THE EFFECT OF VA AN DISCHARGES ON THE MICROBIOLOGICAL WATER QUALITY IN CATCHMENT SYSTEMS: AN ENVIRONMENTAL HEALTH-RELATED IMPACT STUDY.

Dissertation submitted by

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BLOEMFONTEIN
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DECLARATION OF INDEPENDENT WORK

I, MARIëTTE GRIESEL, do hereby declare that this research project, submitted to the Technikon Free State for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work.

This work has not been submitted before to any institution, by myself or any other person, in fulfilment of requirements for the attainment of any qualification.

M Griesel

SIGNATURE OF CANDIDATE

20 April 2001

DATE

ACKNOWLEDGEMENTS

To my Father in Heaven – I thank you for showing me the path and keeping me strong.

To my mom and dad – Words cannot express my love. This one is for you!

To Doctor Paul Jagals – One question comes to mind when I think of your guidance and support, "Would this have been a reality without you?"

To Marcel Basson - Thank you for your love, patience and being close.

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To the National Research Foundation – Thanks for the financial assistance.

To my friends and especially Corinne, Robin and Catherine – I thank you all.

SEL

Die doel van die studie was om die impak van stedelike aflope van die stad Bloemfontein, sowel as uitvloeisel vanuit twee afvalwater behandelingsaanlegte, op die gesondheidsverwante mikrobiologiese kwaliteit van water in die Renosterspruit sub–opvangsgebied, te ondersoek. Die sub-opvangsgebied vorm deel van die Modderrivier opvangsgebied in die Vrystaat, Suid Afrika.

'n "Impak" op die mikrobiologiese waterkwaliteit was, vir doeleindes van hierdie studie, gedefinieer as die vermoë van fekaal besoedelde water om die natuurlike assimilasie kapasiteit van ontvangende water tot so 'n mate te oorkom, dat die kwaliteit van die water ongeskik raak vir gesondheidsverwante huishoudelike-, ontspanning-en landbou gebruike en gevolglik, waterverwante infeksies inhou.

Die gesondheidsverwante mikrobiologiese kwaliteit van die waters was ondersoek deur gebruik te maak van *Escherichia coli*, *Clostridium perfringens* spore en somatiese kolifage as mikrobiologiese indikatore.

Die natuurlike geometriese agtergrondgemiddeldes in die Renosterspruit was 85; 10 en 3 per 100 mL vir *E. coli*, *C. perfringens* spore en somatiese kolifage, onderskeidelik. Hierdie vlakke het daarop gedui dat die water in die Renosterspruit, ongeskik was vir doeleindes van onbehandelde water inname of kontak ontspanning. Die water was wel geskik vir behandeling vir huishoudelike gebruike sowel as die besproeiing van gesondheidssensitiewe oeste en ander landbou gebruike.

Behandelde uitloop van Sterkwater afvalwater behandelingsaanleg het na kommisie 'n beduidende impak op die waterkwaliteit van die Renosterspruit gehad en getalle van indikator organismes het beduidend gestyg. *E. coli*, *C. perfringens* spore en somatiese kolifage se geometriese vlakgemiddeldes in die Renosterspruit was 13 686; 4 003 en 8 923 per 100 mL onderskeidelik. Die vlakke het daarop gedui dat die Sterkwater behandelingsaanleg, gedurende die studie tydperk, nie die statutêre mikrobiologiese vlakke in die uitvloeisel kon handhaaf nie.

Indikator organisme getalle, gemeet in die Bloemspruit, dui op strawwe fekale besoedeling deur diffuse stedelike aflope sowel as uitvloeisel vanuit die Bloemspruit afvalwater behandelingsaanleg. Geometriese vlakgemiddeldes vir *E. coli*, *C. perfringens* spore en somatiese kolifage was so hoog as 59 027; 402 en 9 098 per 100 mL onderskeidelik, wat dui op 'n gewigtige impak.

Die Bloemspruit het, na sam gehad op die waterkwaliteit in die Renosterspruit. Die geometriese vlakgemiddeldes vir *E. coli*, *C. perfringens* spore en somatiese kolifage was so hoog soos 2 240; 154; 4 883 per 100 mL onderskeidelik. Die water in hierdie deel van die Renosterspruit was ongeskik vir huishoudelike, ontspanning en landbou gebruike.

Verder stroom-af het die waterkwaliteit sodanig verbeter dat die water geskik was vir landbou gebruike. Die geometriese vlakgemiddeldes vir *E. coli*, *C. perfringens* spore en somatiese kolifage het afgeneem tot 481; 51; 922 per 100 mL onderskeidelik. Die water was egter steeds ongeskik vir die ander gebruike.

Water in die Modderrivier, stroom-af van die Renosterspruit sameloop, was geskik (na geringe behandeling) vir huishoudelike gebruike, ontspanning en die besproeiing van gesondheidssensitiewe oeste. *E. coli*, *C. perfringens* spore en somatiese kolifage geometriese vlakgemiddeldes van 39; 37; 36 per 100 mL was, respektiewelik, gemeet.

Die water van die Renosterspruit, in die direkte omgewing van Bloemfontein, is ongeskik vir huishoudelike, ontspanning en landbou gebruike. Die waterkwaliteit van die Modderrivier, stroom-af van die Renosterspruit sameloop, het geen impak getoon nie. Die laer gedeeltes van die Renosterspruit het suksesvol die mikrobiologiese besoedeling lading van die stedelike aflope geassimileer. Resultate toon egter dat die gesondheidsverwante mikrobiologiese kwaliteit van die Renosterspruit versleg wanneer dit vergelyk word met vorige studies in dieselfde studie-gebied.

'n Opvangsgebied bestuursprogram, met 'n fokus op omgewingsgesondheid, moet opgestel en geimplementeer word vir die Renosterspruit sub-opvangsgebied sodat die gesondheid van potensiële huishoudelike en ander water gebruikers beskerm en bevorder kan word.

The aim of this study was to assess the impacts that urban run-off, as well as effluent from two wastewater treatment works, from Bloemfontein city, had on the health-related microbiological quality of water in the Renoster Spruit sub-catchment, which forms part of the Modder River catchment (Free State province, South Africa).

"Impact" on the microbiological quality of receiving waters was, for the purposes of this study, defined as the ability of faecally polluted urban discharges to overcome the natural assimilation capacity of receiving waters to the extent that the water quality became unfit for health-related domestic, recreation or agricultural use and water-related infections could therefore, be expected. The health-related microbiological quality of the various waters was investigated by using *E. coli*, *C. perfringens* spores and somatic coliphages as microbiological indicators.

The natural background geometric mean levels for *E. coli*, *C. perfringens* spores and somatic coliphages in the Renoster Spruit were 85; 10; 3 per 100 mL, respectively. This indicated that the water was not suitable for untreated drinking or full contact recreation purposes, but suitable to treat for domestic use, and for irrigation of health-sensitive crops.

The numbers of organisms of the selected indicator groups, increased significantly in the Renoster Spruit directly downstream from the newly constructed Sterkwater wastewater treatment works since treated effluent discharge commenced. The geometric mean levels for *E. coli*, *C. perfringens* spores and somatic coliphages increased to 13 686; 4 003 and 8 923 per 100 mL respectively, which indicated a noticeable impact on the microbiological quality of water in the Renoster Spruit. This was also an indication that Sterkwater could not maintain statutory microbiological levels in effluent for this study period.

The numbers of indicator organisms in the Bloem Spruit system indicated heavy faecal pollution from diffuse urban run-off as well as effluent from the Bloem Spruit wastewater treatment works. Geometric mean levels for *E. coli*, *C. perfringens* spores and somatic coliphages were as high as 59 027; 402; 9 098 per 100 mL respectively, indicating a severe impact.

The Bloem Spruit impacted the quality of water in the Renoster Spruit after confluence. The geometric mean levels for *E. coli*, *C. perfringens* spores and somatic coliphages were as high as 2 240; 154 and 4 883 per 100 mL respectively. The water in this part of the Renoster Spruit was unfit for domestic, recreation and agricultural uses. Further downstream, the water quality improved to a quality suitable for fitness for agricultural uses, but still constituted a risk for other uses. The geometr *E. coli*, *C. perfringens* spores and somatic coliphages decreased to 481; 51 and 922 per 100 mL respectively.

Water in the Modder River downstream from the Renoster Spruit confluence could be used for domestic (after limited treatment), recreation and irrigation of health-sensitive crops. The geometric mean levels for *E. coli*, *C. perfringens* and somatic coliphages were 39; 37 and 36 per 100 mL respectively.

The water of the Renoster Spruit, in the direct vicinity of Bloemfontein, was unfit for domestic, recreational and agricultural purposes. However, the microbiological quality of water in the Modder River, after confluence with the Renoster Spruit, did not reflect any impacts since the lower reaches of the Renoster Spruit appeared to have successfully assimilated the microbiological pollutant load from the urban discharges.

From the study results, it appeared that the health-related microbiological quality of water in the Renoster Spruit deteriorated when compared to results from previous studies on the same waters. This implies that urban pollution of the Renoster Spruit is increasing.

A catchment management plan, with a focus on environmental health, should be designed and implemented for the Renoster Spruit sub-catchment, in order to protect and promote the health of potential water consumers and other categories of water users.

Complete and outstan to be done.

Water Quality Management IV

Assignment to be handed on the **06-April-2001**

The South African Catchment Management Strategy

NB: The report must focus on the application of a catchment management plan to promote public health-related Water Quality.

Study Guide is complete.

Environmental Epidemiology IV

Practical Assignment to be handed on the **06-April-2001**

Select any one risk source related to any one risk agent from the Module literature.

Design a monitoring programme to assist in the assessment of the release potential of the risk source.

NB: The report must not be longer than 25 A4 typed folios – Title page included.

The letter type must be Arial (Tahoma) or Times Roman with font size 12. Line spacing 1.5.

Study Guide – modules 2 & 3 not complete.

Management Practice IV

Practical Assignment to be handed on the **06-April-2001** — 19th.

Formulate an Environmental Health unit in a rural area using the six generic processes.

NB: The report must not be longer than 25 A4 typed folios – Title page included.

The letter type must be Arial (Tahoma) or Times Roman with font size 12. Line spacing 1.5.

Study Guide – Not complete or updated.

Look at **mark list form** for student results control.

Next contact session for Module 2: **17-20-April-2001**

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TECHNICAL CONCEPTS RELATED TO THE STUDY

Agricultural use	Use of water to irrigate crops.
Assimilation capacity	In the water environment, the assimilative capacity of a water body is its natural ability to process wastes, such as microbiological contaminants, without the quality of the water deteriorating beyond the point that its value for human use or ecological sustainability is adversely affected.
Health-sensitive crops	Crops, such as salad-type crops that, when irrigated with polluted water, may pose a risk of water-related infections when eaten raw and uncooked.
Impact	The deterioration of the health-related microbiological quality of water as a result of receiving faecal wastes from a specific source, to such an extent that the water becomes unfit for consumption or use for any other non-potable purposes such as recreation and agriculture.
NOAEL	This refers to the No Observed Adverse Effect Level. The NOAEL refers to the highest contaminant level, e.g. *E. coli*, at which no statistically or biologically significant health effects are detected in a user population.
Non-point (diffuse) sources	Unidentified effluent pollution sources dispersed over an area.
Point sources	Identifiable effluent sources emanating from a single event with potentially quantifiable flows and quality.
Pollution	The introduction of substances or energy to the water environment, at levels that have an unacceptable impact on the water environment or its users.
Water quality suitable for use	Water of a microbiological quality that will not pose a risk of water-related infection to humans when used for agriculture, recreation or domestic purposes such as drinking.
Water-related infections	Diseases associated with contact with contaminated environmental water or with any impurities within the water that are somehow taken in by people.

LIST OF OUTPUTS RELATED TO THE STUDY

CONFERENCE PRESENTATIONS

Papers and posters presented at international conferences:

1 The Water Institute of Southern Africa (WISA). 28 May – 01 June 2000. Biennial conference and exhibition, Sun City, South Africa.

Paper presentation: Infection risk for riparian users of waters in a catchment system receiving urban and wastewater discharges. Griesel M., Jagals P. and Grabow WOK.

2 International Water Association (IWA). July 2000. 1^{st} World Water Congress and Exhibition, Paris, France.

Poster presentation: Infection risk for riparian users of waters receiving treated wastewater and other urban discharges. Griesel M., Jagals P. and Grabow WOK.

Papers presented at national conferences:

1 Suid-Afrikaanse Akademie vir Wetenskap en Kuns, Afdeling Biologie. September 1999. Jaarkongres, Pretoria, Suid Afrika.

Referaat Aanbieder: Bepaling van infeksie risikos vir oewerverbruikers van water in 'n opvangsgebied wat behandelde afvalwater en ander stedelike aflope ontvang. Griesel M., Jagals P. and Grabow WOK.

Papers presented at local events:

1 Faculty of Health and Environmental Sciences, Prestige Research Day. October 1999. Technikon Free State, Bloemfontein, South Africa.

Paper presentation: Assessment of infection risk for riparian users of a catchment system receiving treated wastewater and other urban discharges. Griesel M. and Jagals P.

2 Faculty of Health and Environmental Sciences, Prestige Research Day. October 2000. Technikon Free State, Bloemfontein, South Africa.

Paper presentation: The effect of various urban discharges on water in catchment systems: An Environmental Health related impact study. Griesel M., Jagals P. and Grabow WOK.

PUBLICATIONS

1 Griesel M., Jagals P. and Grabow WOK. (2000). Bepaling van infeksie risikos vir oewerverbruikers van water in 'n opvanggebied wat behandelde afvalwater en ander stedelike aflope ontvang. Uitgebreide uittreksel in Die Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie. Jaargang 19. No.2. Volume 19. ISSN 0254-3486. Junie. Pretoria.

2 Griesel M., Jagals P. and Grabow WOK. (2000). Infection risk for riparian users of waters in a catchment systems receiving urban and wastewater discharges. Conference proceedings: Biennial Conference and exhibition, the Water Institute of Southern Africa (WISA), 28 May-01 June, Sun City, South Africa.

3 Jagals P., Grabow WOK., Griesel M. and Jagals C. (2001). Evaluation of Selected Membrane Filtration and Most Probable Number Methods for the Enumeration of Faecal coliforms, Escherichia coli and Enterococci in Environmental Waters. *Quantitative Microbiology*. In press, Kluwer Academic Publishers, Norwell, Massachusetts.

Chapter 1
INTRODUCTION

All cities in South Africa are presently experiencing rapid population growth and urbanisation arising partly from the extensive political and social changes occurring in the country (Ketley et al., 1996). In the developing world, expanding cities have, however, been reported to contribute to the degradation of natural environmental systems. The urban confluence of industrialisation, waste generation, and concentrated transport systems, compounded by the sheer size of cities, usually pose many environmental health hazards (Medical Research Council (MRC), 1999).

Waste products, generated in cities by the provision of services, through the production and consumption of food as well as other consumer products, are released into the air, soil and water. It is assumed that these environmental elements will assimilate the wastes by converting it to a less hazardous form (MRC, 1999).

In the water environment, the assimilative capacity of a water body is its natural ability to process wastes, such as microbiological contaminants, without the quality of the water deteriorating beyond the point that its value for ecological sustainability, or human uses, is adversely affected (Department of Water Affairs and Forestry (DWAF), 1995; O'Keeffe et al., 1996). This capacity to assimilate wastes, such as microbiological contaminants, plays a significant role to reduce numbers of pathogenic microorganisms in polluted surface waters to levels that are not hazardous to human health.

Surface water streams, with high flow and assimilative capacity, will probably assimilate substantial levels of microbiological contaminants, while a stream with low capacity, which is faecally polluted with high concentrations of faeces (faecally polluted), will probably not assimilate this level of microbiological contaminants sufficient enough to ensure water safety for users. Any increase in waste production, especially faecal pollution, will have a negative or positive impact on such a low-flow stream (Jagals, 2000).

This study was done on the Renoster Spruit, which is a tributary of the Modder River, in the southern parts of the Free State Province, South Africa. The Renoster Spruit is a low-flow peri-urban stream that receives run-off from the Bloemfontein urban areas at various levels of development, as well as final effluent from two wastewater treatment works. The impact that microbiological contaminants from all these sources have on the health-related microbiological quality of water in the spruit was investigated by studying the assimilative capacity of the spruit, and it's tributaries, the Bloem and Fontein Spruits (Figures 1 & 2.1).

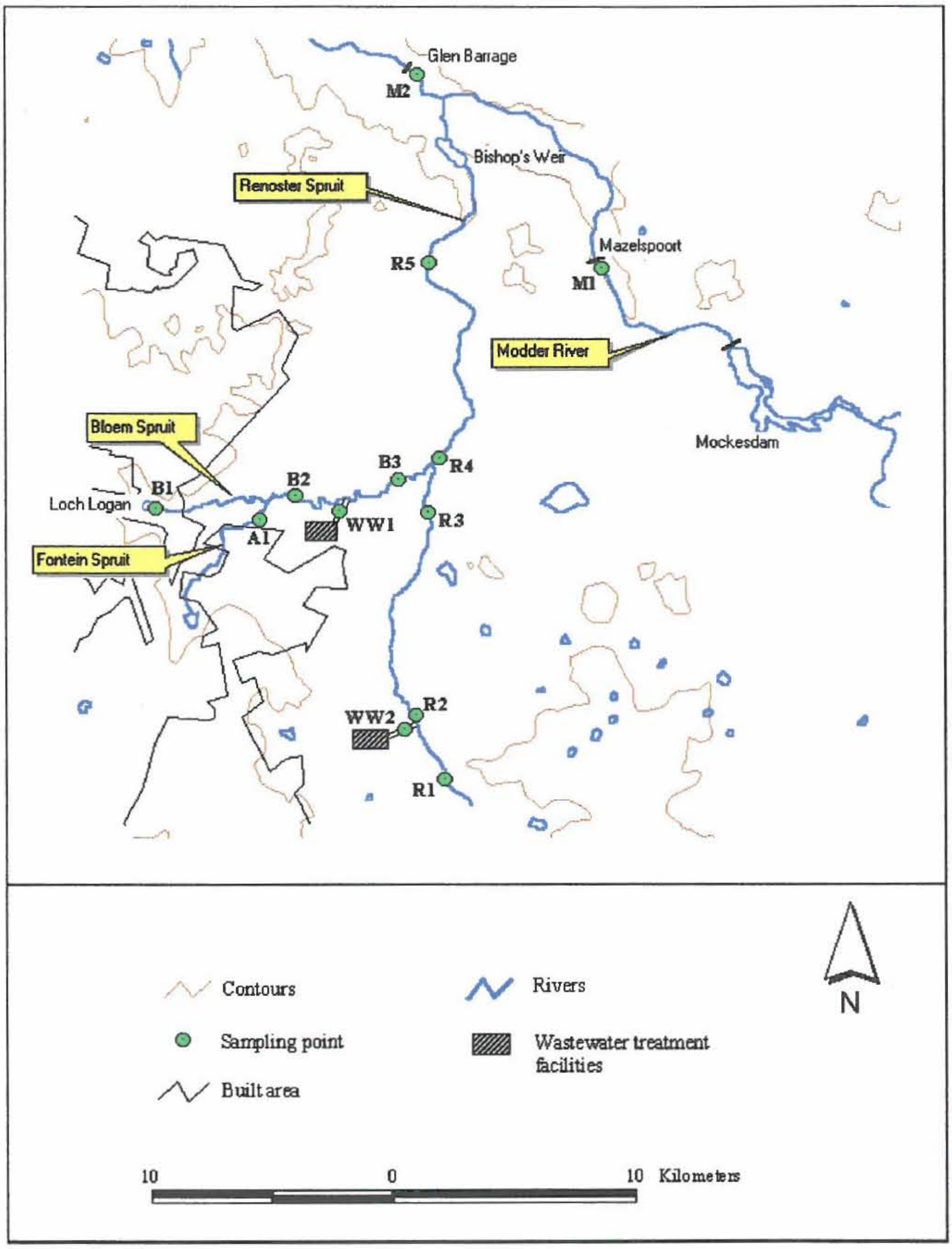

FIGURE 1: The study area indicating the sampling points (also refer to Figure 2.1).

1.1 THE IMPACT OF URBAN DISCHARGES ON THE HEALTH-RELATED MICROBIOLOGICAL QUALITY OF RECEIVING SURFACE WATERS

When microbiological contaminants are released into urban run-off through discharges and rainfall, these contaminants move with the run-off into receiving streams, rivers and groundwater aquifers (Tchobanoglous and Schroeder, 1987; Chapra, 1997). Microbiological pathogens, discharged into surface water, are then usually controlled, or even reduced, by factors such as sunlight, temperature, availability of nutrients, natural die-off and the presence of autochthonous organisms (Geldreich, 1996; Medema et al., 1997). These features are often referred to as the assimilation capacity of the receiving waters (Tchobanoglous and Schroeder, 1987; Chapra, 1997). If, however, surface streams cannot assimilate these contaminants, several impacts on the receiving stream may be observed, one of which can be exceptionally high levels of pathogenic microorganisms.

The term "impact" implies, or is defined as, "to have a (pronounced) effect on" (Oxford English Dictionary, 1991). In this study, the term "impact" refers to the deterioration of the health-related microbiological quality of receiving waters as a result of receiving faecal wastes from a specific source, to such an extent that the water becomes unfit for consumption or use for any other non-potable purposes such as recreation and agriculture.

It was therefore assumed that if the numbers of health-related microorganisms, released at a specific point in the receiving stream, increased to such an extent that the microbiological organisms being released exceeded the numbers of organisms being reduced, the natural assimilation capacity of the water body was exceeded. In such cases, the release can be seen to have an impact on the heath-related microbiological quality of the receiving waters, and the risk of infection from the faecally polluted water may increase.

1.2 THE RISK OF INFECTION FROM FAECALLY POLLUTED WATER

The World Health Organisation (WHO) rates poor water quality, together with inadequate sanitation, as the leading cause of infections and water-related deaths in developing countries (WHO, 1997). Water-related infections and diseases can be associated with contact with contaminated environmental water or with any impurities within the water that are somehow taken in by people (Genthe and Seager, 1996).

Water-related diseases are usually caused by enteric pathogens, transmitted by the faecal-oral route, which implies that organisms are excreted in the faeces of infected individuals and are then ingested by others through faecally contaminated water or food (Grabow, 1996).

In South Africa, Pegram et al. (1[illegible] related infections and diseases to be a real problem in densely populated, under-developed and poorly sanitised rural and urban areas. This causes alarm if it is taken into account that currently more than 8 million people are estimated not to have access to adequate supplies of potable water and nearly 20 million people in South Africa lack basic sanitation (DWAF, 2000).

Rapid urbanisation has resulted in large numbers of people living in overcrowded informal settlements. People in these areas live in sub-standard housing, often with inadequate water supply, sanitation and other basic necessities (Seager, 1995). Poor infrastructure and circumstances often force these people to, directly, or indirectly, use partially treated or even untreated surface water from environmental sources that are of poor microbiological quality (Republic of South Africa, 1994). These are favourable conditions for enteric infections to be transmitted (Levine and Levine, 1994).

The microbiological qualities of water sources, and consequently the health of communities, are influenced by faecal pollution from human settlements (Haiping and Yamada, 1998). Frequently cited sources of microbiological contamination include discharges of treated and untreated sewage, agricultural run-off, storm water run-off from impervious surfaces associated with urban, commercial, or industrial land uses, malfunctioning or poorly-sited wastewater treatment works, and direct deposition of faecal material (Weiskel et al., 1996).

Faecal contamination of aquatic environments can degrade water for human uses, principally because of the introduction of a risk of infection by pathogenic microorganisms. Enteric pathogens such as *Shigella* spp., *Salmonella* spp., *Yersinia* spp., enterohaemorrhagic *Escherichia coli*, *Giardia* spp., *Cryptosporidium* spp. and Hepatitis A viruses can cause diseases ranging from mild eye, skin and respiratory infections to diarrhoea and infectious hepatitis (Sinton et al., 1998).

General practices for safeguarding communities against contaminated water traditionally include treating and disinfecting extracted bulk water before the water is distributed to communities for drinking and other domestic purposes. Sadly, despite substantial resources being put in place to make water more available, more accessible and safer for domestic use, communities in South Africa are still using a variety of waters of poor microbiological quality (Republic of South Africa, 1994).

The National Water Act (Act No. 36 of 1998) aims to protect the national water resources through the management of pollution sources (Van Niekerk, 2000). From a health-related perspective, the deterioration in the quality of South African surface waters must be prevented, managed and controlle : (National Water Act, No. 36 of 1998):

- Availability of water in sufficient quantities to meet basic human needs of present and future generations.
- Availability of water of an acceptable quality to minimise the potential risks of infections associated with the use of untreated waters.
- Availability of water of an acceptable quality at the point of raw water extraction for domestic water treatment.

To minimise faecal pollution of urban run-off, the Department of Water Affairs and Forestry adopted an approach of catchment management in the new National Water Act (Act No. 36 of 1998). This was followed by the implementation of a “Dense Settlements” project in 1999, to identify ways of managing water quality effects caused by specific sources of contamination from densely populated, poorly serviced and poverty stricken communities (DWAF, 1999).

In light of these developments, and in an attempt to gather information that could be used in an urban catchment management program, this study was done to contribute to knowledge about the risk of infection posed by the target streams in the study sector of the Modder River catchment.

1.3 SOURCES OF FAECAL POLLUTION IN THE STUDY AREA

It was decided to focus on the health-related microbiological water quality of the receiving stream because the hazard posed by pathogenic microorganisms to human health outweighed those posed by the chemical quality of water, especially in developing countries (Bern and Glass, 1994; Craun et al., 1994).

Both natural and anthropogenic factors are known to influence the microbiological quality of water sources (American Water Works Association (AWWA), 1990). However, before attempts can be made to control or eliminate pollution, urban catchment management programmes generally begin with the identification of factors that, individually or collectively, affect the quality of the receiving water resources (Pegram, 2000). A similar approach was followed by this study.

Pollution can be defined as an undesirable change in the physical, chemical and biological characteristics of air, water, soil, or food that can adversely affect the health, survival, or activities of humans and other living organisms (Miller, 1998). In terms of this study, pollution was defined as the introduction of substances or energy to the water environment, at levels that have an unacceptable impact on the water environment or its users (Pegram et al., 1998). Anthropogenic sources uman activities that may contribute to pollution of the water environment (Pegram et al., 1998). Human factors influencing the source water quality are categorised as two types (DWAF, 1995):

- Non-point (diffuse) sources that include all other sources (including instream activities), which contribute pollution over a dispersed area and cannot be directly identified.
- Point sources that may be defined as identifiable effluent sources emanating from a single event with potentially quantifiable flows and quality.

The Renoster Spruit receives a variety of urban discharges from the city of Bloemfontein. These discharges include final effluent from two wastewater treatment works (point sources), as well as general surface run-off from the city (diffuse sources).

1.3.1 Diffuse sources of faecally polluted urban run-off

Diffuse (non-point) pollution sources can result from any activities that produce constituents that enter the receiving water body in an intermittent and/or diffuse manner (DWAF, 1995).

Land development and land use have been identified as some of the most significant non-point contributors to catchment degradation and poor water quality (DWAF, 1995). Studies have also shown that urban settlements, at various levels of development, contribute to diffuse pollution of surface waters (Lubout et al., 1997; Haiping and Yamada, 1998). Housing, commercial, as well as industrial structures, often replace wetlands and terrestrial vegetation, covering catchments, to a large extent, with impermeable surfaces (Eichbaum, 1990), thereby reducing the assimilation capacity of receiving surface waters as well as contributing to effective transport of pollutants to the receiving surface water source.

Sources of microbiological pathogens include land-deposited human faecal material from areas with limited or inadequate sanitary facilities, as well as faecal material from warm-blooded domestic animals kept in the urban areas (Geldreich, 1996; Jagals, 2000). Constant polluted run-off can also be identified in areas where sewerage systems are poorly maintained, leading to overflows (Jagals, 1997).

