg)		FOX (30µg)		CTX (30µg)		CRO (30µg)		GM (10µg)		TN (10µg)		AK (30µg)	
	θ		θ		θ		θ		θ		θ		θ		θ		θ		θ		θ		θ	
Umgeni River																								
1	28.5	S	23.5	S	19.0	S	9.00	R	17.0	I	16.5	I	20.5	S	24.5	S	25.5	S	18.0	S	18.0	S	17.0	S
2	34.0	S	23.5	S	18.0	S	-	R	16.5	I	16.5	I	20.5	S	20.5	I	24.0	S	18.5	S	17.0	S	15.5	I
3	28.0	S	21.5	S	-	R	-	R	14.0	I	14.5	R	22.0	S	22.5	I	20.0	I	15.5	S	14.5	I	14.5	R
4	26.0	S	24.0	S	18.0	S	-	R	14.5	I	15.5	I	21.0	S	21.5	I	24.0	S	16.0	S	15.5	S	15.5	I
5	29.5	S	20.5	S	-	R	-	R	18.0	I	17.0	I	22.5	S	23.5	S	24.0	S	15.5	S	16.0	S	15.5	I
6	27.5	S	23.5	S	-	R	-	R	14.0	I	13.5	R	20.5	S	17.0	I	20.5	I	15.5	S	15.0	S	13.0	R
7	31.0	S	18.0	S	20.5	S	8.00	R	18.0	I	20.5	S	23.0	S	25.0	S	29.0	S	17.5	S	17.5	S	17.5	S
8	28.5	S	-	R	-	R	-	R	16.5	I	18.5	S	23.0	S	23.0	S	27.5	S	19.5	S	17.0	S	17.5	S
9	37.5	S	19.5	S	21.0	S	-	R	19.5	S	19.0	S	23.0	S	25.0	S	29.0	S	18.5	S	17.0	S	18.0	S
10	24.0	S	21.0	S	22.5	S	-	R	17.5	I	19.5	S	18.0	S	28.5	S	28.0	S	17.5	S	15.0	S	17.5	S
11	31.5	S	20.0	S	19.5	S	-	R	17.0	I	17.5	I	21.5	S	24.0	S	30.5	S	16.0	S	16.0	S	16.0	I
12	26.5	S	19.0	S	21.5	S	-	R	16.5	I	19.0	S	21.0	S	22.5	I	25.5	S	16.0	S	16.0	S	15.0	I
13	35.5	S	20.5	S	19.5	S	-	R	18.5	I	19.0	S	21.5	S	24.0	S	25.5	S	16.0	S	17.5	S	14.5	R
14	31.5	S	22.5	S	-	R	-	R	18.5	I	16.5	I	23.0	S	23.0	S	24.5	S	21.0	S	16.0	S	16.5	I
15	24.5	S	22.0	S	17.5	S	-	R	19.0	S	21.0	S	24.0	S	20.0	I	21.5	S	17.5	S	16.0	S	13.5	R
16	26.5	S	15.5	I	18.0	S	-	R	19.5	S	18.5	S	18.0	S	22.5	I	29.5	S	15.0	S	17.0	S	12.5	R
17	30.0	S	23.5	S	-	R	-	R	17.5	I	19.5	S	20.0	S	28.0	S	28.5	S	16.5	S	14.5	I	14.5	R
18	32.0	S	24.0	S	-	R	-	R	14.0	I	20.5	S	22.5	S	29.0	S	29.5	S	16.5	S	16.0	S	15.0	I
19	28.5	S	16.0	I	21.0	S	9.50	R	20.5	S	16.5	I	20.0	S	23.0	S	31.0	S	13.0	I	18.0	S	17.5	S
20	32.0	S	22.5	S	19.5	S	-	R	16.5	I	15.5	I	20.5	S	20.0	I	25.5	S	19.5	S	15.0	S	18.0	S
21	25.0	S	-	R	18.5	S	-	R	14.0	I	15.5	I	20.0	S	25.5	S	15.5	I	17.5	S	17.5	S	18.0	S
22	28.0	S	-	R	21.5	S	-	R	16.0	I	15.0	I	23.0	S	26.0	S	25.0	S	22.0	S	14.5	I	12.5	R
23	29.5	S	21.5	S	-	R	-	R	16.0	I	19.5	S	21.0	S	24.5	S	27.5	S	14.0	I	14.0	I	16.5	I
24	37.0	S	18.0	S	16.5	S	-	R	20.0	S	19.0	S	15.0	I	16.0	I	30.5	S	18.5	S	18.5	S	15.0	I
25	38.0	S	15.5	I	14.5	I	8.50	R	18.0	I	20.5	S	23.5	S	20.0	I	32.0	S	20.5	S	16.5	S	20.0	S
26	34.0	S	22.0	S	-	R	8.00	R	14.5	I	13.5	R	22.5	S	25.0	S	28.0	S	19.5	S	16.0	S	18.5	S
27	31.5	S	23.0	S	15.0	I	-	R	15.0	I	16.5	I	16.5	I	29.0	S	29.5	S	15.0	S	14.5	I	13.0	R
28	35.0	S	21.0	S	18.5	S	-	R	17.5	I	16.0	I	22.0	S	24.5	S	27.5	S	16.5	S	17.0	S	16.5	I
29	37.5	S	20.0	S	9.50	R	-	R	18.0	I	17.5	I	21.5	S	28.0	S	26.5	S	19.0	S	18.0	S	14.5	R
30	26.0	S	21.0	S	-	R	-	R	16.0	I	18.0	S	21.0	S	24.5	S	26.0	S	16.0	S	17.0	S	14.0	R
31	35.5	S	20.0	S	22.5	S	-	R	18.5	I	19.0	S	21.0	S	26.0	S	25.5	S	23.0	S	18.0	S	19.5	S
32	34.0	S	20.5	S	20.0	S	-	R	17.5	I	20.5	S	23.5	S	27.5	S	26.5	S	18.5	S	18.0	S	17.5	S
33	30.5	S	-	R	8.00	R	8.00	R	14.5	I	23.0	S	25.0	S	30.0	S	31.5	S	20.0	S	20.5	S	17.0	S
34	34.5	S	-	R	-	R	-	R	13.5	R	19.0	S	21.5	S	27.0	S	24.5	S	20.5	S	20.5	S	21.5	S

Table B14/cont.