The numbers of microbiological organisms in diffuse effluents generally increase during wet weather events, when stormwater run-off carries organisms from urban areas into receiving water bodies (DWAF, 1995; Haiping and Yamada, 1998). In 1996, Weiskel et al. postulated that faecal deposits accumulate on paved or compacted land surfaces during dry periods, with surviving microorganisms becoming entrained in the high-velocity run-off generated by such surfaces during periodic storm events.

Bloemfontein has well-developed -developed urban areas, which generally comprise of residential, commercial and industrial land uses. The areas are characterised by paved surfaces, full waterborne sewage, limited sanitary facilities as well as additional areas of informal settlement with inadequate or no sanitary services.

Other studies done in the area have shown that during dry weather periods, run-off from unconfirmed sources such as blocked sewers and water intensive activities (e.g. washing of premises and vehicles) contained levels of faecal pollution that constitutes a risk of infection according various microbiological water quality guidelines. These pollution levels increased during rain events, when the run-off from all the various urban areas, often contained equivalents, or even greater loads, of faecal indicator organism numbers than those of raw sewage received at wastewater treatment works (Jagals, 1997; Pretorius, 1996).

It is therefore evident that urban run-off is faecally polluted during dry and wet weather events, with the run-off containing even higher loads of faecal pollution during rain (Jagals, 1997; Pretorius, 1996). This study therefore did not investigate, or distinguish, between microbiological water quality of the wet and dry weather urban run-off. The focus instead, was on the impact of the total run-off on receiving surface waters, over a period of 31 months, regardless of season or weather condition.

1.3.2 Treated effluent as potential point sources of faecal pollution

Point sources of faecal water pollution are characterised by a single identifiable source or event (DWAF, 1995). Typical point sources include discharges from wastewater treatment works, industrial discharges, and discharges from hazardous waste works.

Wastewater treatment works are used to treat domestic sewage and other organic wastewaters that vary considerably in microbiological and biochemical quality. These systems are generally designed to reduce or remove organic matter, solids, nutrients, disease-causing organisms and other pollutants associated with the untreated wastewater. Their respective designs generally include barriers, such as chlorination facilities and maturation ponds, to reduce numbers of pathogenic microorganisms in the final effluent (Mancl, 1996). Wastewater treatment works are, therefore, heavily relied upon to safeguard water users against contamination of receiving waters (Watkins and Cameron, 1991).

In South Africa, the National Water Act (Act No. 36 of 1998) has made it mandatory to treat effluents to acceptable standard before returning it to public watercourses. The water-resource and user-protection approaches of the Act ensure that the wastes, before discharge are treated to such a quality that (DWAF, 1998):

- The aquatic ecosystems in th tay protected.
- The water resources are not degraded beyond recovery.
- The water quality requirements of the different water users are satisfied as far as it is reasonably practical.

However, despite the installation of such facilities, increasing evidence is found that conventional wastewater treatment does not safeguard as well as previously thought. Two reasons account for this. Firstly, pathogens, such as viruses and protozoa, previously not studied as intensively as the more "conventional" pathogens, are emerging in the water cycle (West, 1991). It is not certain that these pathogens are continually inactivated by conventional wastewater treatment methods and disinfection. Secondly, despite all efforts, indications are that any water treatment or disinfection system is subject to some degree of intermittent failure (Geldreich, 1996). Treated wastewater effluent may then still contain potentially harmful contaminants, which the user must be aware of in order to minimise detrimental environmental or human health effects (Yates, 1997).

It is reported that pathogenic organisms in treated wastewater have a limited lifespan after discharge into receiving waters (Geldreich, 1976), which implies that riparian water users downstream might not be exposed to excessive risk of infection (Shuval et al., 1986). However, other studies have highlighted the fact that treated wastewater, as well as the receiving waters, in the vicinity of, as well as some considerable distances downstream from effluent discharge points, may still contain considerable numbers of pathogenic microorganisms (Cartwright, 1993; Solo-Gabriele and Neumeister, 1996).

Many of the receiving waters in South Africa are non-perennial streams, with such low flow capacity that treated wastewater, discharged at a particular point, often maintains the stream-flow. As a result, pathogens surviving treatment processes end up in public watercourses, which may not have the capacity to assimilate these.

Two wastewater treatment works, of which one was commissioned during the time of this study, are situated in the study area. As it was suspected that these treatment works discharge final effluent of varying qualities into the waters of the Renoster Spruit sub-catchment area, the effluents were investigated as potential point sources of pathogenic microorganisms.

The recently commissioned Sterkwater wastewater treatment works was built to accommodate the expanding residential areas of Bloemfontein. Wastewater received by this treatment system is put through a series of waste stabilisation ponds, followed by an activated sludge polishing system. The final effluent is chlorinated to reduce numbers of microbiological organisms before … environment. At the time of the study, final effluent of up to 10 ML per day was discharged into the Renoster Spruit (Figure 1). This effluent subsequently became the only constant source of water in the Renoster Spruit.

The Bloem Spruit wastewater treatment works receives domestic sewage from most of the older parts of the city of Bloemfontein. Treated, pond-matured final effluent from this established, fixed-medium, wastewater treatment installation amounts to approximately 60 ML per day, which is discharged into the Bloem Spruit (Figure 1).

1.4 WATER USES IN THE CATCHMENT

The impacts the diffuse and point sources had on the background (natural) levels of microbiological organisms, at the points of discharge, were determined. This was done to determine whether there would be any risk of infection for people using the receiving waters for various potable and non-potable purposes.

The Renoster Spruit supports a range of activities along its course to join the Modder River. From an environmental health perspective, water uses in the Renoster Spruit sub-catchment area can be divided into two main categories (DWAF, 1996a & b; WHO, 1997; Jagals and Steyn, 1999; Jagals 2000):

(1) Consumption of partially/untreated surface water or products that are supported and/or produced by the water in some way or another (irrigation for agriculture).

(2) Contact with water with limited ingestion of the water (washing, bathing and recreational activities).

Although no information is available regarding the quantities of the surface water used by the various water users in the study area, the user practices that might constitute a risk of infection are described as follows:

1.4.1 Consumption and food production

1.4.1.1 *Domestic consumption*

Rapid urbanisation in South Africa has resulted in large numbers of people from rural areas flocking to developed urban and metropolitan cities. Due to circumstances of depravation, these people live in overcrowded and informal settlements, often with inadequate water supply, sanitation and other basic necessities (Seager, 1995). People in these circumstances often use partially/untreated surface water as a source of domestic water.

The section of the Renoster Spruit, selected for this study, reflects this state of affairs on a small but relevant scale. The various selected tributaries of the Renoster Spruit flow through well-developed, as well as poor -developed areas and rural countryside, where it often is the main source of water for farm labourers and people in informal settlements (Jagals, 2000; Pretorius, 1996).

1.4.1.2 *Food production*

For many deprived persons in the informal settlements, fish is harvested for food from surface water and the maturation ponds of the wastewater treatment works. The consumption of the fish generally does not pose a water-related infection risk to the consumer (Van den Heever, 1992), but fishing activities lead to intermediate- and often full contact with the water.

In the rural countryside, the agricultural sector may also use polluted surface waters that match the microbiological quality of poorly treated, and often, raw sewage, to irrigate crops (Jagals, 1997; Jagals and Steyn, 1999). It is especially foods, such as salad-type crops eaten raw, that would pose the biggest threat to human health (Blumenthal et al., 1999).

1.4.2 Contact users

1.4.2.1 *Water contact for domestic purposes*

People living in sub-standard housing with no access to safe potable water, often have no other choice but to use environmental water for washing clothes as well as personal ablution (Pretorius, 1996).

1.4.2.2 *Recreational users*

This group includes water-related activities such as swimming and boating for relaxation (DWAF, 1996b). Depending on actual water contact, recreational activities may be classified as full- (e.g. swimming), intermediate- (e.g. canoeing) and non-contact recreation (e.g. hiking and camping along the riverbed).

The quality requirements for such a variety of water uses represent a synthesis of the needs. The quality criteria set for full-contact recreation, keeping in mind that the water may be ingested, are therefore higher than those set for the other water uses (DWAF, 1996b).

Traditionally people using the water for full-contact recreation and relaxation come from areas where proper sanitation and water supply is provided. They are therefore not subjected to any significant risk of infection from waterborne diseases in their domestic environment. Such persons could inadvertently tend to underestimate health risks associated with their recreational water activities due to the virtual absence of waterborne diseases in their domestic environment (Jagals, 2000)

1.5 HEALTH-RELATED MICROBIOLOGICAL WATER QUALITY

Pathogens in faecally polluted water may cause infectious diseases such as cholera, typhoid and paratyphoid fever, dysentery, gastroenteritis, giardiasis, salmonellosis, hepatitis as well as a variety of other eye, ear, nose and skin infections (WHO, 1997; Sinton et al., 1998; Grabow et al, 1998).

The pathogens include bacteria (e.g. *Shigella* spp., *Salmonella* spp., *Yersinia* spp., *Vibrio* spp. and enterohaemorrhagic *Escherichia coli*), enteric viruses (so-called since these viruses inhabit the intestinal tract of humans) (e.g. Hepatitis A) and protozoa (e.g. *Giardia* spp. and *Cryptosporidium* spp) (DWAF, 1996a; WHO, 1997; Sinton et al., 1998; Grabow et al, 1998).

People using faecally polluted water maybe exposed to these pathogens that might be present in the water. Depending on the various practices and purposes the water is used for, the users might be directly, or indirectly, exposed to risks of infection. These water-related infections might then lead to secondary transmission of the pathogens to others (Geldreich, 1996).

The most reliable approach to measure potential waterborne dangers for human health, posed by microbiological agents of faecal origin, is to directly assess the incidence of such pathogenic organisms in water. Numbers of pathogens in environmental waters are, however, generally too low for feasible direct testing. Most pathogen detection methods are also expensive, complex, time-consuming and hazardous to the health of the analyst. It is therefore more practical, and preferable, to use microbiological organisms that are able to indicate the presence of pathogens in water (DWAF, 1996b; Grabow, 1996).

When such indicator organisms are present in water sources, it implies possible presence of pathogenic microorganisms and the possibility of a health hazard. For this study, the numbers of microbiological indicator organisms, tested in the waters of the study area, were compared to the no observed adverse effect level (NOAEL) stated in various guidelines to indicate risk of infection to users.

1.5.1 Microbiological indicator organisms

Ideally a microbiological indicator organism fulfils a number of criteria (Genthe and Kfir, 1995):

i) It should be present when the pathogen is present.

ii) Should be absent in unpolluted waters.

iii) Sufficiently present in numbers greater than the pathogen it indicates.

iv) Its survival in the environment, and resistance to treatment processes, should be comparable to that of the pathogen.

v) Easy to enumerate, identify

vi) It should not be harmful to human health.

A microbiological indicator suite, comprising *Escherichia coli*, *Clostridium perfringens* and somatic coliphages, was used during this study to indicate faecal pollution. The occurrence of faecal pollution, in turn, indicates the potential presence of microbiological pathogens in the water.

These three organism groups were considered as the best combination because they represent a spectrum of different types of microorganisms, which indicate faecal pollution and the potential presence of pathogenic microorganisms. None of these indicators specifically indicate the presence of any pathogens. They do, however, reflect the survival of certain pathogens (Jagals, 2000). *E. coli* may more realistically reflect the survival of bacterial pathogens such as *Salmonella*, *Shigella*, *Campylobacter* and *Vibrio* than would *C. perfringens* and phages, *C. perfringens* spores may more realistically reflect the survival of cysts and oocysts than the other indicators, and phages may more realistically reflect the survival of viruses than the other indicators.

1.5.1.1 *Escherichia coli* (*E. coli*)

E. coli is a member of the faecal coliform group of microorganisms, which generally inhabit the intestines of warm-blooded animals (SABS, 1984; ISO, 1995). Faecal coliforms indicate the possible presence of pathogens responsible for the transmission of infectious diseases such as gastroenteritis, salmonellosis, dysentery, cholera as well as typhoid fever (SA Water Quality Guidelines, 1996; Jagals 2000). While faecal coliforms are widely used to evaluate the quality of waste water effluents, environmental freshwater sources, sea water at bathing beaches, raw water for drinking water supply and recreational waters (Geldreich, 1976; Jagals et al., 1995; Standard Methods, 1998), it was decided for this study to use only *E. coli* instead of faecal coliforms as an indicator organism group.

Water Quality Guidelines in South Africa (1996) provide various infection risk levels that can be indicated by both faecal coliform and *E. coli* assessments. Of the faecal coliform group, *E. coli* is a more specific indicator of faecal water pollution than the faecal coliform group by itself (SABS, 1984; Grabow, 1996; Jagals, 2000) because *E. coli* has been found to constitute approximately 97% of faecal coliform bacteria in human faeces (Canadian Water Quality Guidelines, 1992; SA Water Quality Guidelines, 1996).

Furthermore, certain members of the faecal coliform group are able to multiply at relatively lower temperatures than that of the intestines of warm-blooded animals and could therefore yield higher counts in water sam is originally added to the particular water environment.

The occurrence of *E. coli* in a stream indicates faecal pollution specifically, as well as the possible presence and survival of bacterial pathogens (Canadian Water Quality Guidelines, 1992; Baudisova, 1997; Jagals 2000). *E. coli* can therefore be used to evaluate the quality of wastewater effluents, river water, raw water for drinking water supply, water used for irrigation and aquaculture as well as water used for recreation (DWAF, 1996b).

1.5.1.2 *Clostridium perfringens (C. perfringens)*

Protozoan parasites such as *Giardia* and *Cryptosporidium* spp. are environmentally resistant parasites commonly found in water. They can cause gastro-enteritis in humans when ingested (Kfir et al., 1995; Gericke et al., 1996). Ingesting cysts or oocysts, excreted in the faeces of infected humans or animals, transmits the parasites. The infection can therefore be transmitted from person to person, through ingestion of contaminated water (drinking water and water used for recreational purposes), food, from animal to person, or by contact with faecally contaminated environmental surfaces (Ongerth et al., 1995; Juranek, 1995).

As *Cryptosporidium* oocysts and *Giardia* cysts have been found in water sources, wastewater and treated effluents in South Africa (Gericke et al., 1996), it is necessary to monitor water for the possible presence of these parasites. However, methods for detection of pathogenic parasites are generally expensive, complex and time-consuming (Venter et al., 1996). Furthermore, due to variable recovery rates achieved by current detection methods, numbers of *Giardia* cysts and *Cryptosporidium* oocysts correlate poorly with bacterial indicators such as *E. coli* (Ferguson et al., 1996).

Studies done by Ferguson et al. (1996) and Medema et al. (1997) have shown that spores of *C. perfringens* survive for periods similar to those for cysts and oocysts in untreated river water. Other studies have also shown that although there is no correlation between the numbers of *C. perfringens* and the protozoan parasites in water samples, *C. perfringens* may prove useful as an indicator of the possible presence and survival of *Cryptosporidium* and *Giardia* cysts and oocysts (Payment and Franco, 1993; Ferguson et al., 1996; Medema et al., 1997; Jagals, 2000). *C. perfringens* were therefore included in the study to indicate the possible presence of protozoan parasites.

1.5.1.3 Somatic coliphages

Viruses feature prominently among the wide variety of pathogenic microorganisms transmitted by water. Faecally polluted water may harbour a wide variety of viruses originating from the human intest , 1996) and as far back as 1991 and 1992, more than 120 types of human enteric viruses have been already identified and enumerated in human faeces (West, 1991; Botero et al., 1992).

The detection methods used for viruses are generally expensive, complex, time-consuming and hazardous for the health of the analyst (Grabow, 1996). It was therefore more practical to use bacteriophages as indicator organisms for the possible presence of pathogenic viruses (Grabow, 2000). Bacteriophages are viruses that infect bacteria (Grabow, 2000). The size and structure of bacteriophages are similar to pathogenic enteroviruses and can be used as model human viruses. Laboratory assay is also much easier (Sakoda et al., 1997).

Borrego et al. (1990) studied the survival and productive infectivity of bacteriophages in natural aquatic environments and concluded that bacteriophages may be considered as good indicators of faecal pollution in natural waters. Although there are several types of bacteriophages, Kfir et al. (1991) reported that somatic coliphages outnumbered other bacteriophages in faecally polluted waters. These bacteriophages also produce larger and clearer plaques (opaque spots seen in the host culture-mat, Appendix C) than all the other bacteriophages and were therefore used in the study to indicate the possible presence of human enteric viruses in water.

1.6 ENVIRONMENTAL HEALTH RISK ASSESSMENT

Water has been rated as one of the most important environmental factors that may influence the health of people (WHO, 1997). While microbiological indicator organisms and their related pathogens do not appear to have a negative impact on the ecological quality of the receiving waters in the Modder River catchment system (Jagals, 2000), an increase in the number of microbiological pathogens in the surface water in the study area may therefore constitute a definite risk of contracting water-related diseases, depending on water use.

An impact on the receiving waters will lead to a risk of infection if the microbiological indicator organisms in the water are present in numbers exceeding particular levels stated in several national and international water quality guidelines.

The assessment of the infection risk done in this study was based on comparing the determined levels of microbiological contaminants in a water source, to the NOAEL of such contaminants stated for instance, in the South African Water Quality Guidelines (1996). This step provided the means to describe the impact of faecal pollution on the health-related microbiological quality of the receiving streams.

1.6.1 Site-specific water qual

Jagals (2000) compiled a site-specific guide (Table 1) for the NOAEL of the indicator organisms used in this study to provide readers with a composite version of the various guidelines. For this study, this guide was used to assess the risk of infection to users of the stream water within the study sectors of the Modder River catchment based on the NOAEL. The numbers of microbiological indicator organisms, detected in the surface waters of the study area, were therefore compared to the various proposed water-use limits to describe the infection risk to consumers.

Table 1: Site-specific guidelines for health related microbiological instream water quality.

Use category		*E. coli* Per 100 mL	*C. perfringens* per 100 mL	Somatic coliphages per 100 mL
Domestic	Drinking	≤ 1[2] Insignificant chance for infection	≤ 1[5] Maximum limit for insignificant risk of infection	≤ 10[3] Low risk of viral infection
	Food Preparation	≤ 1 Faecal coliforms[1] Insignificant chance for infection	Not indicated	Not indicated
	Bathing (contact risk)	≤ 10 Faecal coliforms[1] Insignificant chance for infection	Not indicated	Not indicated
	Laundry (contact risk)	≤ 10 Faecal coliforms[1] Insignificant chance for infection	Not indicated	Not indicated
Agriculture (consumption risk)	Vegetable and salad crops eaten uncooked, sports fields, public parks	≤ 1 000 Faecal coliforms[7] No potential risk No potential viral risk (Shuval et al., 1997)	Not indicated	≤ 1 000 Faecal coliforms[7] No potential risk No potential viral risk (Shuval et al., 1997)
Agriculture (worker contact risk but limited consumption risk)	Cereal crops, industrial crops, fodder crops, pasture and trees	≤ 100 000 Faecal coliforms[7]	Not indicated	Not indicated
Livelihood fishing (consumption risk)	Harvesting impoundments with baited fishing lines	≤ 1 000 Faecal coliforms[7] Fish muscle invasion risk if higher levels	Not indicated	Not indicated
Recreation	Full contact (consumption risk)	≤ 130[4] Risk of gastrointestinal effects expected	≤ 50[6] Maximum limit for insignificant risk of infection with consumption	≤ 20[4] Enteric virus infection risk indicated
	Intermediate contact (contact risk)	≤ 1 000[4] Health effects are indicated	Not indicated	

1. Quality of Domestic Water Supplies - Volume 1: Assessment Guide (WRC, 1998)
2. South African Water Quality Guidelines - Vol.1: *Domestic Use*. (DWAF, 1993)
3. South African Water Quality Guidelines - Vol.1: *Domestic Use*. (DWAF, 1996a)
4. South African Water Quality Guidelines- Vol.2: *Recreational Use*. (DWAF; 1996b)
5. Water Quality Criteria in South Africa (Aucamp and Vivier, 1990)
6. Waterborne Pathogens (Mahin and Pancorbo, 1999)
7. Health guidelines for the use of wastewater in agriculture and aquaculture - Technical Report (WHO, 1989)

Microbiological indicator organis[illegible]ed from current South African and other local and international water quality guides. Regulations for the microbiological quality of receiving treated wastewater bodies in South Africa, currently stipulate that effluent should contain no faecal coliforms per 100 mL. The standard may be relaxed to 1000 faecal coliforms per 100 mL under certain circumstances (Water Amendment Act, Act No. 96 of 1984). There are no instream microbiological standards or guidelines for any distance in the receiving waters after discharge, and it can be assumed that the microbial loading of receiving water bodies, by indicator organisms in effluent discharge, is within the assimilative capacity of such water bodies, if the General Standard is complied to (Venter et al., 1996).

Treated, and often untreated, wastewater is disposed of directly, or indirectly, into rivers and streams. The contaminated river is then used for irrigating various crops, without any national or international microbiological water quality guidelines (Westcot, 1997). The US Environmental Protection Agency (EPA), therefore, recommended that the acceptable guideline for irrigation with natural surface water, including river water containing wastewater discharges, be set at 1000 faecal coliforms per 100 mL (Westcot, 1997).

The UN Food and Agriculture Organisation (FAO) has recently recommended that the 1000 faecal coliforms per 100 mL limit, proposed by the WHO in the WHO Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture (1989), are used as interim irrigation water standard (Westcot, 1997).

The use of *E. coli* as a more specific indicator for the possible presence of faecal pollution in recreational waters has been recognised (DWAF, 1996b), but *E. coli* criteria do not yet exist for the various agricultural uses of water. It is also not common practise to compare *E. coli* and faecal coliform numbers in water samples since these groups represent various different species and strains. However, Jagals (2000) reported that faecal coliform numbers tested in faecally polluted urban run-off as well as in treated wastewater effluents from this study area, may consist of approximately 80% *E. coli*. If this were to be extrapolated to this study, the prescribed *E. coli* numbers would then be 800 cfu per 100 mL in instances where the faecal coliform limit is proposed to be 1000 cfu per 100 mL. This formula is used throughout this dissertation.

The faecal coliform criterion given in the WHO 1989 guidelines, is intended to also serve as a risk limit for indicating the presence of viruses in the agriculture category for irrigation of vegetable and salad crops eaten uncooked as well as sports fields and public parks (Westcot, 1997; Shuval et al., 1997; Blumenthal et al., 1999).

Comprehensive criteria for acce . *perfringens* for most of the water-use categories do not yet exist in reputable guides. Criteria especially do not exist for *C. perfringens* as quantitative indicators of the numbers of protozoan parasites such as *Cryptosporidium* and *Giardia* spp.

For drinking water, Aucamp and Viviers (1990) proposed a guideline limit of ≤1 cfu per 100 mL. This would ensure that an insignificant level of risk would not be exceeded. Mahin and Pancorbo (1999) reported that the State of Hawaii accepted *C. perfringens* to be one of the most reliable indicator groups for faecal contamination in tropical waters, because the tropical environment naturally contains high numbers of traditional bacterial indicators. This state recommends a limit of 50 colony-forming units (cfu) per 100 mL for freshwater.

For this study, the numbers of *C. perfringens* detected in recreational waters were compared to *C. perfringens* criteria used by the State of Hawaii. These were the only guidelines for *C. perfringens* in recreational waters that could be found in literature and were subsequently included in the site-specific guide (Table 1). No guidelines for the other uses could be found.

1.7 RESEARCH AIM AND OBJECTIVES

During the study, the release of microbiological indicator organisms into the Renoster Spruit sub-catchment area, as well as the assimilative capacity of the receiving stream, was investigated. The assimilation capacity of the receiving waters was assessed by comparing the variance in levels of indicator microorganisms released both spatially (unpolluted point to polluted point) and temporally (before and after events at the same point) through discharge events. Aspects such as the hydraulic regime of the target streams fell outside the scope of this study and were therefore not done.

The impact created by the microorganism releases, was translated into statements of potential risk of infection to people that may use the untreated surface waters for domestic purposes as well as recreation and agriculture, based on guidelines values proposed in the site-specific guideline (Table 1).

The aim of the study was, therefore, to assess the impact of various wastewater and urban discharges on the health-related microbiological quality of water in receiving surface watercourses within the catchment area, through empirical water quality measurement and statistical comparison of pollution and non-pollution aspects.

More specific objectives were identified to support the aim. These were:

1) To assess the impact of th[illegible]charged from the recently commissioned Sterkwater wastewater treatment works on the microbiological quality of the Renoster Spruit.

2) To assess the impact of diffuse urban discharges from the various residential areas of Bloemfontein (surface run-off) on the microbiological quality of water in the Fontein and Bloem Spruits.

3) To assess the impact of final effluent from Bloem Spruit wastewater treatment works on the microbiological quality of water in the Bloem Spruit.

4) To determine the compounded impact of diffuse urban discharges from the city of Bloemfontein (surface run-off and discharged effluent from Bloem Spruit wastewater treatment works in the Bloem Spruit) on the microbiological quality of water in the Renoster Spruit.

5) To determine the compounded impact of diffuse urban discharges from the city of Bloemfontein via the Bloem Spruit and final effluent from Sterkwater treatment works on the microbiological quality of water in the Renoster Spruit.

6) To determine the impact of the Renoster Spruit on the microbiological quality of water in the Modder River.

Chapter 2
METHODOLOGY

The release of microbiological indicator organisms from two wastewater treatment works (Bloem Spruit and Sterkwater treatment works) as well as surface run-off from the city of Bloemfontein, into the waters of the Bloem, Fontein and Renoster Spruits, was monitored over a period of 31 months (August 1997 → March 2000). The aim of the study was to determine the impact these releases created on the health-related microbiological quality of water in the Renoster Spruit sub-catchment.

STUDY AREA

The study was conducted in the Renoster Spruit sub-catchment within the catchment of the Modder River (Figures 1 & 2.1). The city of Bloemfontein is situated in this sub-catchment.

2.1 CLIMATE

South Africa is classified as a sub-humid, warm country with an annual water deficiency (i.e., evaporation exceeds rainfall) (DWAF, 1993). The climate in the study area varies from moderate to hot during summer months, to very cold in winter.

Rainfall in South Africa is frequently very seasonal and varies in quantity (DWAF, 1996). The average rainfall in Bloemfontein is 559 mm / year which is well below the world average of 860 mm / year (Midgley et al., 1994). The rainy season generally occurs between October and March. Thunderstorms and soft rains occur in approximately equal quantities. Thunderstorms are characterised by rain and hailstorms. These storms result in high rates of storm water run-off over short periods of time.

2.2 BLOEMFONTEIN CITY

Bloemfontein has well developed modern urban areas, as well as economical and sub-economical residential developments. This is supplemented by well-functioning business sectors and well-developed industrial zones. All these areas are serviced by full waterborne sewerage.

In addition, large areas of low cost residential settlements, with limited sanitary systems, as well as areas of informal settlement with inadequate sanitary services, intersperse and fringe the sub-economical residential areas. In 1999, approximately 18 % of the 144 174 houses in the city were classified as areas with low cost informal housing. Numbers of these houses increase daily (Vogis werksgroep, 1999).

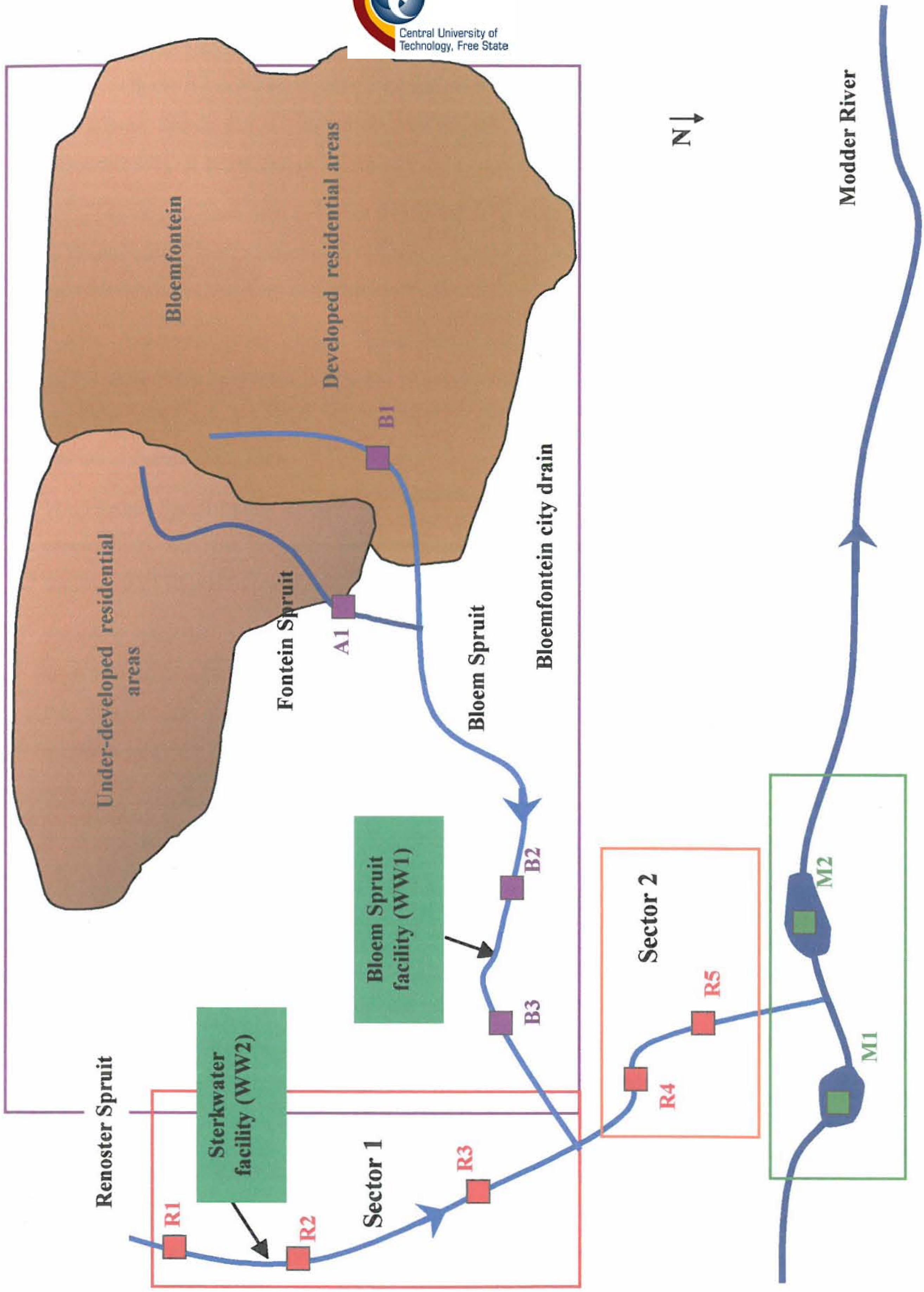

FIGURE 2.1: Diagrammatic representation of the Renoster Spruit sub-catchment area indicating the various sectors and sampling points.

2.3 SAMPLING SITES

2.3.1 Bloem Spruit and Fontein Spruit (Figure 2.1 as well as Figure 1)

The Bloem Spruit and it's tributary, the Fontein Spruit, are urban streams that drain an estimated 80% of storm and other run-off from Bloemfontein into the Renoster Spruit.

Loch Logan is a small dam (±100 000 m^3) that impounds the Bloem Spruit in the central part of Bloemfontein (sampling site **B1**). Loch Logan receives surface run-off from a large part of the well developed western residential suburbs.

Further downstream, the Bloem Spruit receives run-off from residential, central business, industrial, as well as the lesser-developed residential zones of Bloemfontein. Run-off, during and after heavy rainstorms, occurs rapidly into the Bloem Spruit due to extensive paving and cemented canals in the area.

The Fontein Spruit drains surface run-off, containing land deposited faecal materials, from the surrounding low-cost high-density residential and informal zones, into the Bloem Spruit, south-east of Bloemfontein (sampling site **A1**).

Sampling site, **B2,** was selected approximately 300 metres downstream from the Bloem Spruit/Fontein Spruit confluence. This point approximately 3 000 metres downstream from **B1**, was used as a reference point for the city's run-off, before receiving 60-ML/day final effluents from the Bloem Spruit wastewater treatment works (WW1).

Sampling site, **B3,** was approximately 400 metres downstream from this effluent discharge point and represented the majority of urban surface water discharges from Bloemfontein city.

2.3.2 Renoster Spruit (Figures 1 and 2.1)

The Renoster Spruit is historically a low-flow stream up to the point where it receives the water of the Bloem Spruit. Since January 1999, after the commissioning of the Sterkwater wastewater treatment works (WW2), the up-stream had also become perennial from the point where it receives treated effluent from the new works. For the purpose of this study, the Renoster Spruit was divided into two sectors up to the point where it confluences with the Modder River, north-east of Bloemfontein.

Sector 1 included sampling sites **R1** to **R3**. These sites were monitored to assess the impact of the discharges from Sterkwater on the health-related microbiological quality of water in the Renoster Spruit. At the time of the study, no other urban point or diffuse sources of faecal pollution could be identified in this Sector of the Renoster Spruit. Due to limited presence of livestock and human settlement, the small-scale riparian agricultural land use in this sector, was assumed not to contribute markedly to faecal pollution of the Spruit.

R1 represented the unpolluted in an agricultural surrounding scarcely populated by livestock and humans. Since the Bloem, Fontein and the Renoster Spruits are situated in the same geographical and topographical surroundings, the microbiological quality of water sampled at this point was also considered as characteristic for unpolluted stream water in the study area. Results obtained at this headwater sampling point reflected the natural background levels of microbiological indicator organisms for this study.

R2 was approximately one kilometre downstream from R1 and immediately downstream from the final effluent discharge point from the Sterkwater wastewater treatment works. Results obtained at **R2** indicated the possible impact of the discharged final effluent on the health-related microbiological quality of water in the Renoster Spruit.

R3 was approximately eight kilometres downstream from R2 and approximately one kilometre upstream from the confluence with the Bloem Spruit. The site was selected to assess the residual levels of microbiological indicator organisms in the impacted Sector 1 of the Renoster Spruit, before receiving the other urban discharges from Bloemfontein via the Bloem Spruit.

Sector 2 had a similar riparian profile to Sector 1. It included sampling sites **R4** and **R5,** which were used to determine the collective impact of the urban discharges (including treated wastewater effluent from Bloem Spruit and Sterkwater treatment works) from Bloemfontein, on the health-related microbiological water quality of the lower Renoster Spruit.

R4 was the sampling site in the Renoster Spruit, immediately downstream from the point of confluence with the Bloem Spruit. Results from this point reflected the impact of the majority of discharges from Bloemfontein on the microbiological quality of water in the Renoster Spruit, which, before this point, already reflected (at R3) the impact of the Sterkwater discharge.

R5 was the last downstream sampling site in the Renoster Spruit, selected to assess the capacity of the Renoster Spruit to assimilate the microbiological contaminants from all the various discharges from Bloemfontein, before draining into the Modder River.

2.3.3 Modder River (Figures 1 and 2.1)

Sampling sites **M1** and **M2** were selected to reflect the impact of the Renoster Spruit on the health-related microbiological quality of water in the Modder River.

M1 was the reference point in the Modder River at the Mazelspoort Barrage system, which was situated approximately four kilometres upstream from the Renoster Spruit confluence. Results obtained at this point indicated the background water quality of the Modder River.

M2 was the site in the Modc imately 300 metres downstream of the confluence with the Renoster Spruit, used to determine the impact of the Renoster Spruit on the Modder River.

2.3.4 The wastewater treatment works

Two wastewater treatment works are located in the Renoster Spruit sub-catchment area. Their respective positions, relative to the other sample points in the sub-catchment, are shown in Figures 1 and 2.1.

2.3.4.1 *WW1* (*Bloem Spruit wastewater treatment works*)

Final effluent from this established fixed-medium wastewater treatment works, amounts to approximately 60 ML per day. Pond maturation is used as method of disinfection to destruct pathogens before discharge. This discharge often constitutes the major constant source of running water in the Bloem Spruit.

2.3.4.2 *WW2* (*Sterkwater wastewater treatment works*)

This system consists of waste stabilisation ponds, followed by an activated sludge polishing system and was discharging approximately 10 ML per day final effluent at the time of the study. The design of the works includes chlorination of the final effluent as the method of disinfection. This final effluent often provides the only running water in Sector 1 of the Renoster Spruit since the works was commissioned.

2.4 WATER SAMPLING

2.4.1 Sampling program

A sampling program had been established on the following basis:

- Water samples were collected at least once a month, during all seasons, from the Fontein, Bloem and Renoster Spruits. This was to provide the baseline data of ordinary flow water quality. Samples were also taken, at the same time, from the effluent canals emerging from the two wastewater treatment works.

- For the first phase of the study, water samples were collected at least once a month, during all seasons, for 16 months "before" Sterkwater wastewater treatment works was commissioned.

- The second phase included taking samples, biweekly, during all seasons, for 15 months "after" Sterkwater was commissioned, to determine the impact of the final effluent on the health-related microbiological quality of water in the Renoster Spruit.

2.4.2 Sampling methodology

To obtain representative samples, all water samples were collected as near as possible to the middle of the various streams. Homogeneous organism distribution in the various waters was assumed at each sampling point because of stream turbulence. Final effluent samples were collected from the effluent canals, just before the effluents discharged into the receiving water bodies.

Sterile, 500 mL Whirl Packs® were submerged into the water and filled. The samples were placed in cooler bags (7°C-10°C) and transported to laboratories at the Technikon Free State. Samples were analysed within 4-6 hours of collection. Water samples for somatic coliphage assessments were transported overnight to the University of Pretoria (Department of Medical Virology) and analysed within 24–30 hours from collection.

2.5 HEALTH-RELATED MICROBIOLOGICAL WATER QUALITY

2.5.1 Microbiological indicator organism analyses

The Membrane Filtration Technique (Appendix A), (Standard Methods, 1998) was used to enumerate *E. coli* and *C. perfringens* (Appendix B). The Double-layer Plaque Assay method (Appendix C) was used to enumerate somatic coliphages (Grabow, 2000).

2.5.1.1 *Escherichia coli* (*E. coli*)

E. coli are bacterial indicators of definite faecal pollution from humans and warm blooded animals (DWAF, 1996b). These organisms were generally detected by methods that require some elaborate and time-consuming techniques (Jagals., 2000). Recently developments in defined substrate technology for detecting specific bacteria groups such as *E. coli* saw the Chromocult Coliform® Agar developed by the Merck Corporation (1996) (Appendix B). According to Jagals (2000) this media provides a practicable alternative for other more cumbersome methods due to its ability to detect *E. coli* in a single membrane filtration step. Jagals et al., (2001) also compared this method with other similar methods and had found it to be a reliable method to detect *E. coli* in polluted as well as unpolluted surface waters.

2.5.1.2 *Clostridium perfringens* (*C. perfringens*)

C. perfringens were used as indicators of resistant faecal pollution (Ferguson et al., 1996; Medema et al., 1997) and to indicate the presence of the cysts- and oocysts of pathogens such as *Cryptosporidium* and *Giardia* (Payment and Franco, 1993).

To enhance the value of the organism group in order to indicate resistant organisms, water samples were pasteurised (Appendix B) to knock out the background flora in the water samples as well as to be left with predominantly the spores of resistant organisms, including the spores of *C. perfringens.* were then enumerated on supplemented Perfringens (OPSP) Agar (Oxoid Corporation, 1990) (Appendix B).

2.5.1.3 *Somatic coliphages*

Somatic coliphages indicated the potential presence of enteric viruses (Grabow, 2000) and were enumerated by the Plaque Assay method with a Double Agar Layer technique using the *E. coli* strain HTCC 70078 (Grabow et al., 1993) (Appendix C).

2.5.2 Counting the organisms

After incubation for the prescribed periods of time, *E. coli* and *C. perfringens* colonies as well as somatic coliphage plaques, were counted according to the prescriptions, from the respective manufacturer's and other manuals, for each of the organism groups (Appendices B and C).

Bacterial indicator organism numbers were expressed as colony-forming units (cfu) / 100 mL. Somatic coliphage numbers were expressed as plague-forming units (pfu) / 100 mL. The formula for calculation is presented in Appendix A.

2.6 COLONY VERIFICATION (Appendix D)

Non-indicator organisms often find the selectivity of a specific growth medium accommodating, and then grow in the colours prescribed to the analyst for colony identification. These organism colonies are referred to as false positives (Standard Methods, 1998). Dionisio and Borrego (1995), as well as Figueras et al. (1996) found the selectivity of the bacterial growth media to be inconsistent. One of the most common reasons is that many sub-species can be found in the single indicator organism group. To determine the accuracy of true indicator organism numbers, a verification programme, designed by Jagals (2000) using Standard Methods (1998), was followed. The programme is discussed in Appendix D.

2.7 STATISTICAL ANALYSES OF DATA (Appendix E)

Data was tabled in Microsoft Excel® 2000 spreadsheets and transformed to $_{\log}10$ values. The data was then described statistically for the sample size, geometric mean, median and 95% confidence intervals.

The statistical programme SigmaStat® 2 (1997) was used to calculate and test for sample size, normality as well as analyses of variance (ANOVA) between data groups.

SigmaPlot® 6 (2000) was used to plot the data in graphs. The data were displayed with line graphs for spatial appraisal, and in boxplots for statistical appraisal. The spatial appraisals were of the differences in the microbiological water quality of the Renoster Spruit during the first and second phase of the stu[illegible]ts provide visual summaries of the centre of the data, the variation of spread, the skewness (normality) and the presence or absence of unusual values in the data (Helsel and Hirsch, 1995).

2.8 STATISTICAL CONSIDERATIONS

2.8.1 Developing hypotheses (Appendix E)

Water scientists often have prior ideas, called hypotheses, of how they think systems behave. These hypotheses are generally referred to as research hypotheses or can be in the form of problem statements or theories. The primary objective for statistically analysing data is to test whether these prior ideas are of value, based on the evidence provided by the data (Helsel and Hirsch, 1995). To enable scientists to apply statistical methods to test their theories, specific hypotheses are developed to which the outcomes of statistical tests can be related. These are referred to as statistical hypothesis and may appear different in their wording to the original research hypotheses (Katzenellenbogen and Joubert, 1997).

Statistical tests are quantitative techniques, used to determine whether the statistical hypotheses can, or should be, substantiated, modified, or rejected outright (Helsel and Hirsch, 1995). These tests are used to indicate the validity of the original research hypotheses. For this study, various problem statements and related statistical hypotheses were formulated for each of the sections contained in Chapter 3: Results and Discussion.

2.8.2 Minimum sample size (Appendix E)

In order to detect an effect or difference at a specified level of statistical difference, minimum sample sizes were determined. Approaches used to determine sample sizes for this study are discussed in Appendix E.

2.8.3 Analyses of variance (ANOVA) (Appendix E)

Analyses of variance are parametric or non-parametric tests that determine whether groups of data differed significantly in their (or have identical) central values (mean or median, depending on the test) and variation of spread. Microbiological water quality data are seldom normally distributed around the mean (Standard Methods, 1998) and can therefore be assumed to be non-parametrical (Helsel and Hirsch, 1995). For this study, the non-parametric Mann-Whitney Rank Sum and the Kruskal-Wallis ANOVA on Ranks tests were used to test for significant differences between the test groups, beyond what can be attributed to random sampling variation.

The Mann-Whitney Rank Sum Test was selected when two data groups were compared and the Kruskal-Wallis ANOVA on Ranks test was selected when three or more groups were compared (Helsel and Hirsch, 199 ... igmaStat® 2, 1997). The latter, compares results from several different experimental groups (more than 2 sampling points) that may be affected by a single factor (numbers of indicator organisms).

2.9 PROBLEM STATEMENTS AND STATISTICAL HYPOTHESES

Figure 2.2 shows the layout used in Chapter 3: Results and Discussion. Data obtained during the study period were grouped in sections, based on the location of the sampling points.

Data was statistically analysed to address the problem statement within each of the sections, based on whether the relevant statistical hypotheses were accepted or rejected.

2.9.1 Health-related microbiological quality of water in the Renoster Spruit (Section 3.1)

This section gives a general overview of the health-related microbiological quality of water in the Renoster Spruit during the study period.

2.9.2 Microbiological quality of water in Sector 1 (Section 3.2)

Given the profile of the agricultural nature of the land use in the sub-catchment, it was assumed that storm-induced surface run-off had no impact on the health-related microbiological water quality of this Sector of the spruit after rainfall events. The specific objective for this section was therefore only to determine whether the final effluent, from the Sterkwater works, had any impact on the health-related microbiological quality of water in the Renoster Spruit since it was commissioned. This section also includes data on the microbiological quality of the headwater sampling point (R1) as well as on the final effluent discharged from Sterkwater treatment works (WW2) (Figures 1 and 2.1).

Problem statement 1: The impact that effluent from Sterkwater might have on the health-related microbiological quality of water in the Renoster Spruit in this Sector of the Spruit, was not certain.

Effluent from a properly designed, and managed, wastewater treatment works should not contain excessive levels of harmful microorganisms. In theory, therefore, final effluent from Sterkwater should not impact on the health-related microbiological quality of water in the Renoster Spruit to such an extent that the numbers of the microbiological indicator organisms are increased beyond the criteria for safe use of the stream water, proposed in the site-specific guide (Table 1, Chapter 1).

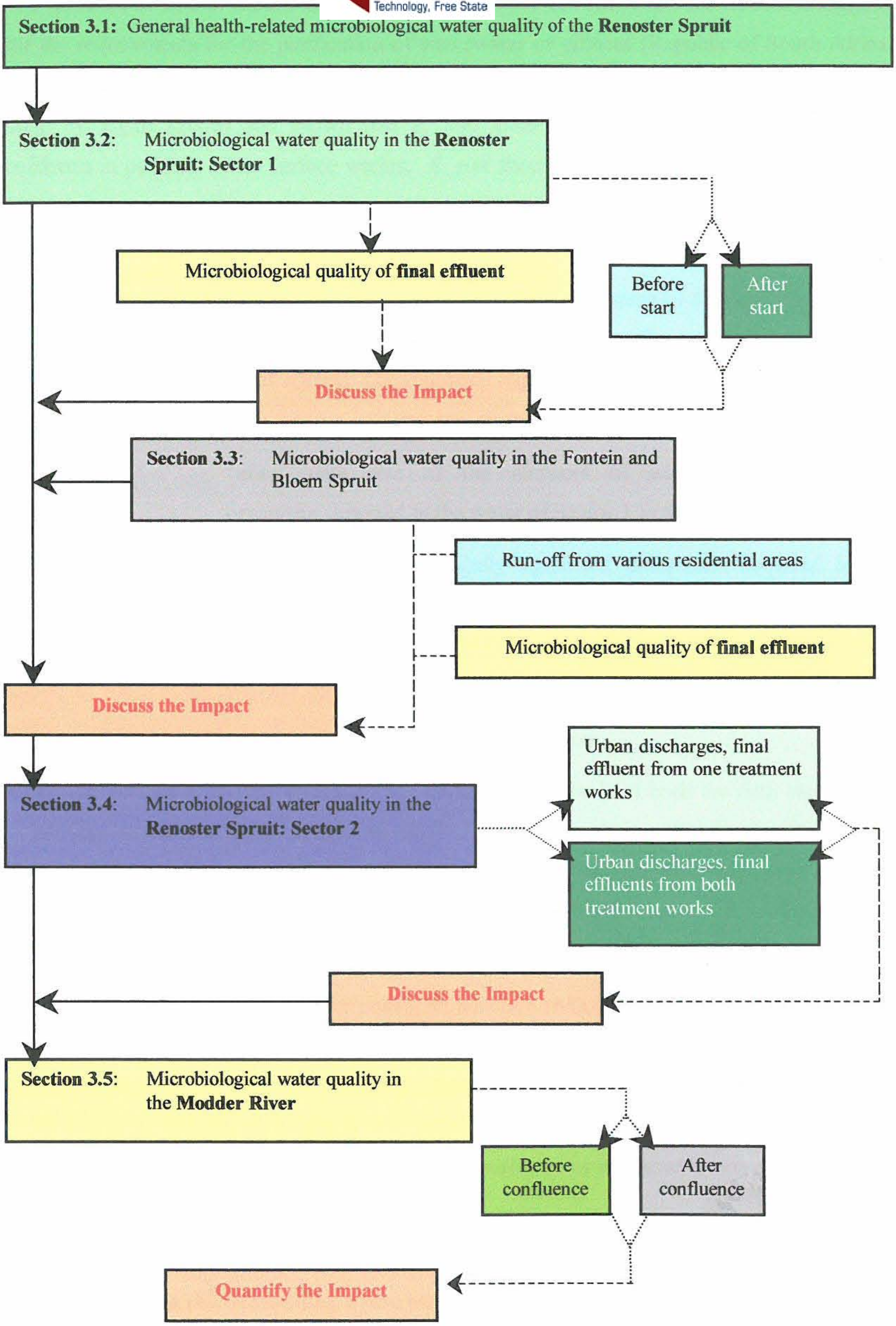

FIGURE 2.2: Outline of results format in Chapter 3.

The numbers of *E. coli* should als per 100 mL, since the *General Standard* for the requirements for the purification of wastewater or effluent (Republic of South Africa, 1984), states that faecal coliforms should not be present in the discharged effluent. Studies done by Jagals (2000) had shown that *E. coli* constitutes approximately 80% of faecal coliforms in polluted urban surface waters. *E. coli* should, therefore, not exceed 800 cfu per 100 mL in instances where a relaxation of the faecal coliform standard (1000 cfu per 100 mL) had been granted.

To test for statistically significant differences in data of all the samples taken during the first and second phase of the study, before and after commissioning, the non-parametric Mann-Whitney Rank Sum Test was applied. The following hypothesis was formulated:

Null hypothesis (H_0): There will be no statistically significant difference in the "before" and "after" data sets of the numbers of microbiological indicator organisms detected in the water of Sector 1 in the Renoster Spruit.

Rejection of the H_0 will indicate that statistically significant differences occurred in the numbers of microbiological indicator organisms, after Sterkwater was commissioned. This will imply that the treatment works had an impact on the health-related microbiological quality of water in the Renoster Spruit.

To quantify any possible impact, the following steps were taken:

- The geometric means (with the 95% confidence interval) of both the data sets, for all three the indicator groups, from all the various sampling points in Sector 1, were compared to the guideline values, in a line graph, to determine whether the various safe limits were exceeded (Section 3.1).

- If apparent that numbers of the indicator groups, in both their respective data sets, exceeded the values (therefore posing an infection risk), the value of comparing the two data sets was to determine whether the "after" data showed a statistically significant difference in the indicator organism numbers. This step would indicate the extent to which the existing risk of infection, indicated by the microbiological test organisms in the Renoster Spruit in this Sector, would be mitigated or increased by the effluent:

  ≠ Should the "before" data not exceed the guideline values, but the "after" data did, the objective of comparing the two data sets was to determine the impact the effluent had to cause a risk of infection, where previously there was none.

  ≠ Conversely, should the "before" data exceed the guideline values, but the "after" data not, the objective of comp[...] sets was then to determine the impact the effluent had to mitigate the risk of infection.

Sampling point R3 (Figures 1 and 2.1) was also use as a downstream reference point, to assess the extent to which the Renoster Spruit assimilated any impacts that the Sterkwater discharge might have had, over distance and time.

2.9.3 Microbiological quality of water in the Bloem Spruit (Section 3.3)

Three specific objectives were set for this section. One of the objectives was to determine whether run-off, from the various residential suburbs of Bloemfontein, had any impact on the health-related microbiological quality of water in the Fontein and Bloem Spruits.

Another objective for this section was to determine whether the final effluent, discharged from the Bloem Spruit wastewater treatment works, had any impact on the health-related microbiological quality of water in the Bloem Spruit, which already contained urban surface run-off. This section also includes results of the microbiological quality of the final effluent.

Problem statement 1: While it is generally assumed that surface run-off from developing urban areas are of a poorer microbiological quality than surface run-off from developed areas, it was uncertain whether this was the situation in the study area.

Null hypothesis (H_0): There will be no statistically significant difference in the numbers of microbiological indicator organisms detected in the water of the Fontein Spruit (A1) and the Bloem Spruit (B1) (Figures 1 and 2.1).

Rejection of the H_0's will indicate that the statistically significant differences in the numbers of microbiological indicator organisms occurred as a result of the varying quality of run-off from the various residential settlements.

Problem statement 2: It was uncertain what the impact of the combined urban run-off (A1 and B1) was on the microbiological quality of the Bloem Spruit before final effluent from the Bloem Spruit works discharged into it.

The Mann-Whitney Rank Sum Test was also applied to test for statistically significant differences between data from B2 and R1 (Figures 1 and 2.1).

Null hypothesis (H_0): There will be no statistically significant difference in the numbers of microbiological indicator organisms detected in the water of the Bloem Spruit sampled at B2 and in the water of the Renoster Spruit at R1.

Rejection of the H_0's will indic[illegible] run-off might have on the numbers of microorganisms in Bloem Spruit before the addition of final effluent from the treatment works.

Problem statement 3: It was uncertain if effluent from the Bloem Spruit wastewater treatment works had an impact on the health-related microbiological quality of water in the Bloem Spruit, already containing the total urban run-off from the city.

The non-parametric Mann-Whitney Rank Sum Test was applied to test for statistically significant differences between the data of sampling points B2 and B3 (Figure 1 and 2.1).

Null hypothesis (H_0): There will be no statistically significant differences in the data of the two sets.

Rejection of the H_0 will indicate that the statistically significant differences in the numbers of microbiological indicator organisms might be due to the following:

- Higher numbers of microbiological indicator organisms at B2, decreased because the effluent actually diluted the numbers.
- The level of microbiological indicator organisms in the effluent was so high that the numbers of organisms determined at B2 were elevated after discharge.

2.9.4 Microbiological quality of water in Sector 2 (Section 3.4)

Sampling points R4 and R5 represented Sector 2 in the Renoster Spruit (Figure 1 and 2.1). The objective of this section was to determine whether the water from the Bloem Spruit had any impact on the general health-related microbiological quality of water in the Renoster Spruit. The impact of the Sterkwater effluent, after the wastewater treatment works commissioned, will also be reflected in the discussion on this section.

Problem statement 1: It was uncertain whether the water from the Bloem Spruit had an impact on the health related microbiological quality of water in the Renoster Spruit, especially before the Sterkwater wastewater treatment works was commissioned.

The Kruskal-Wallis ANOVA of Ranks was applied to test for statistically significant differences between the data of sampling points R3, B3 and R4.

Null hypothesis (H_0): There will be no statistically significant differences in the data of the three sets.

Rejection of the H_0 will indicate t / significant differences in the numbers of microbiological indicator organisms might be due to the following:

- The level of microbiological faecal pollution in the Renoster Spruit, at R3, was so high that the water from the Bloem Spruit actually mitigated the numbers after confluence.
- The level of microbiological faecal pollution in the Bloem Spruit, at B3, was so high, that the numbers of organisms at R4 were elevated after confluence.

***Problem statement 2**:* It was uncertain whether the water from Sector 1 of the Renoster Spruit, which contained the effluent from Sterkwater, added to the impact that water from the Bloem Spruit might have had on the health related microbiological quality of water in the Renoster Spruit.

The Kruskal-Wallis ANOVA of Ranks was applied to test for statistically significant differences between the data of sampling points R3, B3 and R4.

Null hypothesis (H_0): There will be no statistically significant differences in the data of the three sets.