35	30.0	S	20.0	S	19.5	S	-	R	15.0	I	12.0	R	10.0	R	14.0	R	13.0	R	20.0	S	17.0	S	17.5	S
36	36.0	S	20.5	S	23.5	S	-	R	19.5	S	20.5	S	22.0	S	27.5	S	29.0	S	21.0	S	19.0	S	19.5	S
37	29.5	S	25.5	S	18.5	S	-	R	16.5	I	18.5	S	19.5	S	24.5	S	26.0	S	16.0	S	17.0	S	16.0	I
38	35.0	S	-	R	-	R	-	R	16.0	I	18.0	S	21.5	S	21.0	I	21.0	S	19.0	S	17.0	S	18.0	S
39	42.0	S	24.5	S	20.5	S	-	R	18.0	I	19.0	S	20.5	S	25.0	S	26.5	S	21.0	S	18.5	S	18.5	S
40	32.5	S	23.0	S	21.0	S	-	R	16.0	I	20.0	S	22.0	S	25.5	S	26.5	S	20.0	S	18.5	S	18.0	S
41	-	R	20.0	S	18.0	S	-	R	-	R	21.5	S	21.0	S	24.5	S	25.0	S	19.5	S	15.0	S	15.5	I
42	34.0	S	18.0	S	16.5	S	9.00	R	15.0	I	16.5	I	21.5	S	25.0	S	19.5	I	18.0	S	19.5	S	16.5	I
43	27.0	S	24.5	S	19.5	S	-	R	14.5	I	13.5	R	18.5	S	24.5	S	23.5	S	20.5	S	19.0	S	17.5	S
44	27.5	S	17.5	I	-	R	-	R	15.5	I	21.5	S	18.0	S	26.0	S	25.5	S	16.5	S	16.5	S	18.0	S
45	32.0	S	20.5	S	22.5	S	-	R	17.5	I	17.5	I	18.0	S	21.0	I	27.5	S	13.0	I	18.5	S	19.5	S
46	35.0	S	20.5	S	10.0	R	8.50	R	15.0	I	22.5	S	16.0	I	30.0	S	26.0	S	14.5	I	17.0	S	14.5	R
Beaches																								
1	29.5	S	18.5	S	20.5	S	-	R	20.0	S	17.5	I	21.0	S	29.0	S	21.5	S	12.5	R	18.0	S	15.5	I
2	33.5	S	23.5	S	14.5	I	-	R	20.5	S	16.5	I	20.0	S	24.0	S	32.5	S	16.0	S	19.0	S	16.0	I
3	22.5	S	17.5	I	18.5	S	-	R	13.0	R	23.5	S	21.0	S	16.5	I	18.5	I	15.5	S	16.0	S	15.5	I
4	20.5	I	-	R	21.5	S	-	R	-	R	19.5	S	19.5	S	24.0	S	25.5	S	16.0	S	18.0	S	15.5	I
5	31.5	S	23.5	S	15.5	I	-	R	15.5	I	19.5	S	22.5	S	22.5	I	30.5	S	19.5	S	15.0	S	21.5	S
6	32.0	S	20.5	S	15.5	I	-	R	16.0	I	19.0	S	25.5	S	23.5	S	29.5	S	20.0	S	15.5	S	16.5	I
7	38.5	S	20.0	S	8.50	R	-	R	19.5	S	20.5	S	24.5	S	20.5	I	32.0	S	18.5	S	18.5	S	17.5	S
8	40.5	S	21.5	S	-	R	9.00	R	14.5	I	15.5	I	20.5	S	17.0	I	24.5	S	14.5	I	14.5	I	18.0	S
9	26.0	S	24.5	S	18.5	S	-	R	19.0	S	20.5	S	22.0	S	19.0	I	26.5	S	14.0	I	17.0	S	18.5	S
10	23.5	S	20.5	S	22.0	S	-	R	-	R	18.5	S	23.5	S	19.5	I	27.0	S	21.5	S	16.0	S	10.0	R
11	22.5	S	18.0	S	19.5	S	-	R	18.5	I	21.0	S	21.0	S	18.5	I	33.5	S	17.5	S	16.0	S	19.5	S
12	34.5	S	26.5	S	-	R	8.00	R	13.0	R	19.5	S	22.5	S	23.5	S	25.5	S	19.0	S	20.5	S	14.5	R
13	34.5	S	20.5	S	20.0	S	-	R	15.0	I	14.5	R	22.0	S	23.0	S	32.0	S	22.0	S	17.0	S	17.5	S
14	25.0	S	18.5	S	12.0	I	-	R	15.0	I	15.5	I	22.5	S	21.5	I	26.5	S	18.0	S	14.0	I	15.5	I
15	26.5	S	18.0	S	16.5	S	10.0	R	15.5	I	19.0	S	20.5	S	25.0	S	27.5	S	16.0	S	17.0	S	18.0	S
16	24.0	S	22.0	S	-	R	8.00	R	19.5	S	16.5	I	22.0	S	23.0	S	25.5	S	15.0	S	18.5	S	14.5	R
17	33.0	S	16.5	I	18.5	S	-	R	16.5	I	15.0	I	24.5	S	21.5	I	30.5	S	19.0	S	16.5	S	17. 0	S
18	35.5	S	23.0	S	18.0	S	-	R	20.0	S	15.5	I	21.0	S	20.5	I	24.5	S	14.0	I	16.5	S	11.5	R
19	27.0	S	18.5	S	18.0	S	-	R	14.5	I	19.5	S	18.0	S	27.5	S	31.5	S	16.0	S	16.5	S	15.0	I
20	21.0	S	19.0	S	-	R	-	R	16.5	I	14.5	R	18.0	S	16.5	I	29.5	S	13.0	I	16.0	S	13.0	R
21	21.5	S	23.0	S	15.5	I	9.50	R	18.0	I	20.5	S	21.0	S	29.0	S	20.5	I	17.5	S	14.5	I	16.5	I
22	32.0	S	24.0	S	21.5	S	-	R	18.0	I	22.0	S	19.5	S	28.5	S	24.5	S	20.0	S	12.0	R	19.5	S
23	26.5	S	17.0	I	-	R	-	R	19.5	S	17.5	I	20.0	S	22.5	I	25.5	S	15.5	S	15.0	S	16.0	I
24	40.0	S	16.5	I	17.5	S	-	R	22.5	S	21.5	S	25.0	S	24.0	S	27.0	S	19.0	S	13.5	I	14.0	R
25	31.5	S	18.5	S	19.0	S	-	R	15.5	I	19.5	S	17.5	I	18.5	I	18.5	I	18.5	S	19.0	S	13.5	R
26	28.0	S	18.0	S	20.5	S	-	R	13.5	R	21.5	S	22.5	S	26.5	S	20.5	I	13.5	I	17.0	S	12.0	R
27	24.0	S	22.5	S	-	R	8.00	R	23.0	S	21.5	S	22.5	S	24.0	S	31.5	S	15.0	S	17.5	S	19.0	S
28	22.5	S	15.0	I	20.5	S	8.00	R	19.0	S	13.5	R	24.0	S	30.5	S	26.5	S	16.5	S	20.0	S	16.5	I
29	37.5	S	25.0	S	-	R	9.50	R	14.5	I	18.5	S	19.0	S	19.5	I	26.0	S	20.0	S	15.5	S	12.5	R

Table B14/cont.