Rejection of the H_0 will indicate that the statistically significant differences in the numbers of microbiological indicator organisms might be due to the following:

- The level of microbiological faecal pollution in the Renoster Spruit, at R3, was so high that any impact that the Bloem Spruit might have had on Sector 2 of the Renoster Spruit, was increased.
- The level of microbiological faecal pollution in the Bloem Spruit, at B3, was so high, that the water from Sector 1 of the Renoster Spruit had no marked impact on the numbers of organisms at R4.

Sampling point R5 (Figure 1 and 2.1) was also used as a downstream reference point to assess the extent to which the Renoster Spruit assimilated any impacts that the urban point and non-point discharges might have had, over distance and time. Results from R5 were also compared to the headwater quality of R1, to further quantify any potential impact.

2.9.5 Microbiological quality of water in the Modder River (Section 3.5)

This section starts with a description of Modder River at the Mazelspoort and Barrage. The specific objective of this section was to determine whether the water from the Renoster Spruit had any the impact on the health-related microbiological quality of water in the Modder River.

Problem statement 1: It was un[illegible]ater from the Renoster Spruit, which contained the urban point and non-point discharges, had an impact on the health-related microbiological quality of water in the Modder River.

The Kruskal-Wallis ANOVA of Ranks was applied to test for statistically significant differences between the data of sampling points M1, R5 and M2.

Null hypothesis (H_0): There will be no statistically significant differences in the data of the three sets.

Rejection of the H_0 will indicate that the statistically significant differences in the numbers of microbiological indicator organisms might be due to the following:

- The level of microbiological faecal pollution in the Modder River, at M1, was so high that the water from the Renoster Spruit actually mitigated the numbers after confluence.
- The level of microbiological faecal pollution in the Renoster Spruit, at R5, was so high, that the numbers of organisms in the Modder River at M2 were elevated after confluence.

SECTION 3.1: THE HEALTH-RELATED MICROBIOLOGICAL QUALITY OF WATER IN THE RENOSTER SPRUIT

This section gives an overview of the microbiological quality of water in the Renoster Spruit. Emphases are placed on the point and diffuse sources of faecal pollution that influenced the health-related microbiological water quality in the Renoster Spruit during the study period.

Figures 3.1 (a, b and c) illustrate the impact of final effluents from the Sterkwater and Bloem Spruit treatment works, as well as the compounded impact of urban surface run-off, on the health-related microbiological quality of water in the Renoster Spruit. Figure 3.1(a) shows the increase in *E. coli* numbers caused by the discharges from the various point and diffuse sources during the first and second phase of the study.

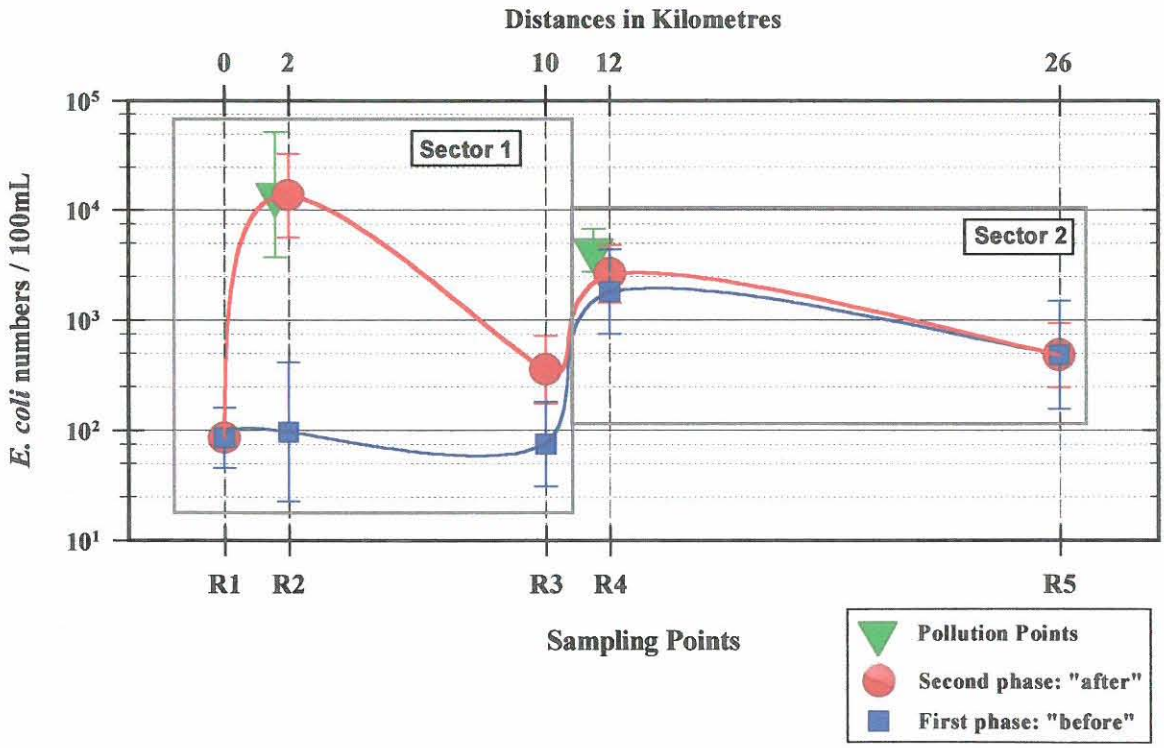

FIGURE 3.1(a): The impact of urban discharges from Bloemfontein city on *E. coli* numbers in the Renoster Spruit during the study period (1997 - 2000).

The immediate impact of these effluents on the geometric mean *C. perfringens* and somatic coliphage levels, at the various sampling points in the Renoster Spruit, are illustrated in Figures 3.1 (b and c).

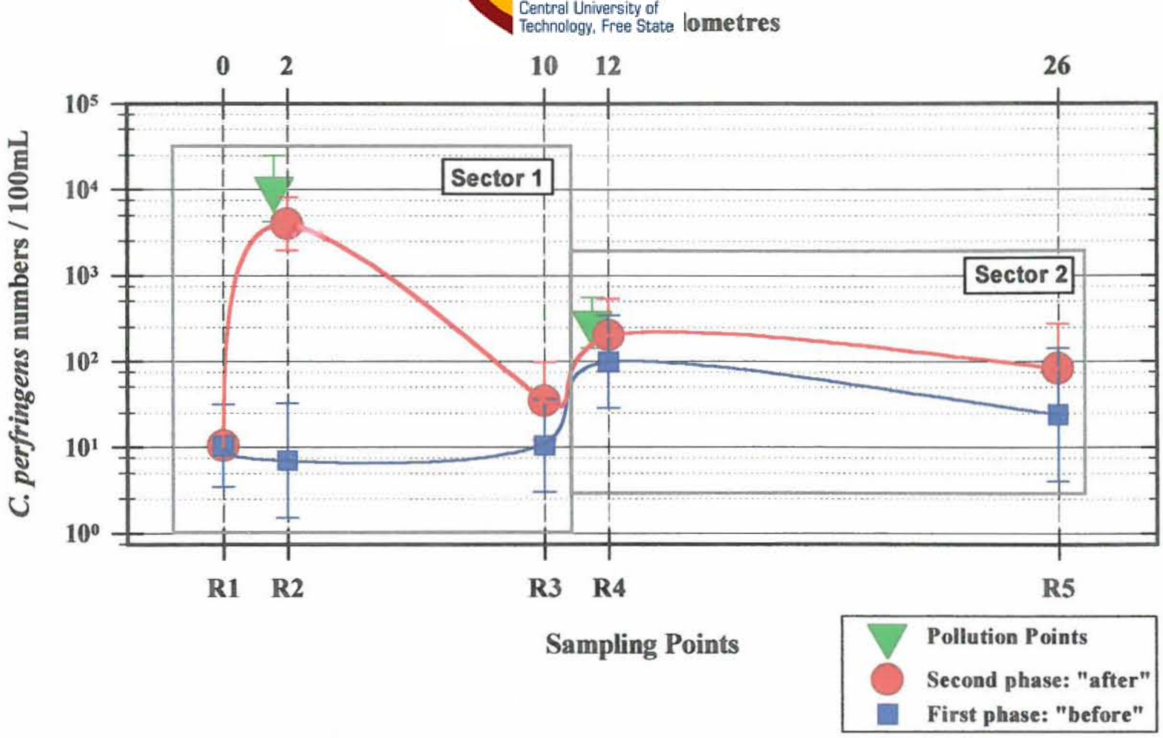

FIGURE 3.1(b): The impact of urban discharges from Bloemfontein city on *C. perfringens* numbers in the Renoster Spruit during the study period (1997 - 2000).

All three graphs show that the indicator organism numbers in Sector 1 of the Renoster Spruit increased since the Sterkwater wastewater treatment works was commissioned.

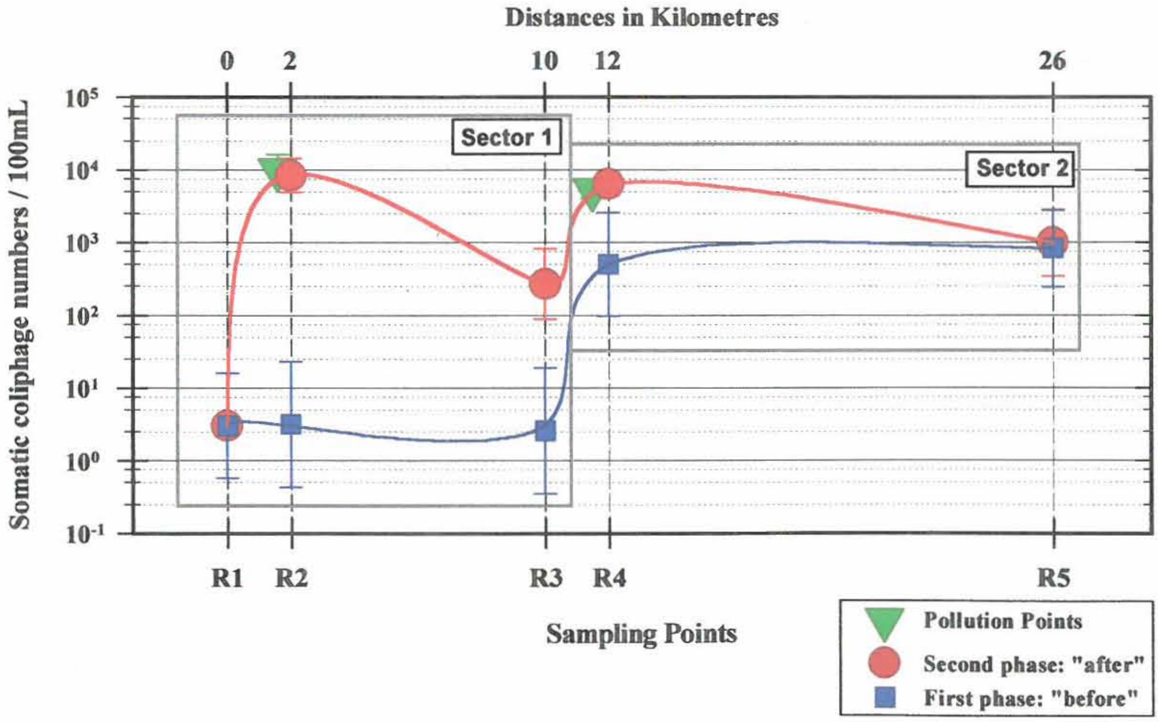

FIGURE 3.1(c): The impact of urban discharges from Bloemfontein city on somatic coliphage numbers in the Renoster Spruit during the study period (1997 - 2000).

The microbiological quality of water from the Renoster Spruit improved downstream from the effluent discharge point, but dete[illegible]er receiving the water from the Bloem Spruit, reflecting the impact of surface run-off as well as final effluent from the Bloem Spruit wastewater treatment works.

SECTION 3.2: MICROBIOLOGICAL QUALITY OF WATER IN SECTOR 1

Land-use in Sector 1 is generally characterised by small-scale riparian agriculture. Livestock and human settlement is limited. The numbers of farm animals in particular were low. No other urban point or diffuse sources of faecal pollution could be identified in Sector 1. Tranter et al. (1996), as well as Marsalek et al. (1994) reported that small-scale riparian agricultural activities of this nature do not contribute meaningfully to faecal pollution of receiving waters. This implies that intra-catchment differences in the microbiological quality of stream water sampling sites could not be related to the intensity of agriculture in the adjacent areas or to climatological events such as rainfall (Tranter et al., 1996). Changes in bacterial water quality do not follow a pattern of highest bacterial concentrations in run-off from areas where livestock farming, and hence faecal input rate, is expected to be most intense, regardless of the event that caused the run-off (i.e. dry or wet weather events) (Tranter et al., 1996). For the purposes of this study, small-scale riparian agriculture was not expected to contribute meaningfully to faecal pollution of receiving waters.

Studies done in the vicinity of the study area by Jagals (1994) had shown that the faecal contamination in run-off from these agricultural areas during, or directly after rainfall events did not differ significantly from the dry weather water quality in the stream. This was probably because sub-surface drainage, as well as the intense vegetative cover in these areas filtered pollutants from run-off before it reached the receiving water (Tchobanoglous and Schroeder, 1987; Chapra, 1997; Pretorius, 1996). This caused the levels of faecal pollution in receiving waters to be low.

Differences in the wet and dry weather water quality in the predominantly agricultural sectors of Sector 1 were not investigated during this study. It was accepted that the water quality would not differ significantly. All the results from both the dry and wet weather periods were included in one data set for Sector 1 to provide an overall view of the water quality. Data from Sector 1 were predominantly used to assess the impact of Sterkwater on the health-related microbiological water quality of the Renoster Spruit after the treatment works commissioned.

3.2.1 Natural microbiological background of the receiving waters (headwater quality)

While the health-related microbiological quality of the water at R1 represents the quality of unpolluted stream water in the Re[illegible] levels were also considered to be typical of the unpolluted quality status of the Bloem and Fontein Spruits, since these waters are from the same geographical and topographical surroundings. The water quality at R1 was, therefore, used as reference for the natural microbiological background quality.

Table 3.2.1 shows the numbers of indicator organisms in the unpolluted Renoster Spruit at R1, as well as those determined at the downstream sampling points R2 and R3. These measurements were done during the first 16 months of the study period (August 1997 until December 1998) and represented the indicator organism numbers in Sector 1 "before" the start-up of the Sterkwater treatment works.

Table 3.2.1: Microbiological indicator organism numbers in Sector 1 before the start of Sterkwater.

	R1 (Headwater quality)	**R2 (1997/8)**	**R3 (1997/8)**	**Statistical comparison**
E. coli	n = 34 Geometric Mean = 85 Min = 5 Max = 2 750 95 % CI = 197	n = 21 Geometric Mean = 97 Min = 0 Max = 1 919 95 % CI = 228	n = 29 Geometric Mean = 75 Min = 0 Max = 34 176 95 % CI = 2 311	No significant difference (P =0.189) H_0 accepted Kruskal-Wallis ANOVA
C. perfringens	n = 33 Geometric Mean = 10 Min = 0 Max = 624 95 % CI = 57	n = 18 Geometric Mean = 7 Min = 0 Max = 586 95 % CI = 68	n = 24 Geometric Mean = 11 Min = 0 Max = 379 95 % CI = 37	No significant difference (P =0.859) H_0 accepted Kruskal-Wallis ANOVA
Somatic coliphages	n = 31 Geometric Mean = 3 Min = 0 Max = 13 000 95 % CI = 1 290	n = 18 Geometric Mean = 3 Min = 0 Max = 600 95 % CI= 92	n = 23 Geometric Mean = 3 Min = 0 Max = 15 200 95 % CI= 1 353	No significant difference (P =0.989) H_0 accepted Kruskal-Wallis ANOVA

It can be seen from Table 3.2.1 that there were no statistically significant differences in the microbiological quality of water sampled at the various sampling points in this Sector of the spruit before Sterkwater was commissioned. The zero-hypothesis is accepted. This implied that the health-related microbiological water quality for Sector 1 varied little in its natural background state before the commissioning of Sterkwater.

3.2.2 Indicator organisms released from Sterkwater

Table 3.2.2 shows the geometric mean indicator microorganism levels in the final effluent discharged from the Sterkwater wastewater treatment works since commissioning in January 1999 until the experimental period ended in March 2000 (15 months).

Table 3.2.2: Indicator organism numbers in the final effluent from Sterkwater.

	E coli	***C perfringens***	**Somatic coliphages**
Final effluent	n = 24 Geometric Mean = 13 908 Min = 206 Max = 3 593 334 95 % CI = 370 585	n = 22 Geometric Mean = 10 384 Min = 76 Max = 319 200 95 % CI= 30 321	n = 19 Geometric Mean = 9 964 Min = 500 Max = 31 000 95 % CI= 4 607

relation to the No Observed A[logo obscures text] els (NOAEL), the most stringent of the various quality guideline limits described in Table 1 (Chapter 1), were used. Maximum limits for drinking water, full-contact recreation and a water quality limit for the irrigation of salad crops are indicated in red, blue and green lines in Figures 3.2.5 (1, 2 & 3) below.

Results from the tables above are summarised in boxplots (Appendix E) in these figures. The geometric means of the data sets are indicated in yellow lines within the boxes, while the 5th and 95th percentiles are indicated with the black dotted end-points. Data for the final effluent, discharged from Sterkwater, are summarised as separate brown boxes inserted in the graphs, to indicate the geometric mean number of indicator organisms released from the works.

3.2.5.1 *Escherichia coli* (*E. coli*)

R2 showed slightly higher background levels of *E. coli* than R1 and R3. Nevertheless, the similarity (Table 3.2.1 indicated no statistically significant differences) of the *E. coli* numbers, determined at all three the sampling points, before discharge from Sterkwater started, is apparent.

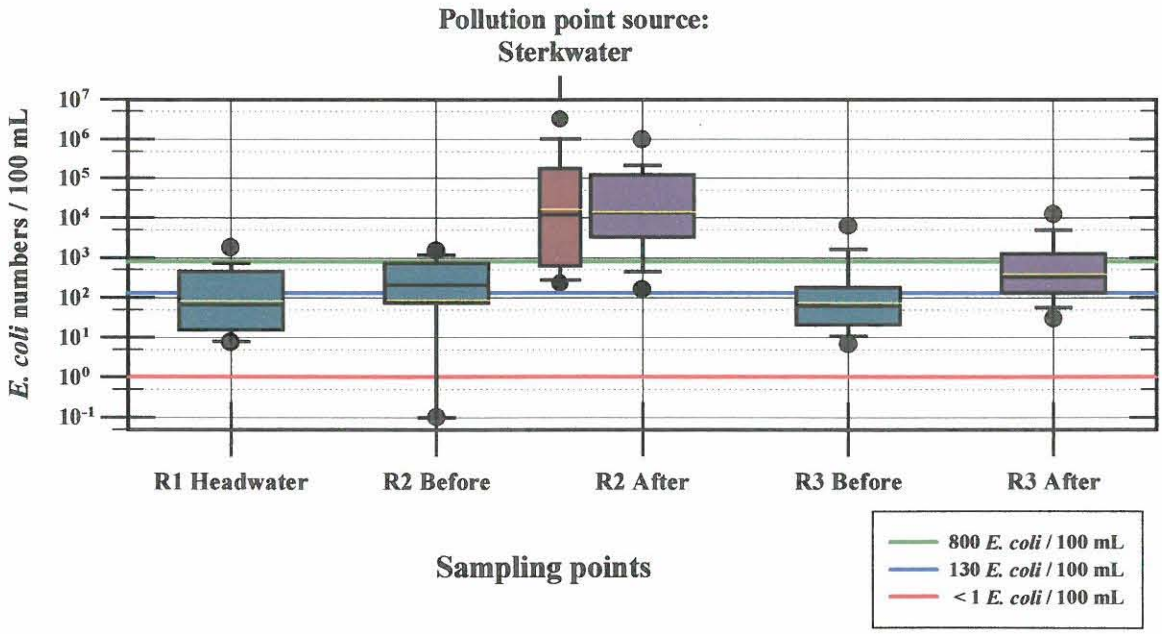

FIGURE 3.2.5.1: *E. coli* numbers detected in the Renoster Spruit: Sector 1.

Faecal coliform counts of 1-10 (1-8 *E. coli*) per 100 mL are reported to be common in pristine waters (WRC, 1998). This implies that the counts at R1 might be slightly higher than what could be expected of unpolluted water, although not excessive. Before discharge from the Sterkwater treatment works commenced, the geometric mean levels of *E. coli* at R1, R2 and R3 were similar to the 96 *E. coli* per 100 mL geometric mean density, determined at an unpolluted sampling site in the St Clair River, Ontario (Marsalek et al., 1994). The Ontario site was selected in an agriculturom major urban effluent discharges such as stormwater and sewage effluents.

Jagals (2000) reported a geometric mean level of 150 faecal coliforms per 100 mL in unimpacted river water in another subcatchment of the Modder River in the area. According to Venter et al., (1996), water suitable for extraction to treat for domestic use, should not have more than 200 faecal coliforms per 100 mL. This implies that the waters of R1, R2 as well as R3 were suitable for raw water extraction before the discharge from Sterkwater commenced.

Although the natural background levels of *E. coli* (at R1) exceeded the NOAEL for drinking purposes (Table 1; Chapter 1), indicated by the red line at <1 *E. coli* / 100 mL, the water at this point appeared, on average, not to exceed recommended limits when used for full or intermediate body contact recreation (the blue line at 130 organisms / 100 mL). A number of data points at the 75^{th} and 95^{th} percentile levels, exceeded the recreational safe limit, which implies that full contact recreational use of this water should generally be discouraged since some occasional risk of infection could be expected.

Before Sterkwater was commissioned, the geometric mean level of *E. coli* at R2 was slightly below the recreational quality limit (the blue line), but the water was generally not suitable for full contact recreation if judged by the numbers of data points exceeding this level. Jagals (1997) had found similar trends in water from other sub-catchments of the Modder River in the area.

Prüss (1998) cautioned against the use of water with high levels of microbiological indicator organisms for recreational purposes. This author concluded that a dose-related increase of health risk in swimmers could be expected with an increase from only a few faecal coliform indicators per 100 mL to about 30 faecal coliform indicators counts per 100 mL. It can, therefore, be expected that only a modest increase in the *E. coli* numbers would impact on the microbiological water quality and would introduce a health risk for recreational users.

At R3, most of the data up to 75^{th} percentile were below the recreational quality limit for full-contact recreation, which implies that 25 percent of the data still exceeded this safe limit.

Before discharge commenced, *E. coli* numbers in the water at R1, R2 and R3 did not exceed the safe limit for agriculture (the green line at 800 *E. coli* organisms / 100 mL). The water was therefore not expected to pose a bacterial risk of infection when used for irrigating vegetable and salad crops eaten uncooked, sports fields or public parks (WHO, 1989; Table 1, Chapter 1).

After discharge commenced, the water at R2, based on *E. coli* numbers as indicators, became totally unsuitable for domestic, r[...] as agricultural use. Although the water quality improved somewhat downstream from R2 towards R3, it was only to the extent that the water became safe to use for crop irrigation with occasional exceedence of the safe limit. The water at R3 remained unsuitable for recreational and domestic use.

3.2.5.2 *Clostridium perfringens* (*C. perfringens*)

Table 3.2.1 showed no statistically significant differences in the data for R1, R2 and R3 before discharge commenced. However, the data for R1 and R2 in Figure 3.2.5.2 showed substantial variance while R3 showed less variance. Jagals (2000) reported similar variability in the occurrence of *C. perfringens* in natural waters. This was probably due to variable numbers of spores in the sample and implied that the numbers of low data points might not be as numerous as reported in this study.

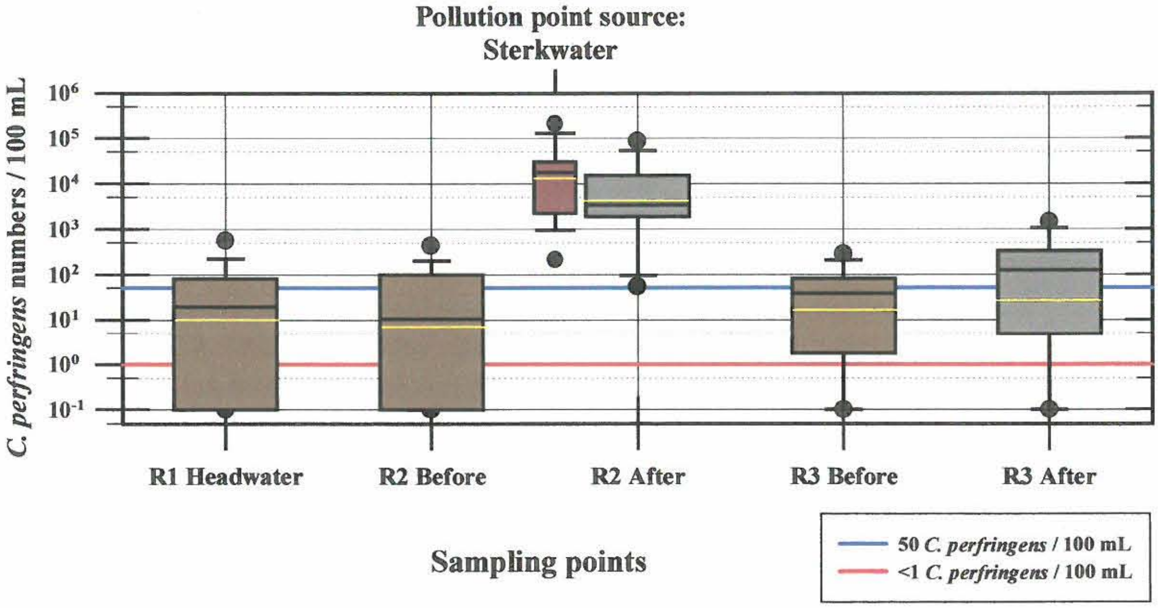

FIGURE 3.2.5.2: *C. perfringens* numbers detected in the Renoster Spruit: Sector 1.

The geometric mean *C. perfringens* levels at R1, R2 and R3, during the first phase of the study, exceeded the NOAEL for domestic purposes (the red line). A risk of infection for full contact recreation was not indicated (Mahin and Pancorbo, 1999 in Table 1, Chapter 1). No agricultural irrigation guideline was included in the site-specific guide proposed in Table 1 (Chapter 1).

The impact of the final effluent discharged from the Sterkwater treatment works, on the numbers of *C. perfringens,* after the discharge commenced, is evident. The water at R2 was rendered unfit for domestic and recreational use. Although the water quality improved towards R3, it would generally still not be suitable for recreational or domestic use.

Results in this sector of the Renoster Spruit were generally similar to those at other sampling points in the area reported by Jag ver than those reported at an "unpolluted" sampling point, as well as a sampling point downstream from treated wastewater discharges, in an Australian river (Ashbolt, et al., 1993; Ferguson et al., 1996). The numbers of *C. perfringens* spores in these studies ranged from 10^1 cfu per 100 mL to as many as 10^4cfu per 100 mL. These numbers were reported to correlate significantly with numbers of *Giardia* spp. detected in the same river. This implied that the presence of *C. perfringens* determined at R2 and even R3 during the second phase of the study, indicated the possible presence of *Giardia* and *Cryptosporidium* spp.

3.2.5.3 *Somatic coliphages*

Before discharge commenced, a considerable number of zero data points at all three sampling points indicated that there were often no culturable coliphages in the water samples. The medians for the three "before" data sets showed a presence of ≤1 in Figure 3.2.5.3.

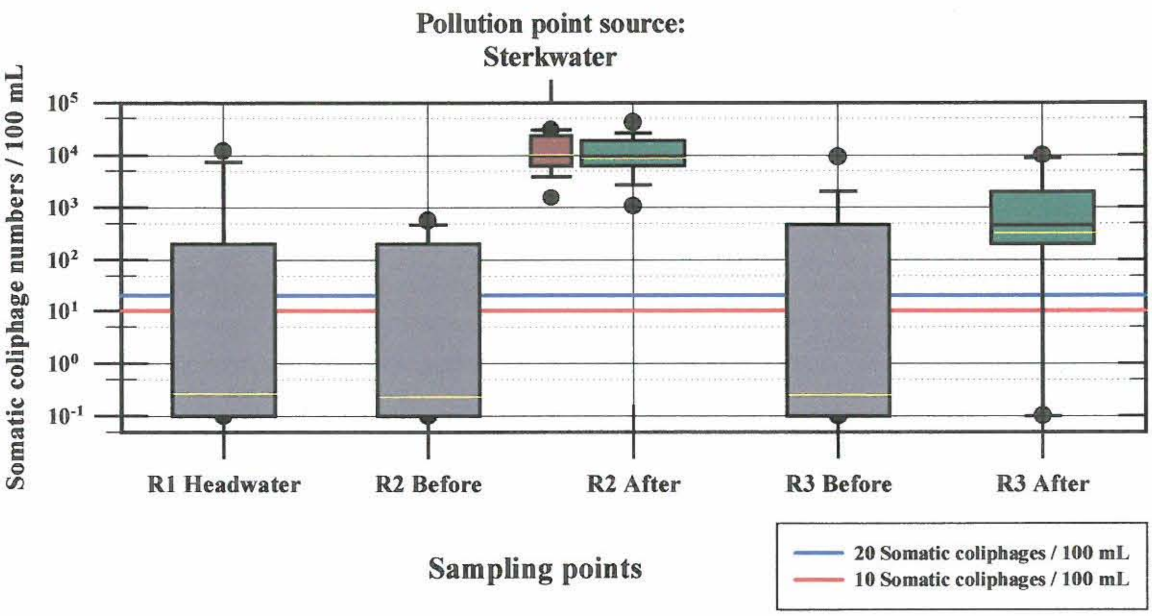

FIGURE 3.2.5.3: Somatic coliphage numbers detected in the Renoster Spruit: Sector 1.

A considerable number of data points at the 75th and 95th percentiles imply that the geometric means could be higher if the ≤1-results were fewer. The geometric mean levels for somatic coliphages at R1, R2 and R3, however, indicated that the water could be expected to be generally safe for domestic as well as recreational use, since negligible virus infection risks were indicated. Occasional increases of the risk of infection can be expected in consumers and bathers since a substantial number of the data points exceeded the red and blue lines

The numbers of somatic coliphages increased dramatically in Sector 1 after discharge from the wastewater treatment works commenced. At R2, geometric mean somatic coliphage level, as well as the 5th percentile, rose to, and remained well above the guideline limits for safe domestic and recreational water u[...] anism numbers decreased towards R3, but the water was still unfit for use.

It is believed that wastewater treatment processes inactivate, and therefore reduce the thousands to hundreds of enteric viruses present in untreated wastewater (Mara et al, 1992; Mahin and Pancorbo, 1999). Grabow et al. (1993) found geometric mean densities of 53 somatic coliphages per 100 mL downstream from wastewater discharges in rivers near Pretoria, South Africa. Geometric mean densities of 46 somatic coliphages per 100 mL upstream and 83 somatic coliphages per 100 mL downstream from wastewater discharges have been reported in the St Clair River, Ontario (Marsalek et al., 1994).

These results are clearly lower than the numbers of somatic coliphages in Sector 1. The high indictor numbers, found in the waters of the study area after the discharge commenced, indicate inefficient treatment at the wastewater treatment works. While, for human use, the water could previously be judged safe from a viral infection risk perspective, the commissioning of Sterkwater rendered the water in this sector of the Renoster Spruit completely unfit for domestic and recreational use. Furthermore, based on the work by Shuval et al. (1997), the levels of *E. coli,* reported in Tables 3.3.1 – 4, also implied that the water contained excessive human viruses, which indicated that health effects could be expected when the water is used for irrigation of crops.

3.2.6 The impact on the Renoster Spruit in Sector 1

The use of the expression “impact” was explained in Chapter 1. In this study, “impact” refers to the deterioration of the microbiological water quality as a result of the release of excessive numbers of health-related indicator organisms from point and non-point faecal pollution sources, into the Renoster Spruit and ultimately into the Modder River.

The original explanation in Chapter 1 also included a statement that read “to such an extent that the receiving water becomes unfit for consumption or use for any of the other potable and non-potable purposes such as recreation and agriculture”. This statement was included to provide clear parameters to identify an impact of a faecal pollution source on the health-related microbiological quality of the receiving waters.

It was expected that the microbiological quality of the unimpacted headwater (the R1 reference point) as well as the quality of water at R2 and R3 (before discharge commenced) would be close to suitable for general use. However, from the discussions in the previous sections, it can be concluded that the natural water quality would generally not be suitable for use in some of the defined uses such as drinking and full contact recreation. The waters of

Sector 1 could generally, however e health-sensitive crops. It can therefore be reasoned that water, not suitable for a certain use in the first place, could not be seen as impacted upon if the quality deteriorated further.

It is a known fact that the more polluted water might be, the more difficult it becomes to treat it effectively for drinking purposes (AWWA, 1990; Venter et al., 1996; Chapra, 1997), be it in a full engineering treatment facility or partial home treatment in areas where people extract the water directly from a open water source (WRC, 1998; Jagals, 2000).

Jagals (2000) stated that international guidelines for acceptable microbiological quality for raw water for drinking water supply differ in their definitions of what constitutes "acceptable". Such guidelines generally use total and faecal coliforms, as well as faecal streptococci, as indicators. Venter et al. (1996) suggested 200 organisms per 100 mL as a maximum faecal coliform limit for raw water prior to treatment. This implies that water sampled at R1, R2 and R3 would be suitable, even for partial treatment such as home treatment. These waters would also, with some marginal exception for R2, be suitable for full-contact recreation.

All this was rendered null and void when the discharge from Sterkwater commenced, which implies a definite impact on the health related microbiological quality of water in Sector 1 of the study section of the Renoster Spruit.

What remains now is to quantify the extent of the impact, in order to report on the severity of the impact. Table 3.2.6.1 shows the log-phase increases in the geometric mean numbers of indicator organisms since the discharges commenced.

Table 3.2.6.1: Increases in mean levels of indicator organisms since discharges commenced.

Geometeric mean numbers in 100 mL	Headwater quality (R1)	R2	R3
E. coli	85	Before = 97 After = 13 686 Log-phase increase = 3	Before = 75 After = 352 Log-phase increase = 2
C. perfringens	10	Before = 7 After = 4 003 Log-phase increase = 3	Before = 11 After = 35 Log-phase increase = 0
Somatic coliphages	3	Before = 3 After = 8 923 Log-phase increase = 3	Before = 3 After = 266 Log-phase increase = 3

The substantial increases were quite evident. The biggest impact can be noted at R2, which is just downstream from the discharge point. Although the level of faecal pollution differed from those reported in the St Clair River, Ontario by Marsalek et al. (1994), the geometric mean log-phase increases in the indicator organism numbers are almost similar. Increase in organism numbers, assessed by A[...]) and Ferguson et al. (1996) in Australian waters, did not compare with the abovementioned studies. This indicated the difference in effectiveness of treatment works to reduce indicator organism numbers in raw sewage.

The stretch of stream between R2 and R3 had shown a remarkable ability to assimilate the release indicator organisms and largely reduced the impact. Table 3.2.6.2 shows that the water quality differed significantly from R2 (higher values) to R3 (lower values) since commencement of discharge.

Table 3.2.6.2: Comparison of indicator organism numbers detected at R2 and R3 during 1999/2000.

	R2	**R3**	**Statistical comparison**
E. coli	n = 35 Geometric Mean = 13 686 Min = 80 Max = 2 208 000 95 % CI = 137 168	N = 40 Geometric Mean = 352 Min = 0 Max = 62 400 95 % CI = 3 128	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
C. perfringens	n = 35 Geometric Mean = 4 003 Min = 46 Max = 115 000 95 % CI = 9 137	N = 40 Geometric Mean = 35 Min = 0 Max = 5 290 95 % CI = 274	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
Somatic coliphages	n = 34 Geometric Mean = 8 923 Min = 700 Max = 45 000 95 % CI =3 861	N = 38 Geometric Mean = 266 Min = 0 Max = 11 800 95 % CI = 1 067	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum

The differences in the water quality means that the water of the Renoster Spruit in Sector 1 could significantly reduce the numbers of indicator organisms released into it within a relatively short distance. The period of time the water flowed from R2 to R3, however, was not investigated during this study. This work was aimed at only broadly investigating the tendencies showed by the impacts. To model the ability of the assimilation capacity of Renoster Spruit more precisely, further investigation is needed and should include flow-rate measurements within the hydraulic regime of the spruit.

The impact could not be fully negated for all the organism groups before the water from the Bloem Spruit, which contained urban run-off as well as treated effluent, discharged into Sector 2 of the Renoster Spruit. This implied that whatever dilution potential the water from Sector 1 might have had, was lost since the discharge from Sterkwater commenced.

SECTION 3.3: MICROBIOLO TY OF WATER IN THE BLOEM SPRUIT

The Bloem Spruit and it's tributary, the Fontein Spruit, drain storm and other run-off from the residential, business and industrial areas of Bloemfontein, as well as final effluent from the Bloem Spruit wastewater treatment works into the Renoster Spruit. Although storm-induced run-off from these types of developments often results in higher faecal pollution of the receiving waters (Grabow et al., 1996; Jagals, 1997; Pretorius, 1996), differences caused by meteorological events were not tested for. Results from a case study of storm water entering Port Phillip Bay, Australia, indicated that during and after wet weather events, all sites sampled were affected by significant human faecal contamination (Leeming et al., 1998). The research group, furthermore, reported elevated levels of faecal contamination during dry weather events (faecal coliforms concentrations ranged from log 3 to log 6, with *C. perfringens* numbers in the range of log 1 and log 4 after rain) (Leeming et al., 1998). Wright et al. (1993) also reported that the microbiological quality of urban storm run-off in the Western Cape region of South Africa was similar throughout the year. In this study, emphasis was, therefore, rather placed on the total picture of point and diffuse sources of faecal pollution that influenced the health-related microbiological quality of water in the Bloem and Fontein Spruits. Results from dry and wet weather periods were collated in one data set to provide an overall view of the water quality. The immediate impact of urban run-off, as well as final effluent from the wastewater treatment works, on the geometric mean *E. coli* levels at the various sample points in the Bloem Spruit, are illustrated in Figure 3.3(a).

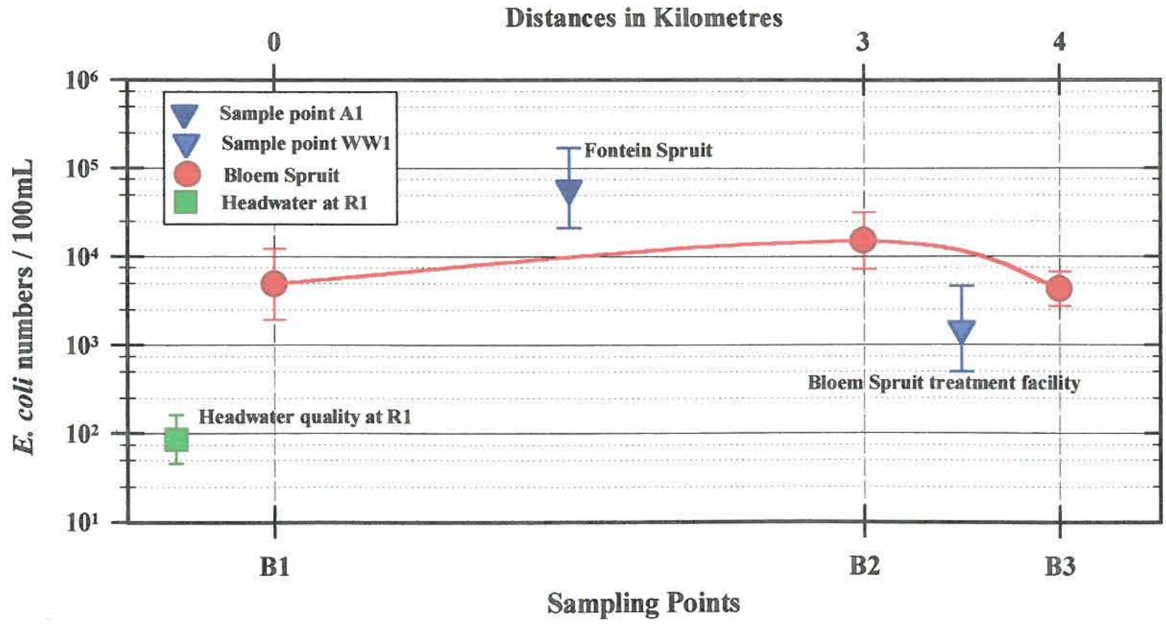

FIGURE 3.3(a): Downstream changes in *E. coli* numbers to illustrate the water quality of the Bloem Spruit.

The numbers of indicator organis[illegible] and A1 were more than one log-phase higher than those reported at the headwater quality in the Renoster Spruit. This indicates the immediate negative impact that run-off from the various residential areas of Bloemfontein had on the health-related microbiological quality of water in the Bloem and the Fontein Spruits.

Sampling point B2 represents the impact from the urban areas. Although the geometric mean *E. coli* level, at this point, was lower than the level received from A1, it was substantially higher than the level at B1, indicating the impact by water quality at A1. Downstream from B2, indicator organism numbers in the Bloem Spruit, decreased as a result of the dilution effect of the final effluent discharged from the Bloem Spruit wastewater treatment works.

The geometric mean *C. perfringens* and somatic coliphage levels showed a similar pattern. The geometric mean indicator organism levels are illustrated in Figures 3.3(b and c).

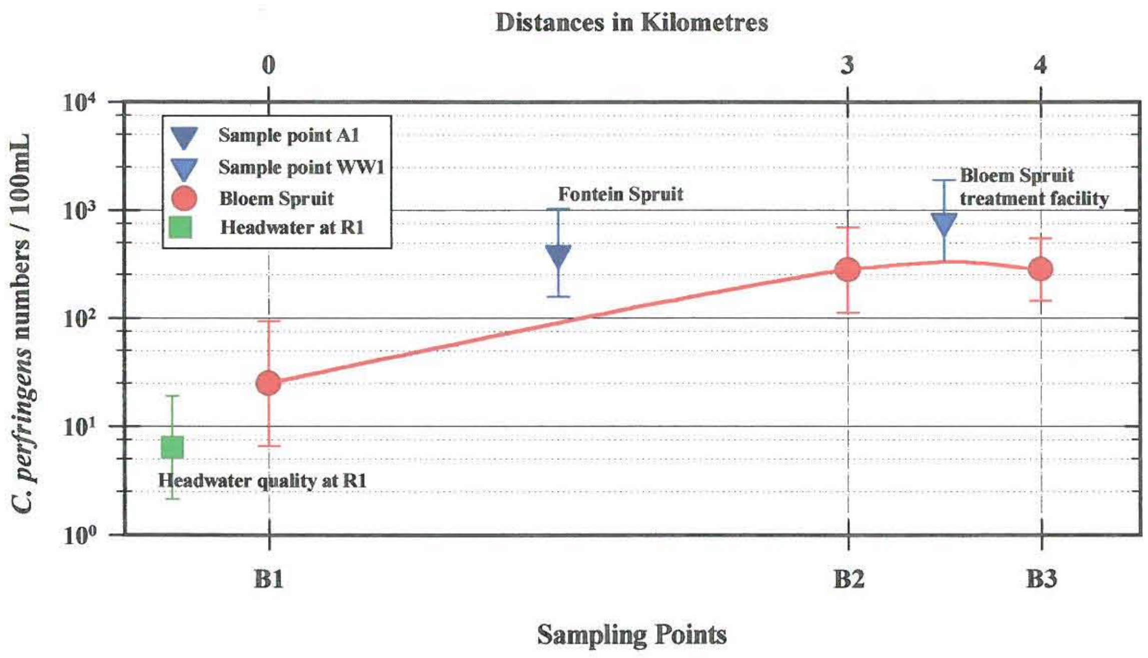

FIGURE 3.3(b): Downstream changes in *C. perfringens* numbers to illustrate the water quality of the Bloem Spruit.

The geometric mean *C. perfringens* density, in the final effluent from the Bloem Spruit wastewater treatment works, was higher than the geometric mean level determined in the Bloem Spruit. It could therefore be expected that the geometric mean *C. perfringens* level at B3 should increase, but this did not happen. The level remained rather constant at this point in the Bloem Spruit, with less variance at B3. This indicated the constant addition of higher levels of *C. perfringens* from the Fontein Spruit and the final effluent.

The same applies for the somatic coliphage densities (Figure 3.3c). Although their numbers in the treated effluent were sligh se in the Bloem Spruit, the numbers of somatic coliphages received from A1 had an impact on the microbiological quality of water in the Bloem Spruit. The data at B2 and B3 varied very little, indicating the constant discharge of similar levels of phages from both the point and non-point pollution sources, as opposed to the higher variances in data recorded at the lesser-polluted headwater and at sampling point B1.

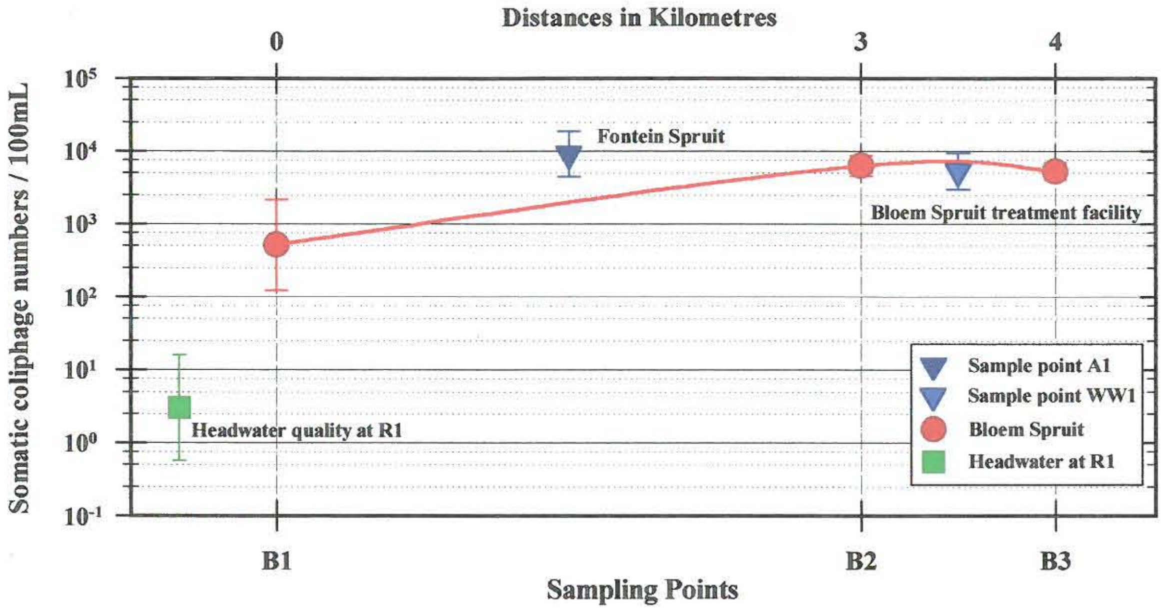

FIGURE 3.3(c): Downstream changes in the somatic coliphage numbers to illsustrate the water quality of the Bloem Spruit.

3.3.1 Microbiological quality of urban surface run-off at B1 and A1

Table 3.3.1 shows the microbiological indicator organisms recorded in the Bloem and Fontein Spruits over the entire study period from August 1997 to March 2000.

Table 3.3.1: Indicator microorganism numbers at B1 in the Bloem Spruit and A1 in the Fontein Spruit.

	B1	A1	Statistical comparison
E. coli	n = 45 Geometric Mean = 4 873 Min = 12 Max = 859 895 95 % CI = 47 600	n = 45 Geometric Mean = 59 027 Min = 0 Max = 11 340 973 95 % CI = 668 414	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
C. perfringens	n = 31 Geometric Mean = 25 Min = 0 Max = 6 900 95 % CI = 516	n = 32 Geometric Mean = 402 Min = 0 Max = 11 730 95 % CI = 835	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
Somatic coliphages	n = 26 Geometric Mean = 516 Min = 0 Max = 41 000 95 % CI = 3 581	n = 27 Geometric Mean = 9 098 Min = 200 Max = 280 000 95 % CI = 20 486	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum

The upper sub-catchment of the Bloem Spruit receives surface run-off from a large part of the city's modern western-style resid major faecal pollution problem in these areas appears to be homeless (street) children living under the enclosed sections of the canals using the stream for toilet practices. The water quality is assessed at B1. Further downstream from B1 (before reaching B2), the flow is supplemented by run-off from business sectors and industrial zones. All these areas are serviced by waterborne sewage systems. The geometric mean indicator levels in the water of this Section of the Bloem Spruit exceeded all the upper safety limits proposed in the on-site guide (Table 1; Chapter 1).

The Fontein Spruit, upstream from A1, drains surface run-off from the surrounding low-cost, high-density residential and informal areas of Bloemfontein. Sanitation in these areas is limited with some areas serviced by waterborne systems, but predominantly, sanitation infrastructure comprises mostly of pit and bucket latrines. The geometric mean indicator levels in this Spruit were substantially higher than those at B1.

The differences between the two sets of data were statistically significant as can be seen in Table 3.3.1. The zero-hypothesis is rejected. This implied that, although diffuse run-offs from the modern urban areas contain faecal indicator organisms in excess of the limits proposed in the on-site guide (Table 1; Chapter 1), run-off from under-developed and informal settlements are of a much poorer microbiological quality.

Results from this study are supported by studies done in the area by Jagals (1997), Koning (1999), in the Western Cape by Wright et al. (1993), and Kienzie et al. (1997) in the Kwa-Zulu-Natal area. All the results showed that run-off from both developed and developing urban settlements constituted a major source of pollution, with the higher level of pollution originating in the lesser-developed areas. The numbers of indicator microorganisms could even be compared to the numbers of indicator organisms in untreated sewage and poorly treated secondary effluent. Jagals (2000) reported that *E. coli* could be found in numbers up to log 7, *C. perfringens* up to log 3, and somatic coliphages up to log 4 in these types of water.

3.3.2 Water quality at B2

To quantify the impact that the various types of urban developments had on the downstream microbiological quality of water in the Bloem Spruit, water quality at B2 was compared to the unpolluted headwater microbiological quality of R1. Results in Table 3.3.2 are for the entire study period.

The numbers differed significantly because of the substantially higher numbers of indicator organisms released with the urban surface run-off. The numbers of indicator organisms in the

Bloem Spruit, downstream from t nents, were similar to those at R2 in the Renoster Spruit, downstream from the Sterkwater effluent discharge point. These results were similar to those reported for raw sewage flushed from developing areas as well as poorly treated secondary effluents (Mara and Oragui, 1985; Jagals, 1994, 1997 & 2000; Grabow, 1996; Koning, 1999).

Table 3.3.2: Comparison of indicator microorganism numbers in unpolluted and polluted receiving waters.

	R1 (Headwater quality)	B2	Statistical comparison
E. coli	n = 34 Geometric Mean = 85 Min = 5 Max = 2 750 95 % CI = 197	n = 61 Geometric Mean = 15 101 Min = 48 Max = 41 600 000 95 % CI = 1 336 305	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
C. perfringens	n = 33 Geometric Mean = 10 Min = 0 Max = 624 95 % CI = 57	n = 58 Geometric Mean = 278 Min = 0 Max = 483 000 95 % CI = 16 523	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum
Somatic coliphages	n = 31 Geometric Mean = 3 Min = 0 Max = 13 000 95 % CI = 1 290	n = 53 Geometric Mean = 6 344 Min = 800 Max = 63 000 95 % CI = 4 501	Significant difference (P = <0.001) H_0 rejected Mann-Whitney Rank Sum

3.3.3 Indicator organism released from Bloem Spruit treatment works

Table 3.3.3 shows the geometric mean indicator organism levels recorded in the final effluent discharged from the Bloem Spruit wastewater treatment works from August 1997 to March 2000.

Table 3.3.3: Indicator microorganism numbers in the final effluent from the Bloem Spruit treatment works.

	E coli	*C perfringens*	Somatic coliphages
Final Effluent	n = 33 Geometric Mean = 1 499 Min = 0 Max = 7 969 500 95 % CI = 473 020	n = 29 Geometric Mean = 792 Min = 0 Max = 38 000 95 % CI = 2 871	n = 22 Geometric Mean = 5 296 Min = 200 Max = 32 800 95 % CI = 3 254

The numbers were much lower than those detected in the water of the Fontein Spruit, which represented run-off from the underdeveloped residential areas of Bloemfontein. When compared to their potential numbers in raw sewage, the relatively higher numbers of *C. perfringens* and somatic coliphages released in the final effluent are probably due to the persistence of these organisms in the water environment (Jagals, 2000). This was especially during periods when the treatment works appeared to have had problems with effective treatment (concluded from the large ranges in the numbers determined). *E coli* bacteria were reported to die off rapidly in the water environment (Venter et al., 1996), which explains their lower numbers.

Numbers of indicator organisms (l effluent implies that this works has the potential to release excessive numbers of *C. perfringens* and somatic coliphages more constantly than it would release *E. coli* at these levels. The Bloem Spruit wastewater treatment works appeared to be a point source of intermediate large release potential at the time of the study, although not of the magnitude of Sterkwater.

3.3.4 Water quality at B3

Table 3.3.4 compares the upstream water quality to the water quality downstream from the effluent discharge point in the Bloem Spruit. The *E. coli* numbers differed statistically while the other indicator microorganism numbers did not.

Table 3.3.4: Indicator microorganism numbers up- and downstream from the final effluent discharge point.

	B2	B3	Statistical comparison
E. coli	n = 61 Geometric Mean = 15 101 Min = 48 Max = 41 600 000 95 % CI = 1 336 305	n = 79 Geometric Mean = 4 277 Min = 50 Max = 1 200 000 95 % CI = 33 099	Significant difference ($P = 0.009$) H_0 rejected Mann-Whitney Rank Sum
C. perfringens	n = 58 Geometric Mean = 278 Min = 0 Max = 483 000 95 % CI = 16 523	n = 65 Geometric Mean = 282 Min = 0 Max = 22 195 95 % CI = 818	No significant difference ($P = 0.734$) H_0 accepted Mann-Whitney Rank Sum
Somatic coliphages	n = 53 Geometric Mean = 6 344 Min = 800 Max = 63 000 95 % CI= 4 501	n = 61 Geometric Mean = 5 272 Min = 400 Max = 62 000 95 % CI= 2 892	No significant difference ($P = 0.621$) H_0 accepted Mann-Whitney Rank Sum

E. coli bacteria have been found to survive for shorter times in the environment than *C. perfringens* and somatic coliphages (Jagals, 2000). This characteristic could explain the marked decrease in the *E. coli* numbers from B2 to B3. The numbers of *C. perfringens* and somatic coliphages remained within the same log-phase, which is indicative of their persistence in the water environment.

Indicator organisms in the water from the Fontein Spruit caused the increases in the Bloem Spruit from B1 down to B2 (Figures 3.3 a, b & c). *E. coli* die off quicker and also received lower numbers contributed from the effluent discharge. Therefore, the *E. coli* decrease from B2 to B3 was statistically confirmed to be more rapid. The *C. perfringens* and somatic coliphage numbers in the Bloem Spruit were substantially inflated by the indicator organism levels in the Fontein Spruit, whereafter these levels were maintained by similar levels of organisms in the effluent. This explains why there were no statistically significant differences in these numbers from B2 down to B3.