30	31.0	S	20.0	S	14.0	I	-	R	19.5	S	23.0	S	16.5	I	23.5	S	19.5	I	19.5	S	15.5	S	17.0	S
31	29.0	S	21.5	S	14.5	I	-	R	23.0	S	19.5	S	20.5	S	21.0	I	28.0	S	14.0	I	13.0	I	17.0	S
Umdloti River																								
1	23.5	S	16.5	I	13.5	I	-	R	17.0	I	19.5	S	23.0	S	26.5	S	29.5	S	15.5	S	14.0	I	12.0	R
2	34.0	S	14.0	I	16.5	S	-	R	24.0	S	14.0	R	20.5	S	23.5	S	25.5	S	21.5	S	17.0	S	14.5	R
3	28.5	S	21.0	S	-	R	-	R	18.5	I	23.5	S	22.5	S	15.5	I	24.0	S	16.0	S	16.0	S	13.0	R
4	29.5	S	23.0	S	-	R	-	R	15.5	I	16.5	I	21.5	S	30.0	S	21.5	S	17.5	S	18.0	S	15.5	I
5	30.0	S	20.0	S	22.0	S	8.50	R	18.0	I	17.0	I	18.5	S	21.5	I	32.0	S	15.0	S	20.0	S	14.0	R
6	20.0	I	15.5	I	14.0	I	-	R	16.5	I	19.5	S	18.0	S	25.5	S	23.5	S	17.5	S	13.0	I	16.5	I
7	33.5	S	13.0	I	14.5	I	-	R	19.0	S	17.5	I	25.0	S	16.5	I	20.5	I	21.5	S	19.0	S	18.5	S
8	35.0	S	24.0	S	15.0	I	-	R	22.5	S	14.0	R	26.0	S	27.5	S	21.0	S	14.5	I	18.0	S	18.0	S
9	27.0	S	17.5	I	19.5	S	-	R	13.5	R	16.5	I	24.5	S	22.5	I	28.5	S	19.5	S	19.0	S	14.0	R
10	21.0	S	25.5	S	23.0	S	9.50	R	16.5	I	21.0	S	19.0	S	29.0	S	25.5	S	20.0	S	18.0	S	10.5	R
11	36.5	S	26.0	S	18.5	S	-	R	18.5	I	16.0	I	15.0	I	17.5	I	26.0	S	20.0	S	15.0	S	16.0	I
12	31.0	S	18.5	S	18.5	S	-	R	16.0	I	19.5	S	19.0	S	24.5	S	30.0	S	21.5	S	17.5	S	13.5	R
13	38.5	S	13.5	I	8.50	R	-	R	23.0	S	24.0	S	18.5	S	19.0	I	19.5	I	13.5	I	20.5	S	19.0	S
14	34.5	S	24.0	S	-	R	-	R	19.5	S	24.0	S	24.0	S	18.5	I	24.5	S	16.5	S	13.5	I	17.0	S
15	40.5	S	19.0	S	20.0	S	9.00	R	20.0	S	23.5	S	22.5	S	31.0	S	21.5	S	14.5	S	14.5	I	17.5	S
16	25.0	S	19.0	S	15.5	I	9.00	R	19.5	S	14.0	R	15.5	I	24.5	S	26.0	S	23.5	S	16.0	S	16.0	I
17	31.5	S	16.0	I	13.0	I	-	R	17.5	I	16.5	I	22.0	S	29.5	S	18.5	I	22.5	S	22.5	S	12.0	R
18	22.5	S	14.0	I	22.5	S	-	R	21.5	S	18.0	S	23.5	S	18.5	I	19.0	I	19.5	S	16.5	S	12.5	R
19	27.0	S	22.5	S	-	R	8.50	R	22.5	S	19.5	S	17.5	I	21.5	I	25.0	S	15.5	S	13.0	I	13.0	R

* Values represent the average of duplicate readings taken from the diameter (θ) of inhibition zones while letters indicate the classification of each isolate as susceptible (S), intermediate susceptibility (I) or resistant (R) according to the interpretative criteria recommended by CLSI (2009). No zone of inhibition is represented by (-).

Antibiotics			
CIP(5µg)	Ciprofloxacin	**FOX (30µg)**	Cefoxitin
C (30µg)	Chloramphenicol	**CTX (30µg)**	Cefotaxime
TS (25µg)	Cotrimoxazole	**CRO (30µg)**	Ceftriaxone
RP (5µg)	Rifampicin	**GM (10µg)**	Gentamicin
NA (30µg)	Nalidixic acid	**TN (10µg)**	Tobramycin
CXM (30 µg)	Cefuroxime	**AK (30µg)**	Amikacin

Table B15: Diameter(s) of inhibition zones (mm) and classification of *E. coli* isolates as susceptible, intermediate susceptibility and resistant.

Isolate	Antibiotics																	
	S (25µg)		T (30µg)		AP (10µg)		PG (10µg)		AUG (30µg)		E (15µg)		TM (5µg)		FC (10µg)		CD (2µg)	
	θ		θ		θ		θ		θ		θ		θ		θ		θ	
Umgeni River																		
1	15.5	S	11.0	R	15.5	I	7.50	R	18.0	S	12.5	R	-	R	-	R	-	R
2	18.5	S	15.5	S	-	R	-	R	18.5	S	10.0	R	-	R	-	R	-	R
3	13.0	I	-	R	-	R	-	R	17.5	I	-	R	-	R	-	R	-	R
4	17.5	S	14.5	I	-	R	-	R	15.0	I	9.00	R	-	R	-	R	-	R
5	20.0	S	-	R	14.5	I	-	R	15.5	I	-	R	-	R	-	R	-	R
6	-	R	-	R	13.0	R	-	R	15.0	I	-	R	-	R	-	R	-	R
7	14.5	I	16.0	S	-	R	8.00	R	17.0	I	8.00	R	-	R	-	R	-	R
8	15.5	S	-	R	15.0	I	-	R	15.5	I	-	R	-	R	-	R	-	R
9	16.0	S	15.0	S	-	R	-	R	19.5	S	-	R	-	R	-	R	-	R
10	18.5	S	18.5	S	-	R	-	R	20.5	S	-	R	-	R	-	R	-	R
11	-	R	-	R	-	R	-	R	17.0	I	11.0	R	-	R	-	R	-	R
12	19.0	S	19.5	S	16.5	I	-	R	18.5	S	-	R	-	R	-	R	-	R
13	14.0	I	-	R	13.5	R	7.00	R	18.0	S	9.00	R	-	R	-	R	-	R
14	-	R	14.5	I	12.0	R	-	R	-	R	-	R	-	R	-	R	-	R
15	-	R	14.0	I	11.5	R	-	R	18.5	S	-	R	-	R	-	R	-	R
16	-	R	18.5	S	-	R	-	R	15.0	I	-	R	-	R	-	R	-	R
17	11.0	R	16.0	S	-	R	-	R	16.0	I	10.5	R	-	R	-	R	-	R
18	16.5	S	7.00	R	-	R	-	R	21.0	S	-	R	-	R	-	R	-	R
19	-	R	12.0	I	-	R	-	R	17.5	I	-	R	-	R	-	R	-	R
20	18.0	S	13.5	I	-	R	-	R	16.5	I	9.50	R	-	R	-	R	-	R
21	18.0	S	16.5	S	-	R	8.00	R	15.5	I	-	R	-	R	-	R	-	R
22	17.0	S	15.5	S	-	R	8.00	R	17.0	I	8.50	R	-	R	-	R	-	R
23	-	R	15.0	S	14.5	I	-	R	16.5	I	10.0	R	-	R	-	R	-	R
24	19.0	S	12.0	I	13.0	R	-	R	17.0	I	-	R	-	R	-	R	-	R
25	15.5	S	19.5	S	17.5	S	-	R	22.0	S	8.50	R	-	R	-	R	-	R
26	16.5	S	-	R	-	R	-	R	16.0	I	-	R	-	R	-	R	-	R
27	14.0	I	18.0	S	11.0	R	-	R	18.5	S	-	R	-	R	-	R	-	R
28	17.0	S	18.0	S	9.00	R	-	R	17.0	I	9.00	R	-	R	-	R	-	R
29	-	R	-	R	12.0	R	-	R	18.0	S	-	R	-	R	-	R	-	R
30	-	R	-	R	-	R	9.00	R	20.0	S	8.50	R	-	R	-	R	-	R
31	12.5	I	19.5	S	-	R	8.50	R	17.5	I	9.00	R	-	R	-	R	-	R
32	16.5	S	15.5	S	18.5	S	-	R	16.0	I	-	R	-	R	-	R	-	R
33	11.0	R	-	R	16.5	I	-	R	-	R	-	R	-	R	-	R	-	R
34	14.5	I	-	R	21.0	S	-	R	14.0	I	8.50	R	-	R	-	R	-	R