3.3.5 Discussion

The levels of indicator organisms in the Bloem and Fontein Spruits were similar to the levels of microbiological indicator organisms detected in raw sewage (Mara and Oragui, 1985; Grabow, 1996; Koning, 1999; Jagals, 2000). Similar counts of up to 10^8 faecal coliforms per 100 mL have also been reported in other faecally polluted urban steams (Jacobs and Ellis, 1991; Venter et al., 1996). This confirms that the Bloem and Fontein Spruits were faecally polluted, and could be described as being impacted upon. Furthermore, indicator organism numbers, reported in studies in the Gauteng province (Freeman et al., 1996) as well as in the Western Cape province, in South Africa (Wright et al., 1993), further supported the approach followed in this study that the Bloem and the Fontein Spruits were faecally polluted throughout the year regardless of rainfall events.

The indicator organism numbers determined in the Bloem and the Fontein Spruits are summarised in Figures 3.3.5.1; .2; .3 below. The indicator organism densities exceeded the numbers recommended in Table 1: Chapter 1, which meant that the water was unfit for domestic, recreational as well as agricultural purposes throughout the year. Water-related infections associated with the use of these waters include infections, ranging from mild eye, skin and respiratory infections to diarrhoea, caused by organisms such as *Shigella* spp., *Salmonella* spp., *Yersinia* spp., enterohaemorrhagic *E. coli*. Protozoan parasites *Giardia* spp., *Cryptosporidium* spp. can cause more severe infections, with Hepatitis A viruses causing Hepatitis (DWAF, 1996b; Sinton et al., 1998).

The data for the final effluent, discharged from the Bloem Spruit wastewater treatment works, were inserted in the graphs as brown boxplots to indicate the level of indicator organisms released from this works. The geometric means of the data sets are indicated with yellow lines within the boxes, while the 5th and 95th percentiles are indicated with the black dotted end-points. The red, blue and green lines indicate the NOAEL for domestic, recreation and agricultural uses respectively.

3.3.5.1 *Escherichia coli* (*E. coli*)

Faecal coliform counts in the range of 10^1 to 10^6 organisms per 100 mL are reported as common in faecally polluted surface waters (WRC, 1998). *E. coli* levels, summarised in Figure 3.3.5.1 show that although the geometric mean *E. coli* level at R1 in the Renoster Spruit were higher than what are expected in unpolluted water, the numbers of indicator organisms counted in the Bloem and Fontein Spruits were clearly higher than the level found at the unimpacted sampling point.

The water at B1, representing r eveloped residential areas, exceeded the NOAEL for drinking (the red line at <1 *E. coli* / 100 mL), recreational (the blue line at 130 *E. coli* / 100 mL) and agricultural (the green line at 800 *E. coli* / 100 mL) purposes. Health effects associated with the use of these waters could therefore be expected.

The numbers of indicator organisms at A1, representing run-off from the underdeveloped residential and informal settlements, were clearly higher than the indicator organism numbers in the run-off from developed areas with sanitary systems. The water at A1 was unfit for domestic, recreational as well as agricultural use because the indicator organism numbers exceeded the levels recommended in Table 1: Chapter 1.

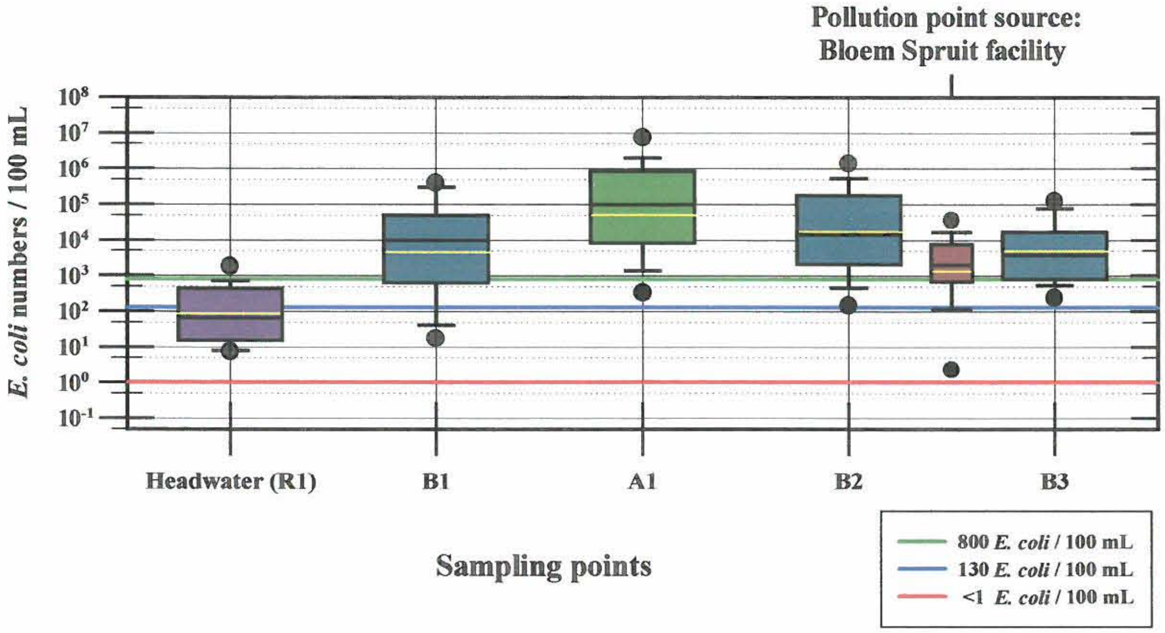

FIGURE 3.3.5.1: *E. coli* numbers detected in the Fontein and Bloem Spruit.

The *E. coli* geometric mean level per 100 mL at A1 was similar to the 10^6 faecal coliforms per 100 mL, reported by Freeman et al. (1996) at a sampling site in the Klipspruit, downstream from one of the largest developing urban areas in South Africa. This research group suggested that a maximum of 10^3 faecal coliforms per 100 mL is acceptable for a river system in a heavily impacted urban catchment. The *E. coli* numbers determined in the Bloem and Fontein Spruits were generally higher than this suggested level throughout the study.

Numbers of *E. coli* counted at A1 were also higher than the reported faecal coliform range between 10^2 to 2.8×10^3 per 100 mL detected downstream from an informal settlement in Gauteng (Grabow et al., 1996), but were similar to the reported *E. coli* numbers of up to 10^5 in urban run-off from Khayelitsha, Cape Town Metropolitan area (Wright et al., 1993).

At B2, the compounded impact of the B1 and A1 discharges, on the health-related microbiological quality of water in the Bloem Spruit, caused most of the data to exceed the

NOAEL for domestic, recreatior [logo obscures text] purposes. The geometric mean level of *E. coli* at this point was similar to the 1.6 x 10^6 faecal coliform per 100 mL geometric mean density reported at sampling sites downstream from the Stanza Bopape village (Pretoria, Gauteng), as well as in the urban Edendale Spruit (Gauteng), South Africa (Grabow et al., 1996). Hsu et al. (1999) reported mean faecal coliform numbers of 3 x 10^5 in rivers in Taiwan.

Although the water quality improved somewhat downstream from B2 towards B3, the levels of indicator organisms still exceeded the NOAEL proposed for all the water use categories in Table 1 (Chapter 1). The microbiological indicator organism numbers at B3, downstream from the point and diffuse sources of faecal pollution, were still higher than the 10^3 faecal coliforms per 100 mL value proposed as an acceptable limit for a system in a heavily impacted urban catchment (Freeman et al., 1996).

3.3.5.2 *Clostridium perfringens* (*C. perfringens*)

Table 3.3.1 showed statistically significant differences in the *C. perfringens* numbers determined in the run-off from the various residential areas of Bloemfontein. These differences in the organism densities at B1 and A1, as well as compared to the headwater quality, at R1 are shown in Figure 3.3.5.2.

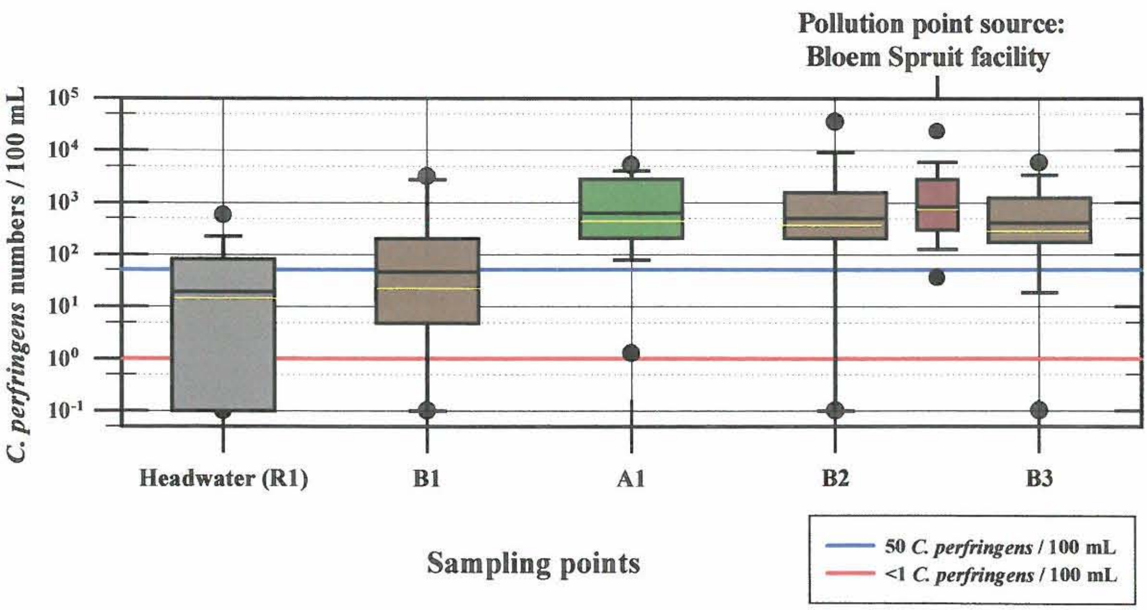

FIGURE 3.3.5.2: *C. perfringens* numbers detected in the Fontein and Bloem Spruit.

The numbers of *C. perfringens* spores counted at B1, A1, B2 and even B3 ranged from 10^1 cfu per 100 mL to as many as 10^3 cfu per 100 mL and were assumed to indicate the possible presence of *Giardia* spp. and *Cryptosporidium* spp (Ferguson et al., 1996). The geometric mean *C. perfringens* levels at all the sampling points in the Bloem and the Fontein

Spruits, exceeded the NOAEL for s (red line at <1 *C. perfringens* / 100 mL).

Up to the 50th percentile of data for B1 were lower than the recreational quality limit for full-contact recreation. The other 50 percent of the data, however, exceeded this safe limit, which implies that the water at B1, was unfit for full-contact recreation. The water at A1, B2 and even B3 was also unfit for recreational purposes, especially when used for swimming.

No agricultural irrigation guideline for *C. perfringens* was included in the site-specific guide proposed in Table 1: Chapter 1.

3.3.5.3 *Somatic coliphages*

Before receiving run-off from the under-developed and informal settlements of Bloemfontein, the geometric mean somatic coliphage level at B1 in the Bloem Spruit already exceeded the NOAEL for domestic and full-contact recreation proposed in Table 1. Geometric mean levels at A1, B2 and B3 further indicated that the water was unfit for domestic and full-contact recreational uses.

The microbiological quality of water sampled before and after receiving final effluent from the Bloem Spruit treatment works, did not differ significantly, which implied that the effluent sustained the somatic coliphage numbers downstream in the Bloem Spruit.

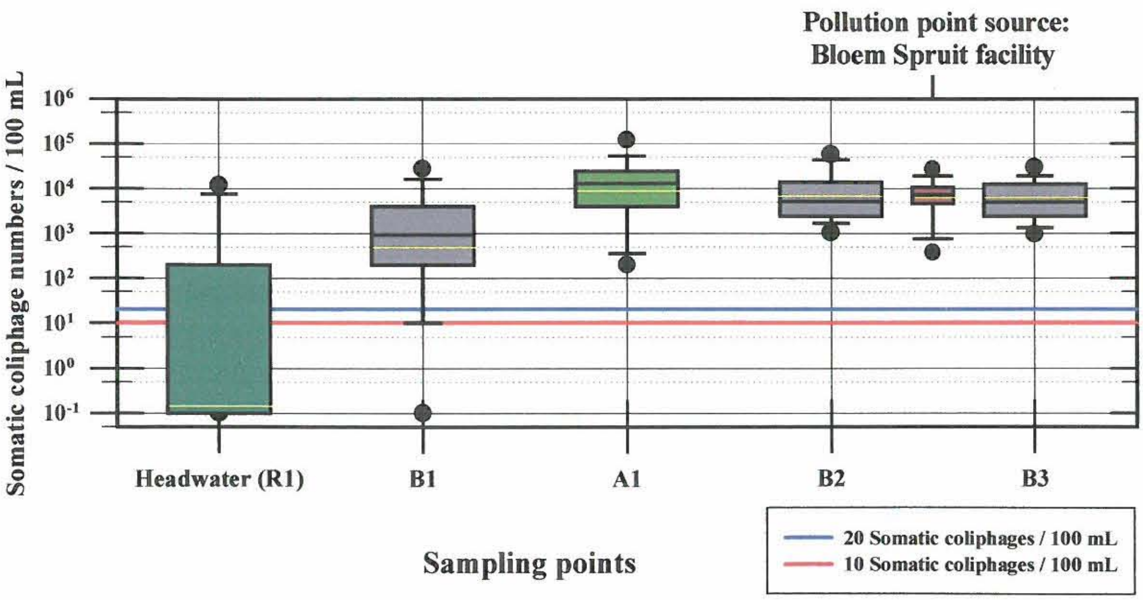

FIGURE 3.3.5.3: Somatic coliphage numbers detected in the Fontein and Bloem Spruit.

As discussed in Section 3.3.5.3 above, the geometric mean level of *E. coli* at B2 (should these be converted to faecal coliform levels) was of similar magnitude when compared to the 1.6 x 10^6 faecal coliforms per 100 mL geometric mean density determined at sampling site downstream the Stanza Bopape village in the Edendale Spruit, South Africa (Grabow et al., 1996). Their results further indic viruses were not isolated in the upstream Edendale Spruit, 70% of samples downstream of the village contained viruses.

The numbers of somatic coliphages at B1, A1, B2 and even B3, during the study period, indicated poorly maintained sanitation systems. It can therefore be concluded that the water of the Bloem and the Fontein Spruits was unfit for domestic and recreational use. Based on the work by Shuval et al. (1997), the levels of *E. coli,* reported in Tables 3.3.1 – 4, also implied that the water contained excessive human viruses, which indicated that health effects could be expected when the water is used for irrigation of crops.

3.3.6 The impact on the Bloem Spruit

It was hypothesised in Chapter 2 that run-off from developed and developing areas would not differ in microbiological quality, nor would run-off from areas serviced by waterborne sewage facilities be faecally polluted. However, from the discussions in this Section, it can be concluded that run-off from urban areas, irrespective of the level of development, are faecally polluted. Sources of indicator organisms in the receiving waters could include land-deposited human faecal material from areas with limited or inadequate sanitary systems, as well as faecal material from warm-blooded domestic animals kept in the developed urban areas (Geldreich, 1996; Leeming et al, 1998; Jagals, 2000). Results from this study are supported by results from other studies that showed that urban settlements, at various levels of development, contribute to diffuse pollution of surface waters (Field and Pitt, 1990; Haiping and Yamada, 1998).

Areas with limited and poorly maintained sewage collection systems were also shown to contain higher indicator organism numbers than run-off from developing areas in the Gauteng area, (Freeman et al., 1996; Lubout et al., 1997) as well as in Kwa-Zulu Natal (Kienzie et al., 1997). In Bloemfontein, as in so many other cities, urbanisation has resulted in substantial numbers of people flocking to the city. People congregate in overcrowded informal housing, often with inadequate water supply, sanitation and other basic necessities. In these circumstances, people often use untreated surface water from environmental sources, directly, or indirectly, for potable and non-potable purposes (Bokako, 2000; Jagals, 2000). Water in the Bloem Spruit as well as in the Fontein Spruit, are rendered unfit for domestic, recreation or irrigation purposes by polluted point and non-point discharges from the city (Jagals, 1997 & 2000; Jagals and Steyn, 1999). These conditions are favourable for enteric infections to be transmitted.

Both these streams could not assimilate the faecally polluted run-offs from the city. It can therefore be concluded that run- ated effluent discharges from the city of Bloemfontein have a significant impact on the health-related microbiological water quality of the Bloem Spruit.

SECTION 3.4: MICROBIOL… TY OF WATER IN SECTOR 2

Land-use within Sector 2 (Figure 2.1, Chapter 2) is similar to the land-use within Sector 1. Differences in the wet and dry weather water quality are also not expected. All the results from both the dry and wet weather periods were collated to provide an overall view of the health-related microbiological quality of water in Sector 2.

In this Section, results from several data points are used to assess the following aspects and their impact on the water of Sector 2:

- The microbiological quality of the water at R3 (discussed in Section 3.2):
  - ≠ Before commissioning of the Sterkwater treatment works to provide, in a sense, a "headwater" quality for Sector 2.
  - ≠ After commissioning of Sterkwater, to provide data of impacted water from Sector 1. This is done to provide baseline data to assess whether Sterkwater contributed significantly to the impact that water from the polluted Bloem Spruit, might have on the water of Sector 2.
- The microbiological quality of the water, at B3, which represents the quality of the urban discharges carried in the Bloem Spruit urban drain (discussed in Section 3.3). These discharges included:
  - ≠ Urban discharges from the informal settlements, developed and developing urban areas of Bloemfontein.
  - ≠ Treated wastewater effluent from the Bloem Spruit wastewater treatment works.
- The microbiological quality of the water at R4, which reflects:
  - ≠ The impact of the water from the Bloem Spruit throughout the period of the study.
  - ≠ Any added impact of water from Sector 1, after Sterkwater was commissioned.
- The microbiological quality of the water at R5, which reflects:
  - ≠ The impact of Bloemfontein city – especially when compared to the microbiological quality of the headwaters of the study environment, as referenced by R1.

3.4.1 Impact of the Bloem Spruit on the Renoster Spruit before Sterkwater started

To quantify the impact of the urban discharges from Bloemfontein (B3), before Sterkwater was commissioned, the water quality at R4 was compared to the water quality at R3 as well as B3. Results, tabled in Table 3.4.1, indicate that the data differed significantly. The zero-hypothesis is rejected.

Table 3.4.1: Mean indicator organism [illegible] 3, B3 and R4 before Sterkwater started.

	R3 (1997/8)	B3	R4 (1997/8)	Statistical comparison
E. coli	n = 29 Geometric Mean = 75 Min = 0 Max = 34 176 95 % CI = 2 311	n = 79 Geometric Mean = 4 277 Min = 50 Max = 1 200 000 95 % CI = 33 099	n = 29 Geometric Mean = 1 797 Min = 12 Max = 98 256 95 % CI = 8 467	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
C. perfringens	n = 24 Geometric Mean = 11 Min = 0 Max = 379 95 % CI = 37	n = 65 Geometric Mean = 282 Min = 0 Max = 22 195 95 % CI = 818	n = 25 Geometric Mean = 99 Min = 0 Max = 3 277 95 % CI = 318	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
Somatic coliphages	n = 23 Geometric Mean = 3 Min = 0 Max = 15 200 95 % CI = 1 353	n = 61 Geometric Mean = 5 272 Min = 400 Max = 62 000 95 % CI= 2 892	n = 22 Geometric Mean = 494 Min = 0 Max = 28 000 95 % CI = 2 569	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA

While Table 3.4.1 shows statistically significant differences between the data sets, it is not clear which data set(s) differed from the other(s). To identify the set(s), all three data sets were compared with the Dunn MCT (multiple-comparison test) procedure described in Appendix E. The results are shown in Table 3.4.1.1.

Table 3.4.1.1: Comparing the R3, B3 and R4 data sets to identify the set(s) that differed significantly.

Comparison	***E. coli***			*C. perfringens*			Somatic coliphages		
	Diff of Ranks	**Q**	**P<0.05**	**Diff of Ranks**	**Q**	**P<0.05**	**Diff of Ranks**	**Q**	**P<0.05**
B3 vs R3	57.801	6.707	Yes	41.912	5.309	Yes	48.274	6.417	Yes
B3 vs R4	12.852	1.491	No	12.660	1.628	No	24.174	3.162	Yes
R4 vs R3	44.948	4.312	Yes	29.252	3.097	Yes	24.101	2.629	Yes

The *E. coli* and *C. perfringens* numbers at R3 differed significantly from those from B3 and R4, the latter two data sets showing no significant differences. Somatic coliphage numbers differed significantly at all three sampling points.

From Table 3.4.1, it can be seen that the values for all three the indicator groups, determined at R3 were considerably lower than those at B3 and R4. This means that the high indicator organism values of B3 (shown in Table 3.4.1) are reflected at R4. Although the values at R4 were lower than those of B3, these differences were not statistically significant. This implies that the lower indicator organism numbers in the water, received from Sector 1, had no significant mitigating influence on the numbers of indicator organisms that discharged from the Bloem Spruit into Sector 2 of the Renoster Spruit.

The exception here was the statistical difference in the numbers of somatic coliphages between B3 and R4. Somatic coliphage numbers were significantly lower at R4 than at B3, which was not the case with the *E. coli* and *C. perfringens* numbers. The phenomenon was probably not due to a dilution effect by water from R3, since this would also have reflected in lower numbers for the other two n groups. The lower values at R4 were probably due to the fact that viruses readily adsorb to solid particles present in the water environment and would, then, tend to settle with the particles (Botero et al., 1992; Grabow, 2000). The water at R3 and R4 flowed far quieter than the waters at B3, where suspended matter, carried in the Bloem Spruit, was still largely in suspension. Samples taken at B3 had more suspended matter in the samples than those of the other two sampling points.

3.4.2 Water quality at R4 since final effluent discharge from Sterkwater commenced

Table 3.4.1 shows the indicator organism numbers at R3, B3 and R4 before Sterkwater started. The water quality data in Table 3.4.2 shows the indicator organism numbers at R3, B3 and R4 Sterkwater since Sterkwater was commissioned. This is presented in this manner to show the additional impact that the discharge from the Sterkwater wastewater treatment works had on the health-related microbiological quality of water in the Renoster Spruit.

Table 3.4.2: Mean indicator organism levels determined at R3, B3 and R4 since Sterkwater was commissioned.

	R3 (1999/2000)	**B3**	**R4 (1999/2000)**	**Statistical comparison**
E. coli	n = 40 Geometric Mean = 352 Min = 0 Max = 62 400 95 % CI = 3 128	n = 79 Geometric Mean = 4 277 Min = 50 Max = 1 200 000 95 % CI = 33 099	n = 40 Geometric Mean = 2 629 Min = 134 Max = 645 334 95 % CI = 31 687	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
C. perfringens	n = 40 Geometric Mean = 35 Min = 0 Max = 5 290 95 % CI = 274	n = 65 Geometric Mean = 282 Min = 0 Max = 22 195 95 % CI = 818	n = 41 Geometric Mean = 201 Min = 0 Max = 25 300 95 % CI = 1 594	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
Somatic coliphages	n = 38 Geometric Mean = 266 Min = 0 Max = 11 800 95 % CI = 1 067	n = 61 Geometric Mean = 5 272 Min = 400 Max = 62 000 95 % CI= 2 892	n = 39 Geometric Mean = 6 353 Min = 800 Max = 140 000 95 % CI = 7 329	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA

Table 3.4.2 shows statistically significant differences between the data sets, but it is not clear which data set(s) differed from the other(s). To identify the set(s), all three data sets were compared with the Dunn MCT procedure (Appendix E) in Table 3.4.2.1.

Table 3.4.2.1: Comparing the R3, B3 and R4 data sets to identify the set(s) that differed significantly.

Comparison	*E. coli*			*C. perfringens*			Somatic coliphages		
	Diff of Ranks	**Q**	**P<0.05**	**Diff of Ranks**	**Q**	**P<0.05**	**Diff of Ranks**	**Q**	**P<0.05**
B3 vs R3	52.600	5.887	Yes	31.431	3.698	Yes	49.953	5.481	Yes
B3 vs R4	10.900	1.220	No	3.987	0.473	No	4.615	0.563	No
R4 vs R3	41.700	4.050	Yes	27.444	2.920	Yes	45.338	5.487	Yes

All three indicator groups at R3 again differed significantly from those determined at B3 and R4, the latter two again showing no significant differences. From the results (shown in

Table 3.4.2), it can be concluded t evel of faecal pollution in Sector 1 of the Renoster Spruit, increased since receiving polluted discharges from Sterkwater, these increases had no influence on the indicator levels at R4. This is because the level of faecal pollution at B3 was so high that even the addition of the polluted water from Sector 1 in the Renoster Spruit could not significantly elevate the indicator organism numbers in Sector 2 of the Renoster Spruit.

3.4.3 The ultimate impact on the Renoster Spruit.

To quantify the severity of the impact by discharges from the poorly maintained Sterkwater treatment works, as well as the overloaded Bloem Spruit treatment works and the heavily polluted urban run-off, on water in Sector 2 of the Renoster Spruit, the collated data from R5 was compared as follows:

- Firstly to the collated data (before and after) at R4, to assess the assimilation capacity of the Renoster Spruit downstream from the urban influence.
- Then to numbers of indicator organisms in the unimpacted waters of the study area, represented by R1.

Table 3.4.3.1: Comparison of collated indicator microorganism numbers at R5 and R4.

	R5	**R4**	**Comparison results**
E. coli	N = 54 Geometric Mean = 481 Min = 0 Max = 60 164 95 % CI = 3 511	n = 69 Geometric Mean = 2 240 Min = 12 Max = 645 334 95 % CI = 18 672	Significant difference (P = < 0.001)
C. perfringens	N = 53 Geometric Mean = 51 Min = 0 Max = 15 640 95 % CI = 642	n = 66 Geometric Mean = 154 Min = 0 Max = 25 300 95 % CI = 1008	Significant difference (P = 0.008)
Somatic coliphages	N = 49 Geometric Mean = 922 Min = 0 Max = 11 300 95 % CI = 939	n = 61 Geometric Mean = 4 883 Min = 0 Max = 140 000 95 % CI = 2 529	Significant difference (P = 0.004)

The geometric mean levels of all the indicator organism groups were significantly lower at R5 than those at R4. This indicated that the Renoster Spruit had the capacity to assimilate the urban pollution loads.

It was, however, not clear whether the assimilation process succeeded to negate the urban impact to the extent that the stream's water was again suitable for the various domestic, recreational and agricultural uses. The information in Table 3.4.3.2 shows this effectiveness.

Table 3.4.3.2: Comparison of indicato[illegible]bers at R5 and R1.

	R5	R1	Comparison results
E. coli	N = 54 Geometric Mean = 481 Min = 0 Max = 60 164 95 % CI = 3 511	N = 34 Geometric Mean = 85 Min = 5 Max = 2 750 95 % CI = 197	Significant difference (P = < 0.001)
C. perfringens	n = 53 Geometric Mean = 51 Min = 0 Max = 15 640 95 % CI = 642	N = 33 Geometric Mean = 10 Min = 0 Max = 624 95 % CI = 57	Significant difference (P = 0.006)
Somatic coliphages	n = 49 Geometric Mean = 922 Min = 0 Max = 11 300 95 % CI = 939	n = 31 Geometric Mean = 3 Min = 0 Max = 13 000 95 % CI = 1 290	Significant difference (P = < 0.001)

The geometric mean levels of all the indicator organism groups at R5 were higher than the levels of indicator organisms in the unimpacted waters of the study area, represented by R1. While it is evident that these levels were significantly higher, it was not clear whether the ultimate impact rendered to water of the Renoster Spruit totally useless for the various water uses. This aspect is discussed in the following sub-section.