Table B15/cont.

35	19.5	S	15.0	S	20.0	S	-	R	-	R	-	R	-	R	-	R	-	R
36	21.0	S	18.0	S	-	R	-	R	-	R	9.00	R	-	R	-	R	-	R
37	13.5	I	-	R	15.5	I	-	R	21.0	S	8.00	R	-	R	-	R	-	R
38	17.0	S	-	R	16.0	I	-	R	-	R	8.00	R	-	R	-	R	-	R
39	16.0	S	-	R	-	R	8.50	R	23.0	S	7.00	R	-	R	-	R	-	R
40	14.0	I	-	R	14.5	I	9.50	R	22.0	S	10.0	R	-	R	-	R	-	R
41	-	R	17.5	S	14.0	I	-	R	18.0	S	-	R	-	R	-	R	-	R
42	13.0	I	16.0	S	-	R	-	R	16.5	I	-	R	-	R	-	R	-	R
43	12.5	I	19.5	S	-	R	-	R	20.0	S	-	R	-	R	-	R	-	R
44	-	R	20.0	S	14.0	I	-	R	17.5	I	-	R	-	R	-	R	-	R
45	14.5	I	-	R	-	R	-	R	-	R	9.50	R	-	R	-	R	-	R
46	-	R	-	R	-	R	10.0	R	21.0	S	-	R	-	R	-	R	-	R
Beaches																		
1	16.5	S	-	R	-	R	8.50	R	19.0	S	-	R	-	R	-	R	-	R
2	15.5	S	-	R	15.5	I	-	R	14.5	I	8.50	R	-	R	-	R	-	R
3	-	R	-	R	16.0	I	-	R	16.0	I	-	R	-	R	-	R	-	R
4	15.5	S	-	R	-	R	-	R	11.0	R	-	R	-	R	-	R	-	R
5	-	R	-	R	14.5	I	-	R	9.00	R	-	R	-	R	-	R	-	R
6	-	R	-	R	14.0	I	-	R	18.0	S	-	R	-	R	-	R	-	R
7	17.0	S	20.5	S	-	R	-	R	20.5	S	-	R	-	R	-	R	-	R
8	-	R	-	R	-	R	-	R	11.0	R	-	R	-	R	-	R	-	R
9	11.0	R	-	R	14.0	I	7.50	R	16.0	I	8.00	R	-	R	-	R	-	R
10	16.5	S	8.50	R	-	R	-	R	19.0	S	-	R	-	R	-	R	-	R
11	17.0	S	11.5	R	-	R	-	R	17.0	I	-	R	-	R	-	R	-	R
12	15.0	S	15.5	S	11.5	R	-	R	19.5	S	-	R	-	R	-	R	-	R
13	11.5	R	15.0	S	15.0	I	-	R	15.5	I	10.5	R	-	R	-	R	-	R
14	9.00	R	15.0	S	-	R	-	R	-	R	-	R	-	R	-	R	-	R
15	-	R	15.0	S	14.5	I	7.00	R	-	R	-	R	-	R	-	R	-	R
16	9.00	R	14.0	I	-	R	-	R	19.5	S	-	R	-	R	-	R	-	R
17	14.0	I	12.5	I	-	R	-	R	20.0	S	-	R	-	R	-	R	-	R
18	13.5	I	16.0	S	17.0	S	-	R	19.5	S	-	R	-	R	-	R	-	R
19	15.5	S	18.0	S	-	R	-	R	-	R	-	R	-	R	-	R	-	R
20	17.5	S	19.5	S	19.0	S	10.5	R	21.5	S	-	R	-	R	-	R	-	R
21	14.5	I	11.0	R	11.0	R	-	R	15.5	I	-	R	-	R	-	R	-	R
22	14.5	I	11.0	R	16.0	I	-	R	19.5	S	-	R	-	R	-	R	-	R
23	15.5	S	-	R	19.5	S	-	R	11.5	R	-	R	-	R	-	R	-	R
24	15.0	S	13.5	I	18.0	S	-	R	14.0	I	-	R	-	R	-	R	-	R
25	11.5	R	21.0	S	-	R	-	R	19.0	S	-	R	-	R	-	R	-	R
26	9.50	R	12.5	I	-	R	-	R	13.5	R	10.0	R	-	R	-	R	-	R
27	16.5	S	16.5	S	15.5	I	8.50	R	-	R	-	R	-	R	-	R	-	R
28	8.50	R	14.5	I	-	R	-	R	16.0	I	-	R	-	R	-	R	-	R
29	16.0	S	19.8	S	-	R	-	R	8.00	R	-	R	-	R	-	R	-	R

Table B15/cont.

30	17.5	S	-	R	-	R	8.00	R	19.0	S	-	R	-	R	-	R	-	R
31	13.5	I	19.5	S	-	R	8.00	R	20.0	S	10.0	R	-	R	-	R	-	R
Umdloti River																		
1	13.5	I	19.0	S	15.0	I	8.50	R	15.5	I	-	R	-	R	-	R	-	R
2	15.5	S	20.0	S	9.50	R	7.50	R	14.0	I	-	R	-	R	-	R	-	R
3	17.0	S	16.0	S	16.5	I	7.00	R	10.0	R	-	R	-	R	-	R	-	R
4	22.0	S	14.5	I	-	R	-	R	20.0	S	8.00	R	-	R	-	R	-	R
5	11.0	R	11.5	R	-	R	-	R	13.5	R	-	R	-	R	-	R	-	R
6	15.0	S	18.5	S	20.0	S	9.00	R	13.0	R	-	R	-	R	-	R	-	R
7	17.5	S	12.5	I	18.0	S	-	R	11.5	R	-	R	-	R	-	R	-	R
8	20.0	S	16.5	S	17.0	S	-	R	19.0	S	-	R	-	R	-	R	-	R
9	10.5	R	15.0	S	17.0	S	-	R	14.5	I	9.00	R	-	R	-	R	-	R
10	13.0	I	12.0	I	19.5	S	10.5	R	16.5	I	-	R	-	R	-	R	-	R
11	21.0	S	-	R	19.5	S	-	R	18.0	S	-	R	-	R	-	R	-	R
12	21.0	S	-	R	15.0	I	-	R	21.5	S	-	R	-	R	-	R	-	R
13	14.0	I	17.5	S	-	R	-	R	11.5	R	-	R	-	R	-	R	-	R
14	18.0	S	16.0	S	-	R	-	R	-	R	-	R	-	R	-	R	-	R
15	16.5	S	-	R	-	R	8.50	R	-	R	-	R	-	R	-	R	-	R
16	-	R	18.5	S	19.0	S	-	R	12.5	R	-	R	-	R	-	R	-	R
17	20.5	S	16.5	S	16.5	I	7.00	R	17.0	I	-	R	-	R	-	R	-	R
18	12.0	I	21.0	S	-	R	-	R	21.0	S	10.5	R	-	R	-	R	-	R
19	14.5	I	20.0	S	16.0	I	-	R	9.50	R	-	R	-	R	-	R	-	R

* Values represent the average of duplicate readings taken from the diameter (θ) of inhibition zones while letters indicate the classification of each isolate as susceptible (S), intermediate susceptibility (I) or resistant (R) according to the interpretative criteria recommended by CLSI (2009). No zone of inhibition is represented by (-).

Antibiotics			
S (25µg)	Streptomycin	**E (15µg)**	Erythromycin
T (30µg)	Tetracycline	**TM (5µg)**	Trimethoprim
AP (10µg)	Ampicillin	**FC (10µg)**	Fusidic acid
PG (10µg)	Penicillin G	**CD (2µg)**	Clindamycin
AUG (30µg)	Augmentin	-	-

Table B16: Diameter(s) of inhibition zones and classification of *V. cholerae* isolates as susceptible, intermediate susceptibility and resistant.

Isolate	Antibiotics																			
	CIP (5µg)		C (30µg)		TS (25µg)		RP (5µg)		NA (30µg)		CXM (30µg)		FOX (30µg)		CTX (30µg)		CRO (30µg)		GM (10µg)	
	θ		θ		θ		θ		θ		θ		θ		θ		θ		θ	
Umgeni River																				
1	29.5	S	11.0	R	-	R	18.0	I	24.5	S	18.5	S	14.5	R	20.0	I	20.5	I	10.5	R
2	28.5	S	10.5	R	-	R	14.0	R	25.5	S	21.0	S	15.5	I	19.5	I	20.0	I	14.0	I
3	26.5	S	10.0	R	-	R	17.5	I	25.5	S	16.5	I	16.5	I	21.5	I	21.0	S	14.5	I
4	21.0	S	10.0	R	-	R	14.0	R	21.5	S	13.0	R	14.5	R	22.5	I	21.5	S	12.5	R
5	30.5	S	11.0	R	-	R	14.5	R	23.5	S	20.5	S	14.0	R	19.5	I	18.0	I	16.5	S
6	23.5	S	10.0	R	-	R	17.0	I	26.0	S	11.5	R	11.5	R	22.5	I	20.5	I	13.0	I
7	25.0	S	9.50	R	-	R	14.0	R	23.5	S	13.5	R	13.0	R	15.5	I	17.0	I	13.5	I
8	29.5	S	9.00	R	-	R	15.5	R	26.0	S	14.0	R	12.0	R	17.5	I	19.0	I	13.0	I
9	21.0	S	11.0	R	-	R	15.5	R	24.0	S	14.5	R	17.0	I	23.5	S	25.0	S	15.0	S
10	30.0	S	11.5	R	-	R	14.5	R	25.0	S	20.5	S	15.0	I	21.0	I	24.0	S	18.0	S
11	29.5	S	12.5	R	-	R	17.5	I	25.0	S	20.0	S	20.5	S	11.0	R	14.0	I	16.5	S
12	30.0	S	13.5	I	-	R	19.5	I	-	R	21.0	S	16.5	I	25.5	S	27.5	S	18.5	S
13	24.0	S	15.5	I	27.5	S	13.5	R	28.5	S	11.5	R	14.0	R	12.5	R	24.0	S	21.0	S
14	26.0	S	19.5	S	-	R	14.0	R	12.0	R	12.5	R	15.5	I	19.5	I	20.0	I	17.0	S
15	24.5	S	10.5	R	-	R	16.5	R	25.5	S	-	R	13.5	R	20.5	I	18.0	I	15.0	S
16	27.0	S	9.50	R	-	R	13.5	R	24.5	S	16.0	I	10.5	R	16.5	I	21.0	S	20.5	S
17	29.5	S	13.0	I	-	R	12.0	R	23.0	S	18.5	S	17.0	I	15.5	I	15.0	I	16.5	S
18	28.0	S	14.5	I	-	R	11.5	R	-	R	19.0	S	16.5	I	22.0	I	14.5	I	16.0	S
Beaches																				
1	24.5	S	12.5	R	18.5	S	14.5	R	19.5	S	20.5	S	13.5	R	23.5	S	20.5	I	16.5	S
2	27.5	S	10.5	R	-	R	15.0	R	23.5	S	19.5	S	16.5	I	21.5	I	19.5	I	14.5	I
3	26.5	S	9.50	R	-	R	18.5	I	26.5	S	16.5	I	18.0	S	18.5	I	20.0	I	13.5	I
4	24.5	S	11.5	R	-	R	11.5	R	21.5	S	14.5	R	14.5	R	16.0	I	24.5	S	10.5	R
5	30.5	S	16.5	I	-	R	10.5	R	25.5	S	20.5	S	9.00	R	21.0	I	15.5	I	18.5	S
6	22.5	S	12.5	R	-	R	16.5	R	18.5	I	11.5	R	12.5	R	24.5	S	21.0	S	20.5	S
7	28.5	S	9.50	R	-	R	16.0	R	-	R	18.5	S	16.5	I	24.0	S	19.0	I	14.5	I
8	29.0	S	12.5	R	-	R	20.5	S	13.5	R	-	R	13.0	R	19.5	I	16.5	I	21.0	S
9	19.5	I	18.5	S	-	R	12.5	R	20.5	S	13.5	R	20.5	S	17.5	I	17.5	I	16.5	S
10	23.5	S	11.5	R	-	R	14.5	R	24.5	S	19.5	S	11.5	R	21.0	I	21.5	S	15.5	S
11	20.5	S	16.5	I	17.0	S	13.0	R	12.5	R	17.5	I	18.5	S	23.5	S	28.5	S	12.5	R
12	29.5	S	8.50	R	-	R	15.5	R	-	R	21.5	S	12.5	R	18.5	I	25.5	S	19.5	S
13	21.5	S	10.5	R	22.0	S	18.5	I	-	R	18.5	S	9.50	R	22.5	I	19.0	I	20.0	S
14	30.0	S	9.50	R	-	R	17.0	I	23.	S	22.0	S	-	R	15.5	I	15.0	I	17.5	S
15	18.5	I	10.5	R	-	R	22.0	S	25.5	S	12.5	R	19.5	S	16.5	I	16.5	I	11.5	R

Table B16/cont.