3.4.4 Discussion

The numbers of indicator organisms in the stream water of Sector 2 initially rose substantially after confluence with the Bloem Spruit, which contained urban run-off and treated wastewater discharge. The indicator organism numbers reported for the Renoster Spruit downstream from Bloemfontein, were similar to those reported for the Klein Modder River, which drained similar discharges from other large urban developments into the Modder River elsewhere in the study catchment (Jagals, 1994; Grabow et al., 1996). Similar results were reported from studies done in other South African provinces such as Gauteng (Kfir et al., 1991; Wimberley, 1993; Freeman et al., 1996; Grabow et al., 1996), Western Cape (Wright et al., 1993), Kwa-Zulu Natal (Gericke et al., 1996) as well as in Sydney, Australia (Ferguson et al., 1996) Arlington, United States of America (Field and Pitt, 1990) and in Japan (Haiping and Yamada, 1998).

The results further indicated that although Sterkwater had an impact on the health-related microbiological quality of water in the Renoster Spruit in Sector 1, the increase in the indicator organism numbers had no significant influence on the indicator densities at R4, because the level of faecal pollution at B3 in the Bloem Spruit was so high that the addition of the polluted water from Sector 1 could not significantly elevate the indicator organism numbers in Sector 2. This impli[...]gical pollutant loads, associated with the urban run-off from the study area, was generally so high that even sub-standard treated effluent, added to the run-off, had no significant effect, albeit to mitigate or to elevate on the impact.

Based on results from studies in Canada (Payment and Franco, 1993), London, United Kingdom (Jacobs and Ellis, 1991), in Australia (Ashbolt et al., 1993), in Finland (Rajala and Heinonen-Tanski, 1998), as well as the results from this study, it can be concluded that, in a sense, faecal pollution levels in urban run-off exceed the faecal pollution levels of sub-standard treated effluents, in a similar manner as would storm water do in combined sewer systems typically found in northern hemisphere cities and towns.

The indicator organism numbers at R4 in Sector 2 were compared to the NOAEL described in Table 1 (Chapter 1) for all types of water users. Organism numbers indicated that the water at this point was unfit for domestic, recreational as well as agricultural use.

The results are summarised separately for each organism group in the following Sections. Boxplots are used in Figures 3.4.4.1 .2 & .3. The levels of indicator organism release from Sterkwater are indicated with a brown-coloured box placed between the "before" and "after" commencement data summarised for R3. This is to reflect the remaining impact caused by Sterkwater on the water at the end of the stream section of Sector 1, which was then released into Sector 2. Note that this is a diagrammatic representation and does not imply that Sterkwater discharges downstream from R3. The sequential representation of the Sterkwater impact is reflected in Section 3.2.

The collated data of the organism numbers, discharged by the Bloem Spruit, is inserted in the graphs as a separate brown-coloured box to show the levels of indicator organisms released by this pollution source into the Renoster Spruit.

3.4.4.1 *Escherichia coli* (*E. coli*)

Figure 3.4.4.1 shows that, although the geometric mean *E. coli* level, at R3, increased after the commissioning of the Sterkwater treatment works, it is evident that it did not add any significant impact to the impact made by water of the Bloem Spruit on the Renoster Spruit water quality as determined at R4.

This reflects the severity of the impact caused by the high numbers of *E. coli* released into Sector 2 by the Bloem Spruit.

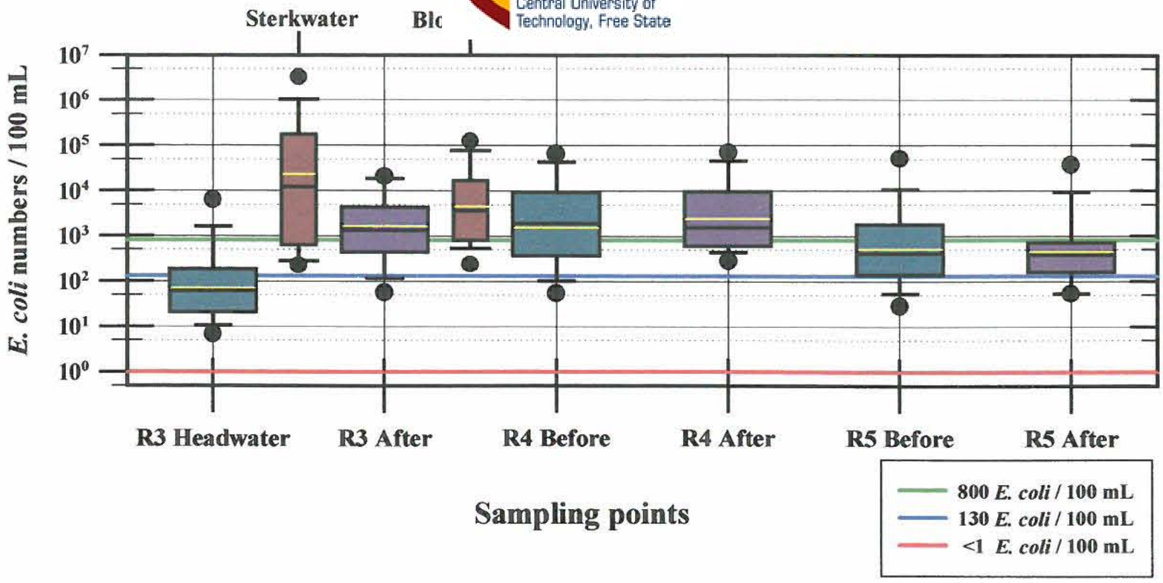

FIGURE 3.4.4.1: *E. coli* numbers detected in the Renoster Spruit: Sector 2.

Results from this study were similar to the 10^4 per 100 mL geometric mean faecal coliform level reported by Venter et al. (1996) in the Riet Spruit, downstream from developing urban areas in Gauteng, as well as the *E. coli* levels, which averaged between logs 3 and 4 per 100 mL, reported in the Slang Spruit in Kwa-Zulu Natal by Pillay and Terry (1991).

Ellis and Wang (1995) reported a faecal coliform geometric mean density of 7.9 x 10^3 per 100 mL at a sampling site in the Pymmes Brook catchment downstream from urban and wastewater discharge points from the city of London. These London results were also similar to results reported by Ellis in 1986 (average faecal coliform density of 6.4 x 10^3 per 100 mL).

These results suggest that, for reasons not discussed, the UK Rivers recovered slowly, and therefore showed insufficient dilution or assimilation capacity when receiving the high pollution load of urban run-off water. By contrast, the Renoster Spruit showed signs of more rapid recovery rates. While the geometric mean *E. coli* level at R4, before and after Sterkwater was commissioned, exceeded the NOAEL for all the water uses, the geometric mean *E. coli* level at R5 decreased to within the NOAEL for agricultural use such as irrigating crops eaten uncooked. The water quality, however, still remained unfit for human consumption and recreation. Judging by the number of data points exceeding the agricultural safe limit (the green line), use of this water for agriculture should also be discouraged.

3.4.4.2 *Clostridium perfringens* (*C. perfringens*)

C. perfringens numbers, determined in Sector 2, are summarised in Figure 3.4.4.2. The similarity of the indicator organism numbers at R4, before and after commissioning of the Sterkwater wastewater treatment works, is apparent.

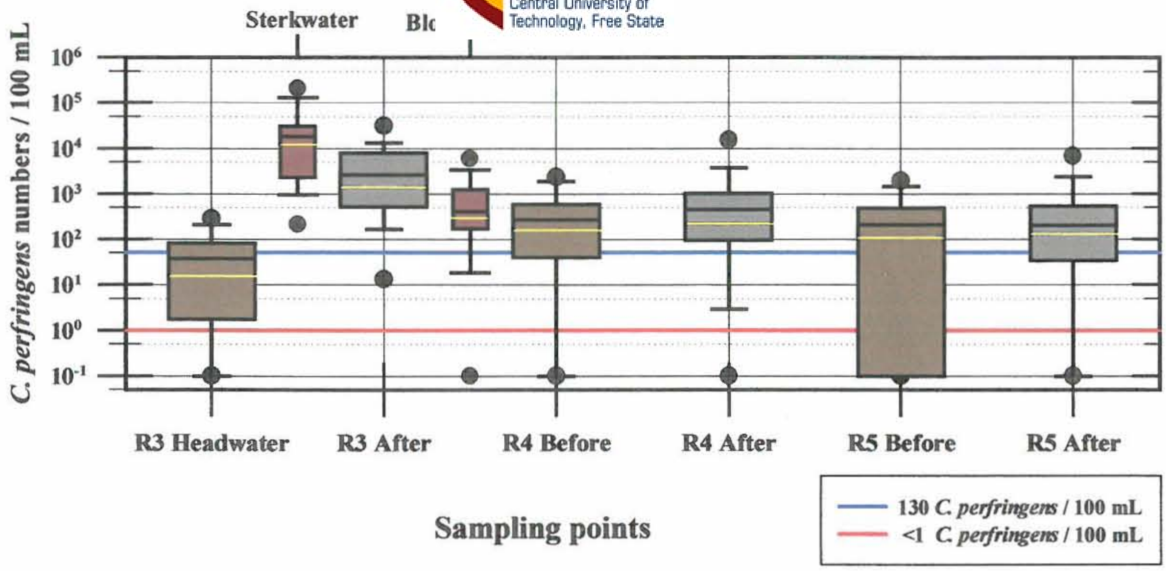

FIGURE 3.4.4.2: *C. perfringens* numbers detected in the Renoster Spruit: Sector 2.

Results indicated that the increase in the geometric mean *C. perfringens* level at R3, after the commissioning of Sterkwater, were not evident at R4, because of the high numbers of *C. perfringens* released into Sector 2 after the Renoster/Bloem Spruit confluence.

Water at R4, before and after commissioning of Sterkwater, was unfit for domestic and recreational use. The results further indicated that although the *C. perfringens* numbers at R5, after Sterkwater started, showed less variance, the water was still not suitable for neither recreational nor domestic use. These results could not be compared with other studies, since none could be found for similar situations, even after intensive literature searches in international and national databases.

3.4.4.3 *Somatic coliphages*

The slight increase in the geometric mean somatic coliphage numbers at R4, before and after the commissioning of Sterkwater is shown in Figure 3.4.4.3. Somatic geometric mean coliphage levels in Sector 2, at sampling points R4 and R5, remained well above the guideline limits for safe domestic and recreational water use even before Sterkwater was commissioned. Indicator organism numbers decreased towards R5, but the water was still unfit for use.

Kfir et al. (1991) found somatic coliphages of up to 6^3 pfu per 100 mL in the polluted Apies River in Pretoria, South Africa. These numbers are similar to the levels reported for this study. By contrast, geometric mean levels of up to 85 pfu per 100 mL somatic coliphages, reported by Marsalek et al. (1994), in the St Clair River, Ontario, which receives discharges from several wastewater treatment works, combined sewer overflow outfalls and storm sewer outfalls, were lower than the results reported for this study. This implies that the management of the urban discharges in the O might be more effective than that of the reported South African catchments.

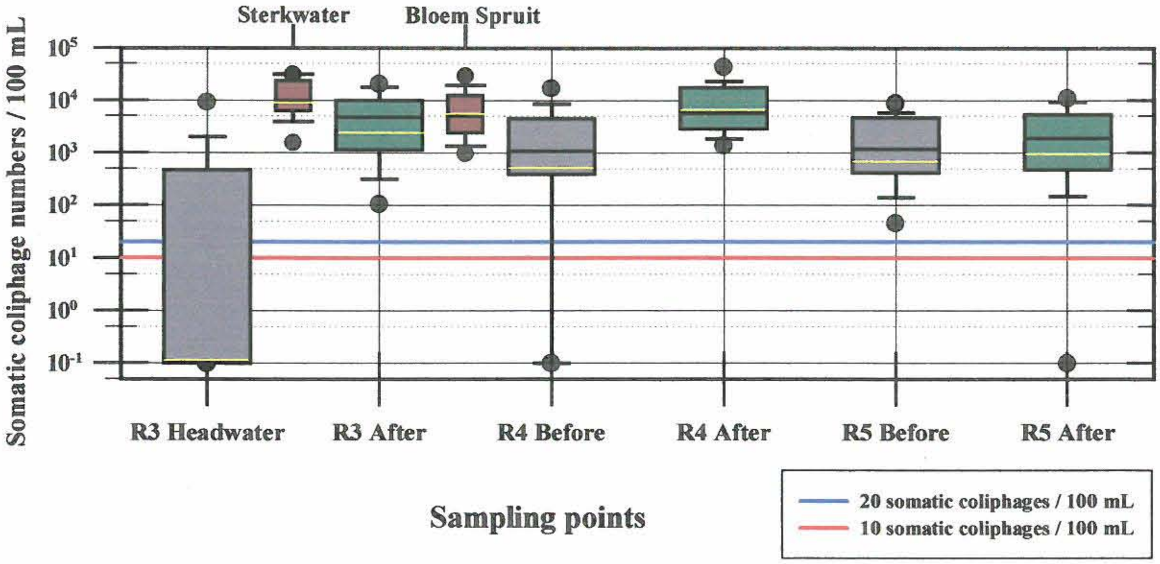

FIGURE 3.4.4.3: Somatic coliphage numbers detected in the Renoster Spruit: Sector 2.

In conclusion, the levels of somatic coliphages in the water of Sector 2 exceeded the guideline limits (Table 1: Chapter 1) for safe domestic and recreational water use. The levels of *E. coli,* reported in Tables 3.4.1 and 2, further suggested that the levels of *E. coli* exceeded maximum numbers recommended for water to be used for the irrigation of crops (Shuval et al., 1997).

3.4.5 The impact of urban discharges on the Renoster Spruit in Sector 2

The high indicator organism counts found in the waters of the study area, especially during the second phase, indicated a poorly maintained Sterkwater treatment works, an overloaded Bloem Spruit treatment works, as well urban run-off heavily polluted with faecal material.

The water of Sector 2 of the Renoster Spruit was severely impacted upon by Bloem Spruit and to a lesser extent by the water from Sector 1, which carried the poorly treated effluent from the Sterkwater treatment works.

The health-related microbiological water quality, at R1 would, although not suitable for direct human consumption, was suitable for raw water extraction for purposes of full treatment, as well as partial treatment such as home treatment (Discussed in Section 3.1). These waters would also be suitable for full-contact recreation and unrestricted irrigation of health-sensitive agricultural crops.

The impact of the various urban discharges on the water of Sector 2 was so severe that, even after some natural impact assimilation between R4 and R5 appeared to have taken place, the water was still not suitable to be [illegible] and recreational purposes. Considering the *E. coli* levels (Venter et al, 1996), this water was not even suitable for the purposes of treatment for human consumption. The water became, on average, suitable to be used for irrigation of health-sensitive crops, although the high levels of indicator organisms in more that 50% of the samples suggested that the use of this water for health-sensitive crops be discouraged.

SECTION 3.5: MICROBIOLC TY OF WATER IN THE MODDER RIVER

In this Section, the impact of the Renoster Spruit (R5) on the health-related microbiological quality of water in the Modder River (M1 & M2) was assessed:

- The combined microbiological quality of the water at R5 provided baseline data for the impacted Renoster Spruit (discussed in Section 3.4).
- The microbiological quality of water in the Modder River was determined:
  - ≠ Before confluence with the Renoster Spruit to provide the "headwater" quality for the river (M1).
  - ≠ Downstream from the confluence with the Renoster Spruit (at M2) to assess whether water of the Renoster Spruit had an impact on the water of the river.

The areas surrounding this section of the Modder River are, like Sector 1 of the Renoster Spruit, mostly used for agricultural purposes. Human settlement and livestock are also limited. No other urban point or diffuse sources of faecal pollution could be identified that influenced the quality of the river in this area. Data from both dry and wet weather periods were collated to provide an overall view of the health-related microbiological water quality.

3.5.1 Impact of the Renoster Spruit on the Modder River

To quantify the impact of the Renoster Spruit (R5), the water quality at M1 was compared to the quality at M2 as well as R5. Results, tabled in Table 3.5.1, indicate that the data differed significantly. The zero-hypothesis is rejected.

Table 3.5.1: Comparison of indicator organism numbers at M1, R5 and M2.

	M1 (1997/2000)	**R5 (1997/2000)**	**M2 (1997/2000)**	**Statistical comparison**
E. coli	n = 46 Geometric Mean = 20 Min = 0 Max = 56 000 95 % CI= 2 381	n = 54 Geometric Mean = 481 Min = 0 Max = 60 164 95 % CI= 3 511	n = 39 Geometric Mean = 32 Min = 0 Max = 32 000 95 % CI= 1 692	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
C. perfringens	n = 48 Geometric Mean = 4 Min = 0 Max = 1 150 95 % CI= 67	n = 53 Geometric Mean = 51 Min = 0 Max = 15 640 95 % CI= 642	n = 37 Geometric Mean = 9 Min = 0 Max = 1 035 95 % CI= 61	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA
Somatic coliphages	n = 37 Geometric Mean = 0 Min = 0 Max = 300 95 % CI= 19	n = 49 Geometric Mean = 922 Min = 0 Max = 11 300 95 % CI= 939	n = 36 Geometric Mean = 1 Min = 0 Max = 300 95 % CI= 30	Significant difference (P =<0.001) H_0 rejected Kruskal-Wallis ANOVA

While Table 3.5.1 shows significant differences between the data sets, it is not clear which data set(s) differed from the other(s). To identify the set(s), all three data sets were compared with the Dunn MCT procedure (A sults are shown in Table 3.5.1.1.

Table 3.5.1.1: Multiple comparison test procedure results (Dunn's Method).

Comparison	*E. coli*			*C. perfringens*			Somatic coliphages		
	Diff of Ranks	Q	P<0.05	Diff of Ranks	Q	P<0.05	Diff of Ranks	Q	P<0.05
M1 vs M2	5.365	0.612	No	9.781	1.118	No	5.705	0.689	No
R5 vs M2	51.896	6.126	Yes	25.431	2.969	Yes	52.686	6.787	Yes
R5 vs M1	46.530	5.843	Yes	35.212	4.420	Yes	6.787	7.581	Yes

All three indicator groups at R5 differed significantly from those at M1 and M2, the latter two sampling points showed no statistically significant differences. From the results (shown in Table 3.5.1), it can be concluded that although the level of faecal pollution in the Modder River increased slightly after receiving the polluted water of the Renoster Spruit, these increases did not cause statistically significant differences in the indicator organism levels at M1 and M2. The larger water mass in the Modder River diluted the higher levels of microbiological indicator organisms discharged by the Renoster Spruit.

3.5.2 Discussion

The results are summarised separately for each organism group in the following Sections. Boxplots are used in Figures 3.5.2.1 .2 & .3. The levels of indicator organism release from the Renoster Spruit, at R5, are indicated separately in these Figures. This is to reflect increases, although generally not significant, in the indicator organism numbers downstream of the Renoster Spruit/Modder River confluence.

3.5.2.1 *Escherichia coli* (*E. coli*)

Figure 3.5.2.1 shows that, although the geometric mean *E. coli* level, at M2, increased after confluence with the Renoster Spruit, this was not statistically significant.

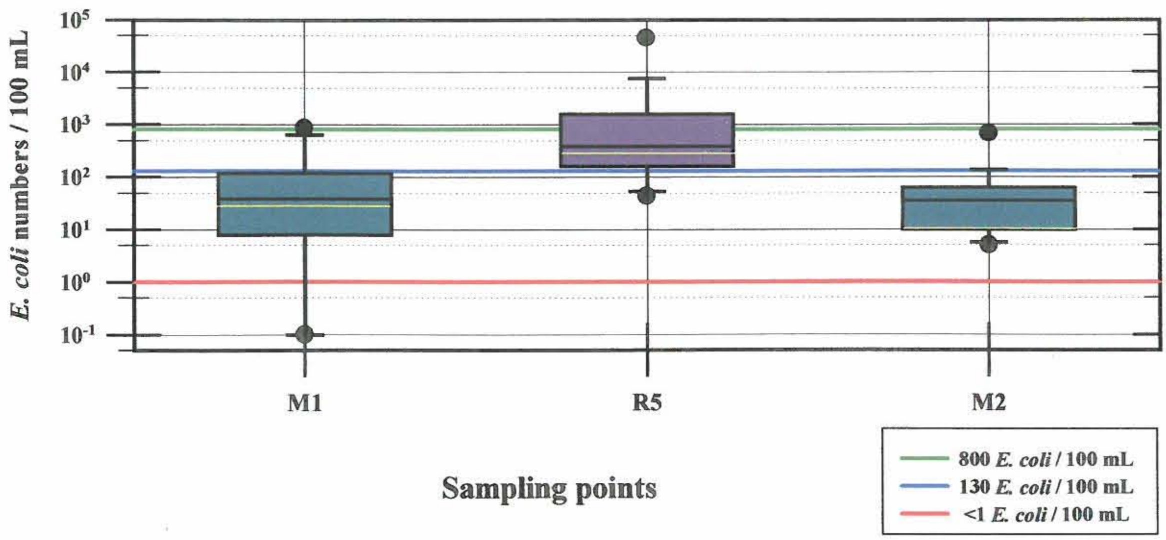

FIGURE 3.5.2.1: *E. coli* numbers detected in the Modder River.

The *E. coli* geometric mean level o be less than the 85 *E. coli* per 100 mL at R1 and the 96 *E. coli* per 100 mL geometric mean density reported at unpolluted sampling sites in the St Clair River (Marsalek et al., 1994).

The geometric mean *E. coli* level at M1 was higher than the proposed safe limit for drinking water but did not, on average, exceed guideline limits recommended for recreation and agriculture. A number of data points at the 75th and 95th percentile levels, however, exceeded the recreational safe limit (the blue line), which implies that full contact recreational use of this water should be discouraged.

While the geometric mean *E. coli* level at M2 exceeded the NOAEL for drinking purposes as well as full or intermediate body contact recreation, the water at this point was not expected to cause health effects when used for unrestricted irrigation of health-sensitive crops. The water could therefore be used for irrigating vegetable and salad crops eaten uncooked, sports fields or public parks.

3.5.2.2 *Clostridium perfringens* (*C. perfringens*)

Figure 3.5.2.2 shows that, although the geometric mean *C. perfringens* level at M2 showed no statistically significant differences to the numbers counted at M1, the data for M1 and M2 showed considerable variance. Jagals (2000) reported similar variability in the occurrence of *C. perfringens* in unpolluted river water. The data variability was probably due to variable numbers of vegetative spores in samples.

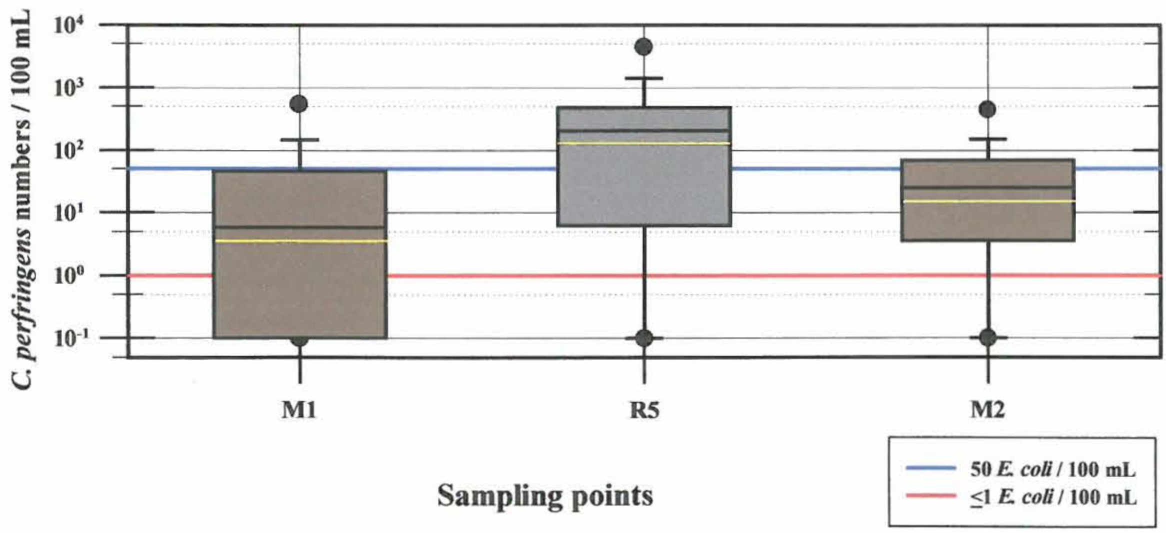

FIGURE 3.5.2.2: *C. perfringens* numbers detected in the Modder River.

The geometric mean *C. perfringens* level at M1 exceeded the NOAEL for domestic purposes proposed for this indicator organism in Table 1. A risk of infection, after full contact recreation, however, was not indicated when the geometric mean level was compared to the

NOAEL in Table 1 (Chapter 1). igation guideline was included in Table 1 and the occurrence of *C. perfringens* could therefore not be evaluated from an agricultural risk perspective.

The water at M2 was also unfit for domestic use, with slightly higher values than what was reported at M1. The levels of *C. perfringens* in water at M2, when compared to the criteria in Table 1, did not exceed guideline limits recommended for full or intermediate body contact recreation. A number of data points at the 75th and 95th percentile levels, exceeded the recreational safe limit (the blue line), which implies that full contact recreational use of this water should be discouraged.

3.5.2.3 *Somatic coliphages*

A considerable number of zero data points indicated that there were no culturable coliphages in the water sampled in the Modder River. The medians for both data sets, showed a presence of ≤ 1 pfu / 100 mL in Figure 3.5.2.3. A considerable number of data points towards the 75th and 95th percentiles imply that the geometric means could be higher if the number of ≤ 1 were less.

The geometric mean levels for somatic coliphages at M1 and M2, however, indicated that the water could be expected to be safe for domestic as well as recreational use.

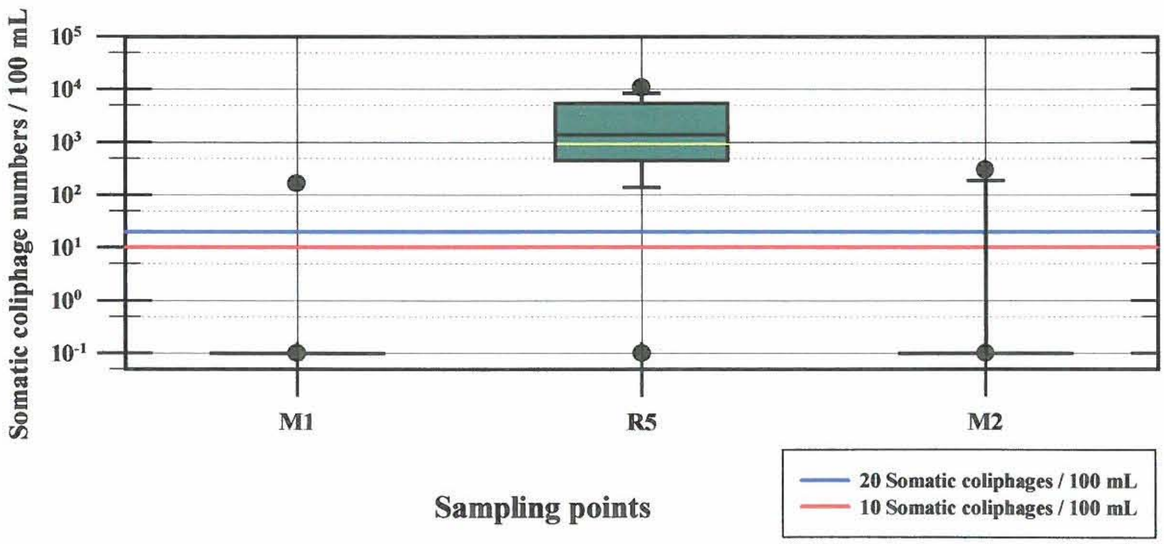

FIGURE 3.5.2.3: Somatic coliphage numbers detected in the Modder River.