16	24.5	S	13.5	I	-	R	11.5	R	14.5	I	19.5	S	12.0	R	14.5	R	14.5	I	9.50	R
17	16.0	I	16.5	I	-	R	12.5	R	27.0	S	17.5	I	16.5	I	12.5	R	15.0	I	10.5	R
18	27.5	S	14.5	I	-	R	19.5	S	12.5	R	13.5	R	12.0	R	14.5	R	22.0	S	13.0	I
19	26.5	S	19.0	S	11.5	I	21.0	S	-	R	21.0	S	16.0	I	23.0	S	23.0	S	16.5	S
20	23.5	S	13.5	I	-	R	11.0	R	26.5	S	20.0	S	14.5	R	19.0	I	21.0	S	12.5	R
21	14.5	R	10.0	R	-	R	13.5	R	28.5	S	11.5	R	-	R	17.0	I	20.5	I	11.5	R
22	30.0	S	10.5	R	-	R	11.5	R	23.5	S	12.5	R	19.5	S	13.0	R	18.5	I	13.5	I
23	28.0	S	11.5	R	-	R	19.0	I	-	R	18.0	S	22.0	S	13.5	R	20.0	I	18.0	S
24	25.5	S	8.50	R	16.0	S	17.5	I	17.5	I	17.0	I	17.6	I	17.5	I	24.5	S	14.5	I
25	26.5	S	9.50	R	18.5	S	15.0	R	24.5	S	22.5	S	15.5	I	24.5	S	17.5	I	12.0	R
26	18.5	I	8.50	R	-	R	17.5	I	22.0	S	18.5	S	18.0	S	16.5	I	-	R	17.5	S
27	31.5	S	14.0	I	-	R	15.0	R	24.5	S	14.5	R	13.5	R	22.5	I	-	R	13.0	I
28	25.5	S	13.5	I	-	R	18.5	I	21.0	S	16.5	I	8.50	R	-	R	25.5	S	20.0	S
29	24.5	S	10.5	R	-	R	20.5	S	19.5	S	13.0	R	23.5	S	16.0	I	23.5	S	16.0	S
30	16.5	I	9.50	R	12.5	I	16.5	R	-	R	20.0	R	-	R	20.0	I	20.0	I	12.5	R
31	28.5	S	10.5	R	-	R	19.5	I	24.0	S	17.5	I	12.0	R	15.0	I	15.5	I	14.5	I
32	24.5	S	10.0	R	-	R	15.5	R	25.5	S	13.5	R	14.5	R	18.0	I	21.5	S	18.5	S
33	26.5	S	8.50	R	-	R	15.0	R	20.0	S	18.0	S	21.5	S	24.0	S	18.5	I	11.5	R
34	32.0	S	10.5	R	-	R	11.0	R	26.5	S	-	R	15.0	I	19.5	I	21.0	S	13.5	I
35	22.5	S	11.5	R	-	R	13.5	R	22.5	S	21.0	S	-	R	19.0	I	21.5	S	-	R
Umdloti River																				
1	17.5	I	11.0	R	-	R	17.0	I	-	R	20.5	S	22.0	S	17.5	I	21.5	S	18.0	S
2	25.5	S	12.5	R	-	R	21.0	S	26.5	S	-	R	19.0	S	13.5	R	20.5	I	13.0	I
3	29.5	S	14.5	I	13.5	I	18.0	I	21.5	S	22.0	S	11.5	R	22.5	I	18.58	I	19.0	S
4	26.5	S	10.0	R	18.0	S	-	R	18.5	I	19.5	S	24.0	S	16.5	I	23.5	S	11.0	R
5	23.5	S	11.5	R	15. 5	I	20.0	S	17.0	I	12.5	R	16.5	I	10.5	R	19.5	I	8.50	R
6	29.0	S	8.50	R	-	R	19.5	I	21.5	S	16.5	I	13.5	R	21.5	I	17.5	I	17.5	S
7	25.5	S	11.5	R	-	R	17.5	I	-	R	18.0	S	12.5	R	14.5	R	16.5	I	20.0	S
8	32.0	S	9.50	R	-	R	19.0	I	24.0	S	21.0	S	8.50	R	9.50	R	20.5	I	16.0	S
9	20.5	I	8.50	R	-	R	16.5	R	27.5	S	15.5	I	14.5	R	18.5	I	22.5	S	25.5	S
10	27.0	S	8.50	R	23.0	S	18.5	I	-	R	11.5	R	9.50	R	21.0	I	15.5	I	14.5	I
11	27.0	S	8.50	R	-	R	-	R	20.5	S	19.5	S	13.0	R	15.5	I	24.0	S	16.0	S
12	23.5	S	16.0	I	-	R	22.0	S	22.5	S	-	R	17.5	I	12.5	R	18.5	I	15.5	S
13	24.5	S	12.5	R	-	R	17.0	I	25.5	S	14.0	R	20.0	S	9.00	R	15.0	I	10.5	R
14	30.0	S	14.5	I	-	R	20.0	S	29.0	S	18.0	S	18.5	S	8.50	R	21.5	S	9.00	R
15	28.5	S	13.5	I	-	R	13.0	R	25.0	S	13.5	R	19.5	S	18.0	I	25.5	S	16.5	S
16	18.0	I	11.5	R	-	R	-	R	21.0	S	16.5	I	14.5	R	21.5	I	21.0	S	14.0	I
17	31.5	S	9.00	R	-	R	16.5	R	23.5	S	19.0	S	16.5	I	13.5	R	17.5	I	23.0	S
18	30.5	S	15.5	I	-	R	19.5	I	22.5	S	12.5	R	8.50	R	15.0	I	20.5	I	17.5	S
19	20.0	I	10.0	R	-	R	12.0	R	24.5	S	20.0	S	15.0	I	20.58	I	16.0	I	18.5	S
20	16.5	I	8.00	R	25.5	S	10.5	R	26. 5	S	13.5	R	20.0	S	12.5	R	23.5	S	10.0	R
21	28.5	S	10.5	R	-	R	12.0	R	-	R	15.0	I	12.5	R	16.5	I	15.5	I	20.5	S

Table B16/cont.

22	29.0	S	13.0	I	-	R	21.5	S	23.0	S	11.0	R	14.0	R	12.5	R	24.0	S	13.5	I
23	26.5	S	8.00	R	-	R	13.5	R	24.5	S	-	R	8.00	R	-	R	20.5	I	22.5	S
24	21.5	S	11.0	R	-	R	17.5	I	28.5	S	19.5	S	11.5	R	12.5	R	20.5	I	16.5	S
25	29.5	S	14.5	I	-	R	19.0	I	25.0	S	17.5	I	15.5	I	-	R	20.0	I	11.5	R
26	19.5	I	9.50	R	27.0	S	17.0	I	-	R	14.0	R	19.0	S	20.5	I	19.5	I	18.0	S
27	27.5	S	12.0	R	-	R	20.0	R	16.5	I	16.0	I	9.50	R	19.0	I	18.5	I	21.0	S
28	24.0	S	11.5	R	-	R	18.5	I	14.5	I	14.0	R	16.5	I	17.5	I	20.0	I	9.50	R
29	15.5	R	10.0	R	-	R	16.0	R	27.0	S	14.5	R	13.5	R	15.0	I	21.5	S	19.5	S

* Values represent the average of duplicate readings taken from the diameter (θ) of inhibition zones while letters indicate the classification of each isolate as susceptible (S), intermediate susceptibility (I) or resistant (R) according to the interpretative criteria recommended by CLSI (2009). No zone of inhibition is represented by (-).

Antibiotics			
CIP(5µg)	Ciprofloxacin	**CXM (30 µg)**	Cefuroxime
C (30µg)	Chloramphenicol	**FOX (30µg)**	Cefoxitin
TS (25µg)	Cotrimoxazole	**CTX (30µg)**	Cefotaxime
RP (5µg)	Rifampicin	**CRO (30µg)**	Ceftriaxone
NA (30µg)	Nalidixic acid	**GM (10µg)**	Gentamicin

Table B17: Diameter(s) of inhibition zones and classification of *V. cholerae* isolates as susceptible, intermediate susceptibility and resistant.

Isolate	Antibiotics																			
	TN (10µg)		AK (30µg)		S (25µg)		T (30µg)		AP (10µg)		PG (10µg)		AUG (30µg)		E (15µg)		MUP (5µg)		TM (5µg)	
	θ		θ		θ		θ		θ		θ		θ		θ		θ		θ	
Umgeni River																				
1	13.5	I	14.5	R	20.0	S	21.0	S	19.0	S	19.0	R	22.0	S	14.5	R	13.5	R	-	R
2	13.0	I	15.0	I	-	R	9.00	R	10.0	R	-	R	12.0	R	12.0	R	14.5	S	-	R
3	11.5	R	13.0	R	-	R	10.0	R	11.0	R	11.0	R	10.5	R	15.0	R	15.5	S	-	R
4	16.0	S	18.5	S	-	R	10.0	R	13.0	R	10.5	R	14.5	I	9.00	R	12.5	R	16.5	S
5	10.5	R	15.0	I	17.5	S	10.5	R	14.5	I	9.50	R	13.0	R	15.5	R	-	R	-	R
6	11.5	R	12.5	R	-	R	9.00	R	11.5	R	16.0	R	13.0	R	15.5	R	13.5	R	-	R
7	12.5	R	10.5	R	-	R	8.50	R	10.5	R	-	R	12.5	R	14.0	R	15.5	S	-	R
8	13.5	I	14.5	R	18.0	S	13.5	I	10.0	R	18.5	R	9.00	R	14.5	R	15.0	S	-	R
9	16.0	S	16.5	I	14.5	I	11.5	R	15.5	I	10.5	R	10.5	R	11.0	R	15.5	S	14.0	I
10	24.5	S	15.0	I	-	R	9.50	R	12.5	R	10.0	R	18.0	S	13.0	R	12.5	R	11.5	I
11	20.0	S	20.0	S	-	R	20.0	S	19.5	S	12.5	R	10.5	R	15.5	R	25.0	S	15.0	I
12	15.0	S	15.5	I	-	R	14.5	I	16.5	I	15.5	R	8.50	R	18.0	I	20.5	S	-	R
13	14.0	I	15.0	I	-	R	10.5	R	18.5	S	9.50	R	14.0	I	24.0	S	-	R	-	R
14	10.5	R	9.50	R	-	R	8.00	R	20.0	S	11.0	R	13.5	R	13.0	R	12.5	R	-	R
15	22.5	S	19.0	S	-	R	12.5	I	19.0	S	-	R	17.0	I	12.5	R	14.5	S	-	R
16	12.5	R	15.5	I	-	R	16.0	S	21.0	S	-	R	9.00	R	9.50	R	13.5	R	-	R
17	15.5	S	16.0	I	-	R	20.0	S	11.5	R	16.0	R	10.5	R	17.5	I	14.5	S	-	R
18	20.0	S	14.0	R	-	R	8.00	R	13.0	R	14.5	R	13.5	R	20.0	I	16.5	S	-	R
Beaches																				
1	11.5	R	21.0	S	-	R	11.5	R	12.5	R	10.5	R	21.0	S	11.5	R	12.5	R	14.5	I
2	16.5	S	19.0	S	-	R	15.5	S	19.5	S	17.5	R	19.0	S	13.5	R	20.5	S	-	R
3	13.0	I	20.0	S	-	R	13.5	I	10.5	R	-	R	21.5	S	18.5	I	18.5	S	13.0	I
4	12.5	R	18.5	S	-	R	18.5	S	20.5	S	9.50	R	-	R	16.5	I	11.5	R	10.0	R
5	9.00	R	14.5	R	15.5	S	16.5	S	12.5	R	16.5	R	18.5	S	10.5	R	13.0	R	-	R
6	10.5	R	12.0	R	-	R	12.5	I	13.0	R	11.5	R	20.0	S	8.50	R	16.5	S	11.5	I
7	12.0	R	15.0	I	-	R	8.50	R	16.5	I	15.5	R	8.50	R	13.0	R	21.5	S	-	R
8	11.5	R	9.50	R	-	R	20.5	S	13.5	R	-	R	15.5	I	12.5	R	12.0	R	-	R
9	15.5	S	20.5	S	-	R	17.5	S	10.5	R	9.00	R	19.5	S	14.5	R	19.5	S	-	R
10	13.5	R	10.5	R	-	R	9.50	R	18.5	S	-	R	20.5	S	11.5	R	23.5	S	-	R
11	10.5	R	11.5	R	-	R	10.5	R	14.5	I	16.5	R	20.0	S	15.5	R	14.5	S	-	R
12	15.0	S	13.5	R	-	R	-	R	21.5	S	19.5	R	20.5	S	20.5	I	-	R	-	R
13	16.5	S	14.0	R	-	R	15.5	S	19.5	S	14.0	R	13.0	R	18.5	I	10.5	R	-	R
14	17.5	S	19.5	S	-	R	18.0	S	17.5	S	17.5	R	8.50	R	21.0	S	9.00	R	9.50	R
15	8.00	R	15.5	I	-	R	21.5	S	14.5	I	9.50	R	22.0	S	12.5	R	-	R	-	R

Table B17/cont.

16	14.5	I	14.0	R	13.5	I	14.5	I	14.5	I	20.0	R	9.50	R	9.00	R	21.5	S	-	R
17	20.5	S	14.5	R	-	R	14.0	I	10.5	R	14.5	R	16.0	I	16.5	I	17.5	S	-	R
18	9.50	R	16.5	I	-	R	15.5	S	15.0	I	19.0	R	9.50	R	19.5	I	-	R	-	R
19	20.0	S	10.0	R	16.5	S	19.0	S	22.0	S	11.0	R	16.5	I	14.5	R	18.5	S	-	R
20	8.50	R	13.5	R	-	R	13.5	I	10.0	R	16.5	R	17.5	I	10.5	R	15.5	S	-	R
21	11.0	R	20.5	S	-	R	9.50	R	20.0	S	16.5	R	9.50	R	20.5	I	10.0	R	-	R
22	15.5	S	12.5	R	-	R	20.0	S	18.5	S	9.00	R	13.5	R	11.0	R	25.5	S	14.0	I
23	17.0	S	20.0	S	-	R	16.5	S	10.5	R	8.50	R	20.0	S	8.50	R	-	R	-	R
24	16.0	S	19.5	S	-	R	11.5	R	11.0	R	17.0	R	-	R	15.0	R	14.0	S	-	R
25	11.0	R	11.0	R	19.5	S	14.5	I	16.5	I	15.0	R	-	R	12.5	R	16.5	S	-	R
26	10.5	R	12.0	R	17.0	S	15.5	S	15.0	I	18.0	R	8.00	R	22.0	S	13.5	R	-	R
27	14.5	I	16.0	I	-	R	8.50	R	15.5	I	13.5	R	22.0	S	9.50	R	19.0	S	-	R
28	13.5	I	15.0	I	-	R	12.5	I	9.50	R	9.00	R	21.5	S	17.5	I	12.5	R	-	R
29	14.5	I	11.0	R	-	R	19.0	S	17.5	S	16.5	R	14.5	I	13.5	R	9.50	R	-	R
30	19.5	S	14.5	R	-	R	14.0	I	-	R	12.0	R	15.0	I	11.5	R	17.5	S	-	R
31	11.0	R	19.5	S	-	R	18.5	S	-	R	20.0	R	18.5	S	15.5	R	22.5	S	-	R
32	15.5	S	13.0	R	-	R	-	R	21.0	S	13.5	R	12.5	R	20.5	I	13.5	R	-	R
33	20.5	S	9.00	R	-	R	10.5	R	16.5	I	-	R	17.0	I	14.0	R	17.5	S	-	R
34	18.5	S	10.5	R	14.0	I	22.0	S	18.5	S	11.0	R	14.5	I	19.5	I	11.5	R	-	R
35	10.5	R	20.0	S	-	R	16.5	S	14.0	I	12.0	R	16.5	I	17.5	I	20.5	S	-	R
Umdloti River																				
1	11.5	R	16.5	I	16.5	S	13.5	I	19.5	S	11.5	R	18.0	S	18.5	I	14.5	S	-	R
2	14.0	I	13.5	R	14.5	I	16.5	S	9.50	R	10.0	R	16.5	I	16.0	I	12.5	R	-	R
3	15.5	S	20.0	S	-	R	9.50	R	13.5	R	14.5	R	21.5	S	13.0	R	13.5	R	-	R
4	16.0	S	13.5	R	-	R	11.5	R	18.5	S	9.50	R	20.0	S	17.5	I	16.5	S	13.0	I
5	13.5	I	12.5	R	-	R	14.0	I	-	R	10.5	R	20.0	S	12.5	R	11.5	R	11.5	I
6	14.5	I	13.0	R	-	R	12.0	I	17.5	S	15.5	R	19.5	S	16.5	I	8.00	R	16.5	S
7	15.0	S	14.5	R	18.5	S	13.5	I	21.5	S	11.5	R	20.5	S	11.0	R	16.0	S	12.0	I
8	12.5	R	15.0	I	-	R	-	R	20.5	S	8.50	R	10.5	R	19.0	I	14.5	S	-	R
9	13.0	I	12.5	R	17.5	S	19.0	S	8.00	R	-	R	18.0	S	13.5	R	10.5	R	-	R
10	11.5	R	14.0	R	-	R	14.5	I	14.5	I	16.5	R	-	R	18.0	I	8.50	R	-	R
11	16.5	S	16.5	I	20.5	S	16.5	S	16.5	I	10.0	R	-	R	9.00	R	13.5	R	12.5	I
12	18.0	S	12.5	R	-	R	21.0	S	13.0	R	9.00	R	-	R	-	R	20.5	S	-	R
13	14.5	I	11.5	R	-	R	17.5	S	-	R	12.5	R	22.5	S	-	R	15.0	S	-	R
14	14.5	I	21.0	S	-	R	-	R	13.5	R	8.50	R	21.0	S	20.0	I	10.5	R	-	R
15	21.0	S	19.5	S	-	R	15.5	S	18.5	S	15.5	R	20.0	S	15.5	R	11.5	R	-	R
16	14.5	I	15.0	I	-	R	13.5	I	-	R	19.5	R	19.0	S	14.5	R	13.5	R	-	R
17	17.5	S	17.0	S	11.5	R	10.5	R	17.0	S	8.00	R	18.5	S	11.5	R	-	R	15.5	I
18	19.0	S	20.0	S	-	R	19.5	S	12.5	R	20.5	R	15.0	I	12.5	R	9.00	R	-	R
19	15.5	S	13.0	R	-	R	17.0	S	9.00	R	15.0	R	22.0	S	16.5	I	12.0	R	-	R
20	13.5	I	19.0	S	-	R	17.0	S	11.5	R	17.5	R	21.0	S	-	R	14.5	S	-	R
21	17.5	S	13.5	R	-	R	12.5	I	20.0	S	14.0	R	-	R	15.5	R	9.50	R	-	R

Table B17/cont.

22	18.0	S	11.0	R	-	R	22.5	S	-	R	9.50	R	-	R	10.0	R	15.5	S	-	R
23	11.5	R	18.5	S	15.5	S	8.00	R	-	R	19.0	R	14.5	I	19.5	I	12.5	R	-	R
24	10.0	R	12.5	R	-	R	10.5	R	-	R	10.5	R	17.5	I	14.5	R	11.0	R	-	R
25	16.0	S	16.5	I	-	R	20.0	S	14.5	I	11.5	R	13.0	R	13.5	R	13.5	R	-	R
26	18.5	S	20.5	S	-	R	11.0	R	12.0	R	8.00	R	23.0	S	17.0	I	23.0	S	-	R
27	18.5	S	17.5	S	14.0	I	13.5	I	-	R	15.5	R	-	R	19.5	I	10.5	R	-	R
28	15.5	S	21.5	S	-	R	8.50	R	16.0	I	12.5	R	9.00	R	21.0	S	16.5	S	-	R
29	20.0	S	12.0	R	-	R	15.5	S	11.5	R	18.5	R	23.0	S	12.5	R	11.5	R	11.0	I

* Values represent the average of duplicate readings taken from the diameter (θ) of inhibition zones while letters indicate the classification of each isolate as susceptible (S), intermediate susceptibility (I) or resistant (R) according to the interpretative criteria recommended by CLSI (2009). No zone of inhibition is represented by (-).

Antibiotics			
TN (10µg)	Tobramycin	**PG (10µg)**	Penicillin G
AK (30µg)	Amikacin	**AUG (30µg)**	Augmentin
S (25µg)	Streptomycin	**E (15µg)**	Erythromycin
T (30µg)	Tetracycline	**MUP (5µg)**	Mupirocin
AP (10µg)	Ampicillin	**TM (5µg)**	Trimethoprim

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