3.5.3 Impact on the Modder River

The numbers of all three the indicator organism groups, at M1 and M2, were compared to the NOAEL described in Table 1 (Chapter 1) for all types of water use categories. The numbers of bacterial indicator organisms in [illegible]fection when used untreated for domestic purposes except for somatic coliphages at M1 and M2, in which cases the untreated water was fit for domestic use. The water was suitable for raw water extraction based on the criteria (200 faecal coliforms per 100 mL) proposed by Venter et al. (1996). Levels of *C. perfringens* and somatic coliphages exceeded maximum numbers recommended for water intended for human consumption. The water of the Modder River was generally also safe for recreational and agricultural purposes. The water could therefore be used for irrigating vegetable and salad crops eaten uncooked, sports fields, golf courses as well as public parks.

The water of the unpolluted Modder River generally assimilated the water from the polluted Renoster Spruit effectively to such an extent that any possible impact on the health-related microbiological water quality in the Modder River was negated.

Chapter 4
SUMMARY and CONCLUSION

The aim of this study was to assess the impact of various treated effluents, as well as other urban discharges, on the health-related microbiological quality of water in several streams of the Renoster Spruit sub-catchment. This sub-catchment is situated in the Bloemfontein area within the Modder River catchment (Free State province, South Africa).

Based on in-stream quality criteria, proposed in a site-specific water quality guide compiled for the purposes of this study (Table 1; Chapter1), these impacts were then qualified as varying levels of infection risks to people using these untreated surface waters for domestic as well as recreation and agricultural purposes.

For this study, a point or non-point pollution source was said to have an impact on the health-related microbiological quality of the receiving waters if it exceeded the natural assimilation capacity of the receiving water body. The assimilation capacity of a water body was, therefore, defined as the ability of a water body to receive, and "absorb", microbiological pollutants without the in-stream microbiological water quality being rendered unfit for health-related domestic, recreation or agricultural use.

The health-related microbiological quality of the various waters was investigated by using *E. coli*, *C. perfringens* spores and somatic coliphages as microbiological indicators. These three organism groups were considered as the best combination because they represent a spectrum of different types of microorganisms, which indicate faecal pollution and the potential presence and survival of pathogenic microorganisms. In addition, *E. coli* may more realistically reflect the survival of bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Vibrio* spp. than *C. perfringens* and phages. Furthermore, *C. perfringens* spores may more realistically reflect the survival of cysts and oocysts than the other indicators, and phages may more realistically reflect the survival of viruses than the other indicators. However, none of these indicators specifically indicate the presence of any pathogens.

To achieve the aim of the study, specific objectives were identified (Chapter 1). The objectives, as well as the outcomes of the investigations towards attaining these objectives, are summarised as follows:

- The natural background levels indicator organisms in the water of Sector 1 in the Renoster Spruit (Chapter 3: Section 3.2), before the newly constructed Sterkwater wastewater treatment works [illegible]l, indicated that the stream water was not suitable, without treatment, for uses such as drinking and full contact recreation. The water could, however, be used as a raw water source for treatment for domestic use, as well as the irrigation of health-sensitive crops without risk of infection to consumers.

- The commissioning of Sterkwater had a noticeable impact on the health-related microbiological quality of water in Sector 1 of the Renoster Spruit. The numbers of indicator organisms in this sector of the Renoster Spruit increased significantly since Sterkwater began discharging effluent (Chapter 3: Section 3.2).

- The abovementioned impact was because the Sterkwater wastewater treatment works did not achieve the desired levels of indicator organism reduction in the effluent discharged for the period of this study (Chapter 3: Section 3.2).

- The faecal coliform standard, stipulated in the *General Standard* (Republic of South Africa, 1984), would have been exceeded (for the period of this study). This was based on the numbers of *E. coli* counted in the final effluent, converted back to faecal coliforms.

- The levels of indicator organisms, determined in the Bloem and Fontein Spruits, receiving urban discharges, were often similar to the levels of microbiological indicator organisms detected in raw sewage (Chapter 3: Section 3.3). This indicated that these urban discharges had an impact on the health-related microbiological quality of water in these urban drains.

- Although not as high as was determined in the Sterkwater effluent, the numbers of *E. coli* (converted back to faecal coliforms) in the final effluent from the Bloem Spruit treatment works, indicated (Chapter 3: Section 3.3) that the faecal coliform standard, stipulated in the *General Standard* (Republic of South Africa, 1984), would also have been exceeded.

- The health-related microbiological quality of the water in the Renoster Spruit, especially at the beginning of Sector 2, was severely impacted upon by the Bloem Spruit. Water from Sector 1, carrying treated effluent from Sterkwater, added, but to a lesser extent, to the pollution of the water in Sector 2 (Chapter 3: Section 3.4). The numbers of microbiological indicator organisms, counted at this point in the stream, indicated that the water was unfit for domestic, recreation and agricultural uses.

- Further downstream in Sector 2, the water of the Renoster Spruit assimilated the polluted upstream water to such an extent that the health-related microbiological water quality improved, at least to [illegible]s for agricultural water uses (Chapter 3: Section 3.4). This indicated that approximately eight kilometres of the Renoster Spruit, including several impoundments constructed in this stream, had the ability to assimilate microbiological faecal pollution from the city of Bloemfontein.

- The water in the Modder River upstream as well as downstream from the confluence with the Renoster Spruit, could, with a negligible risk of infection to consumers, be used for domestic, recreation and the irrigation of health-sensitive crops (Chapter 3: Section 3.5). This indicated that water from the Renoster Spruit had no impact on the health-related microbiological quality of water in the Modder River.

From the study results, it can be concluded that despite measures, such as the implementation of effluent standards by the Department of Water Affairs and Forestry, to prevent and control microbiological pollution of South African surface waters, the quality of surface waters in the study area was severely impacted upon by urban discharges. In fact, the health-related microbiological quality of this part of the Modder River catchment appears to be deteriorating when compared to results from the same waters, reported by Jagals (1994).

Furthermore, not only did the Renoster Spruit, as an apparently natural environmental water resource, present a human health risk associated with the direct and indirect domestic use of the water, but the water quality problems would also increase the costs of treating the water to potable standards.

THE WAY FORWARD

Future Research

- The natural background levels of the health-related microbiological quality of water in Sector 1 in the Renoster Spruit (Chapter 3: Section 3.2), before the newly constructed Sterkwater wastewater treatment works was commissioned, indicated that the stream water was not suitable, without treatment, for uses such as drinking and full-contact recreation. The water could, however, be used as raw water source for treatment for domestic use, as well as the irrigation of health-sensitive crops without risk of infection to consumers. More research, and increased funding would be needed to investigate this.

- Proper health-related impact assessments should be designed and executed before wastewater treatment works are upgraded or newly constructed. This should include guidance on the operational practices during the start-up phases of such treatment works.

- Proper community develop ould be designed and implemented to facilitate proper sanitation practices in urban areas with, as well as without suitable sanitation infrastructure.
- Studies, investigating factors such as the impact of impoundments, natural organism die-off, sedimentation-entrapment of organisms, availability of nutrients and other biotic factors, and the influence of autochthonous organisms, should be encouraged. This would be to explain the reduction of microbiological indicator organism numbers such as were determined in the Renoster Spruit. Such information could then be used to aid catchment management towards improving the water quality.
- Epidemiological, as well as risk assessment type investigations should be done in the area to establish whether the application and use of the water, in the areas where risk was indicated, indeed had the effect predicted by this study.

Catchment management

The Department of Water Affairs and Forestry and the Water Research Commission (1999) currently recognise catchments as the basic management units for managing surface water quality. A catchment management plan therefore includes strategies necessary to manage water quality as well as quantity within a catchment. These strategies consequently outline, quantify and prioritise land-use activities and processes within the catchment that might contribute to surface water quality problems.

Part of the strategies to manage water quality are to outline, quantify and prioritise the processes by which point or diffuse sources of faecal pollution impact on the water quality in any catchment. These processes are divided into four generic elements (Pegram et al., 1998), which include natural or anthropogenic classification of the sources. These are integrated to define cause-and-effect relationships within the catchment. The four processes are then used, usually in a continuum, to describe and also to manage, the path that microbiological contaminants, such as those investigated in this study, follow from point of production to the point at which they impact on the use of the water. These processes are:

① **Production**

The process refers to the production or generation of microbiological indicator organisms at their point of origin. Production, therefore, occurs before the microbiological contaminant reaches the aquatic environment and before these contaminants are mobilised away from the point of origin.

Numbers of microbiological indica his sub-catchment are generated at point sources such as wastewater treatment works that encounter operational and design problems. This appeared to be the problems encountered by the Sterkwater or Bloem Spruit treatment works. Generation of microbiological indicator organisms can also be spread wider, as a non-point source of pollution, over the various economic strata of residential areas. Production management strategies should, therefore:

- Prevent, or minimise, through process optimisation, the numbers of microbiological organisms discharged from wastewater treatment works such as the Sterkwater and Bloem Spruit treatment works.
- Optimise sanitation through engineering and educational intervention measures.

② **Delivery**

To date, advanced production management strategies, such as water quality-related storm water management, are generally not implemented in South Africa, especially in and around cities. Most catchment management plans, address only the delivery of quantities of stormwater, regardless of the quality of the discharge (wastes).

Delivery of wastes refers to the process of introducing stormwater and other discharges that contain excessive numbers of microbiological contaminants, to the aquatic environment in the form of a point, or non-point source discharge. As such, delivery management occurs after the production of the microbiological pollutant, but before these reach the aquatic environment. Management at the point of delivery would involve:

- Delivery management at point sources, usually in the form of implementing and enforcing effluent standards, which are aimed to prevent excessive microbiological waste loads from reaching receiving waters.
- Prevent, minimise, or treat, polluted urban surface run-off before, or as soon as it reaches surface water bodies. Promoting natural assimilation in artificial wetlands or other methods such as land infiltration rather than run-off can achieve this. This will slow the rate at which the microbiological contaminants reach the environment, and allow for natural die-off or uptake within the soil.

③ **Transport**

In cases where it is not possible to prevent urban discharges of unacceptable quality to reach catchment drains, the reduction of the microbiological pollutant load could be addressed and managed though application of effective contaminant transport management.

Transport is the third element [illegible] – effect relationship, and refers to the movement and fate of microbiological contaminants in receiving streams and rivers. Management of contaminant transport requires the use of the assimilative capacity of the particular stream or river. In cases were the assimilative capacity of such a stream is assessed to be effective authorisation may allow relaxation of permit conditions to discharge an effluent. However, if no assimilative capacity exists, transport management must be aimed at creating assimilative capacity. This can be done in several ways:

- Water may be released from unpolluted upstream impoundments to further dilute pollutants.
- The chemical, physical and biological processes that remove or transform pollutants could be enhanced (for example by placing wetlands or cascade weirs within the river channel).
- More stringent effluent standards could be set for upstream discharges.

Management of the transport elements, therefore, focuses on the fate of the microbiological contaminants once already in the aquatic environment. These measures are consistent with source directed management. Nevertheless, it may be necessary to manage the transport process in highly impacted catchments where flow in the rivers is dominated by effluent.

④ **Use**

This is the final element of the cause-and-effect relationship. It refers to the management of water use, rather than managing the in-stream water quality. Use management may require:

- Treatment to make the water fit for its intended use. Although it is recognised that some form of water treatment may be inevitable, particularly for potable use, management at the point of use may require improved treatment facilities. Use management may also involve altering irrigation practices to minimise the impact of poor water quality on health-sensitive crops.
- Avoidance of poor quality water. The use of alternative water sources, the erection of signs warning against poor water quality can also be considered as use management.

Management of water quality at the point of use can only be attempted after impacts on water quality have been noted. It is, therefore, a form of symptomatic management.

In conclusion it can be said that a catchment management plan, based on the abovementioned, should be drawn up and implemented for the Renoster Spruit sub-catchment. Such a management plan should form part of a larger Modder River Catchment Management Plan.

The plan should, apart from the m [illegible] it could contain, such as quantity as well as ecological quality management, provide for a definite focus on the management of environmental health-related water quality in this catchment, in order to protect and promote the health of potential consumers and other types of users.

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Appendix A
EQUIPMENT, TECHNIQUES and PROCEDURES

MEMBRANE FILTRATION

Equipment and procedures for bacteriological analysis by membrane filtration were based on methods described by SABS (1984 and 1987), Millipore Corporation (1992) and Standard Methods (1998).

1 EQUIPMENT

1.1 Filter and Vacuum assembly

The membrane filtration system consisted of the following equipment:

- Three Millipore® three-place PVC manifolds.
- Nine glass 47mm diameter Millipore® filter holder sub assembly comprising:
  - ≠ Glass funnels (± 250-mL capacity).
  - ≠ Fritted glass base support for filter membrane.
  - ≠ Clamp to secure funnel on base after loading filter membrane.
- Two one-litre vacuum filter glass flasks for trapping moisture before vacuum pump.
- Two EDWARDS® 1.5 Two-Stage 220/240 V 50/60 Hz vacuum/pressure pumps.
- Silicone rubber tubing for connecting the assembly.

1.2 Pipettes

Finn® adjustable pipettes with sterile disposable tips for pipetting 1 mL of sample were used. Errors in calibration were checked not to exceed 2.5%. Larger volumes were dispensed with standard graduated glass pipettes.

1.3 Membrane filters

Sterile Millipore® HA-type 0.45-µm pore size membranes were used. The membranes were 47mm in diameter, white and grid marked.

1.4 Incubators

1.4.1 Labocon incubators with circulating air (fan induced) were used. Temperatures varied within 0.5°C accuracy, especially within stacks of incubated plates.

1.4.2 Water baths (25 L) with uniformly distributed heating elements in the steel inner jacket, to ensure constant temperature distributions, were used. The baths were equipped with gabled covers to aid temperature maintenance within 0.2°C of setting.

2. TECHNIQUES and PR

2.1 Sterilisation

Steam sterilisation of equipment was done in an autoclave at 121°C/ 15 psi for 15 minutes. Each glass assembly was separately wrapped in tin foil and sterilised before each completed session of filter plating.

Dry sterilisation of equipment was done in an oven at 180°C for 10 minutes. Dry sterilisation was done between each sample filtration session.

Forceps were decontaminated by immersion in alcohol and flamed before every handling.

2.2 Phosphate buffer

Stock phosphate and stock magnesium chloride solution were prepared according to Standard Methods (1998).

Sterile working solutions of buffer were made up by adding 1.25 mL of phosphate solution (34g KH_2PO_4/500 mL distilled water) and 5 millilitre of magnesium chloride solution (81.1g $MgCl_2.6H_2O$/ 1000 mL distilled water) to 1 litre of reagent grade water.

Steam sterilisation of the phosphate buffer was done in an autoclave at 121°C/ 15 psi for 15 minutes.

2.3 Dilutions

The raw water and wastewater-related samples was vigorously shaken to achieve homogenous organisms distribution within the sample volume. All samples were filtered in triplicate (3 filters) per dilution. Dilutions were made up to ideally achieve counts of between 20 to 60 colonies per plate (Standard Methods, 1998).

Undiluted sample applications varied between 10 mL and 100 mL. Sample volumes of 10 mL was pipetted onto the filter. Sample volumes of 100 mL were firstly decanted into sterile 100 mL measuring cylinders before being poured into the funnel.

1 mL of the undiluted surface water samples, or sample dilutes of up to 10^{-4} were pipetted onto the filter. For first run-off, especially after long dry periods as well as for wastewater related samples, dilutions of up to 10^{-6} were prepared and used. The following dilution procedure was followed:

- Empty test- tubes were autoclaved and sterilised.
- 10 x 1 mL extractions from various areas and depths in the sample were aseptically taken from the sample to the prepared empty test-tube.
- 1 mL of this sample mixture was aseptically transferred to a prepared 9 mL volume of

sterile phosphate buffer to c ted sample.

- 1 mL of the 10^{-1} dilute was aseptically transferred to another 9 mL volume of sterile phosphate buffer to provide a 10^{-2} dilution.
- Subsequent dilutions were made up in a similar manner.

2.4 The technique

Three sets of Millipore® three-place vacuum manifolds, complete with filter holder sub-assemblies were used. Vacuum was created by the electric vacuum pump evacuating through a dual moisture trap system comprising 1-litre capacity vacuum flasks.

Before each session of filter plating, each glass assembly was separately wrapped in tin foil and sterilised in the autoclave at 121°C/ 15 psi for 15 minutes. Between each sample filtration session, constant dry sterilisation and decontamination of the glass sub-assemblies were achieved by placing the glass sub-assemblies in an oven at 180°C for 10 minutes.

Sterile phosphate buffer was used for diluting the samples and rinsing the funnels after filtration (Standard Methods, 1998). Filter plating of the same sample was done in decreasing dilution order to eliminate contamination between dilutions.

The membrane filters were loaded, grid side up, onto the fritted glass support base of the funnel holder with sterile forceps. Thereafter, the funnel was clamped onto the filter base.

The sample was re-mixed by vigorously shaking the sterile whirlpack for several seconds. 20 – 30 mL of the sterile buffer was poured into the funnel and a given volume of sample, depending on the pollution, was pipetted into the buffer with a Finn® adjustable pipette.

All samples portions suspended in dilution were prepared before filtering. The diluted sample was then filtered within 20 minutes to avoid inactivation or multiplication of organisms in the dilution.

Vacuum was applied while slightly swirling the manifold unit to ensure uniform suspension of the sample in the volume of buffer during filtering. The funnel walls were then rinsed (3 times) with approximately 30 mL of sterile phosphate buffer. The buffer was drawn into a syringe and ejected against the top walls of the funnel to ensure that the sample was rinsed of and sucked through the filter.

Vacuum was broken and the membrane lifted with sterile forceps. The membrane was placed grid side up, onto the prepared selective medium in the petri dishes. Care was taken to ensure no trapped air under the membrane. The dishes were marked and incubated invertedly (Millipore Corporation, 1992; Standard Methods, 1998). The incubation temperatures and times for each of the bacteriologic m groups are described in Appendix B.

2.5 Counting

After incubation for appropriate periods of time, colonies were counted according to the prescriptions for each group of indicator organism group grown on the selective media. Colonies of *E. coli* and *C. perfringens* hosted by membrane filters after incubation were counted under a ZEISS® stereomicroscope. To achieve reliable statistical quantification, the final count per 100 mL of sample was calculated as follows (Standard Methods, 1998):

$$\frac{\dfrac{[(\text{Sum of organisms filter 1} + \text{filter 2} + \text{filter 3}) / 3] \times 100}{\text{Sample size}}}{\text{Sample dilute}}$$

This formula was programmed in Microsoft Excel®, 2000 spreadsheets (Appendix E). The analyst entered:

(1) The count from each of the three filters on the plate.

(2) The sample size (maximum 1 mL for diluted samples).

(3) The dilutions expressed as 0.1; 0.01; etc. (minimum 1ml for undiluted samples).

Counts of *E. coli* and *C. perfringens* were calculated and expressed as colony forming units (cfu) per 100 mL. Somatic coliphage counts were calculated and expressed as plague forming units (pfu) per 100 mL.

BACTERIOLOGICAL INDICATOR ANALYSIS

» Media, reagents and procedures

1 *Escherichia coli* (*E. coli*)

1.1 Enumeration by means of chromogenic substrate agar

E. coli were enumerated by means of the membrane filter technique (Appendix A), on Chromocult Coliform® Agar (Merck Corporation, 1996). Enumeration was done in triplicate on 90 mm petri dishes.

Preparation of the Chromocult Coliform® Agar (Merck Corporation, 1996):

- 26.5 g of the powder were suspended in 1-litre of distilled water.
- The mixture was gently boiled in a flowing water bath while gently being stirred until the powder was totally dissolved.
- The media was cooled to 40-50°C and the Cefsulodin solution (10 mg in 2 mL of distilled water) was added to the 1-litre of medium by gently shaking to homogenise. (Cefsulodin solution was added to knock out the expected accompanying flora, especially *Pseudomonas* spp. and *Aeromonas* spp.)
- The liquid was poured into 90 mm petri dishes, 5 mm in depth.
- The media does not require autoclaving.
- Fresh plates, sealed in the plastic bags for moisture retention were stored in the dark, at < 8°C.
- Unused plates were discarded after 6 months.

Incubation: The plates were inverted and incubated at 37°C for 24 hours, ± 2 hours.

Identification: *E. coli* colonies appeared in various shades of dark blue to violet (Merck Corporation, 1996) (FIGURE 1).

Confirmation: Cultured isolates were confirmed on API® 20E (bioMérieux, 1998) as described in Appendix D.

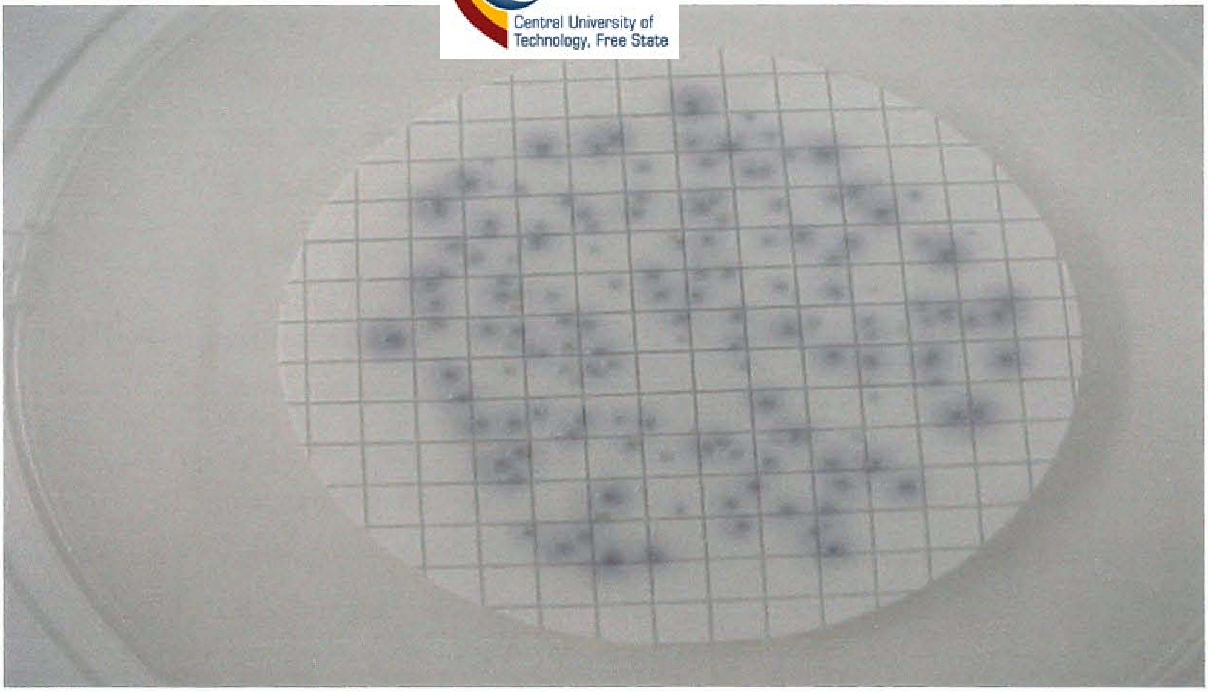

FIGURE 1: *E. coli* colony morphology.

2 *Clostridium perfringens* (*C. perfringens*)

2.1 Enumeration by means of Perfringens (OPSP) Agar

C. perfringens was enumerated by means of the membrane filtration technique (Appendix A), using supplemented Perfringens (OPSP) Agar (Oxoid Corporation, 1990). Enumeration was done in triplicate on 90 mm petri dishes.

Preparation of the Perfringens (OPSP) Agar (Oxoid Corporation, 1990):

- 22.8 g of the powder were added to 500 mL distilled water, the mixture was boiled gently to dissolve the powder.
- The mixture was autoclaved at 121°C for 15 minutes.
- After cooling to 50°C, rehydrated supplements A (SR76) (Sodium sulphadiazine) and B (SR77) (Oleandomycin phosphate and Polymyxin B) were added to give a high degree of selectivity and specificity for *C. perfringens*.
- The mixture was mixed and poured into 90 mm diameter petri dishes, 5 mm in depth.
- After cooling, the plates were stored in plastic bags, to maintain moisture content, in darkness, at <8°C.
- Unused plates were discarded after 2 weeks.

Pasteurisation: Samples (presumably containing *C. perfringens* spores) were pasteurised by placing the san bath at 75°C for 10 minutes (Oxoid Corporation, 1990; Ferguson et al., 1996). Sample pasteurisation knocks out the background flora that might interfere with the growth of *C. perfringens* on the plates, but does not harm the vegetative spores of *C. perfringens*.

Incubation: The plates were inverted and incubated anaerobically in an incubator at 37°C for 44-48 hours. Laminated foil envelope Oxoid Gas Generating Kits with an inner container holding tablets of sodium borohydride, sodium bicarbonate and tartaric acid was used to produce atmospheres of 95% hydrogen and 5% carbon dioxide through the catalytic removal of available oxygen in the jar.

Identification: *C. perfringens* colonies appeared as black colonies (Oxoid Corporation, 1990) (FIGURE 2).

Confirmation: Cultured isolates were confirmed on Rapid ID® 32A galleries (bioMérieux, 1994) as described in Appendix D.

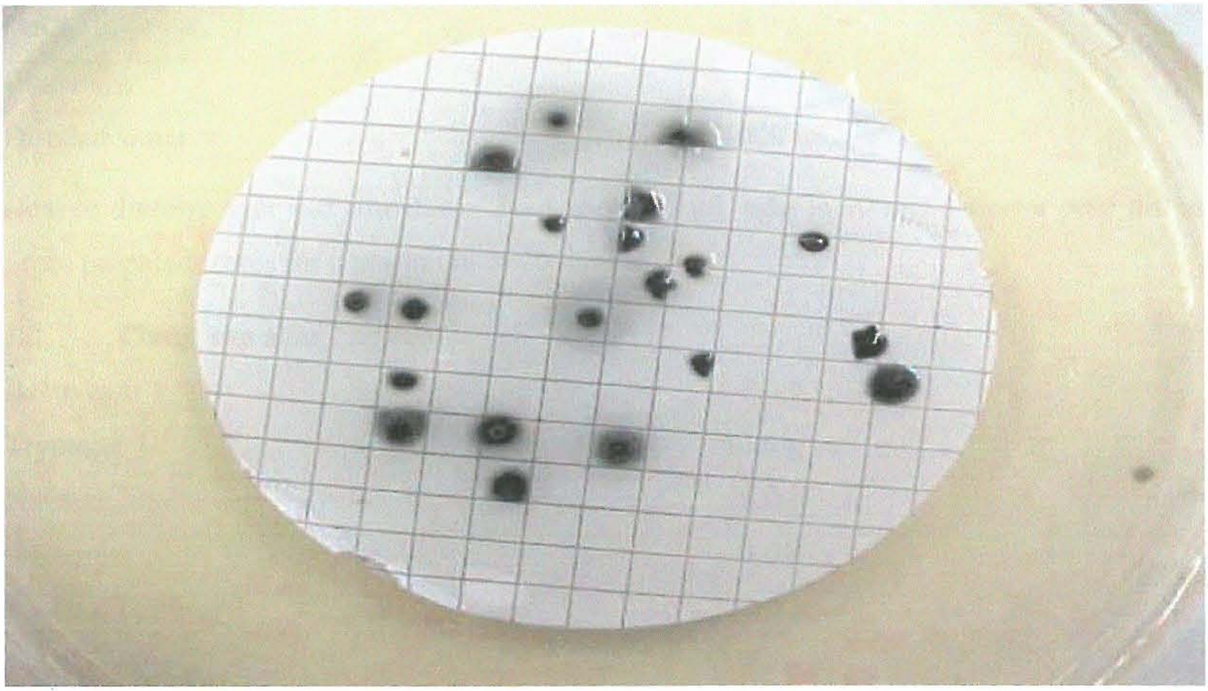

FIGURE 2: *C. perfringens* colony morphology.

PHAGE ANALYSIS

» Media, reagents and procedures

Somatic coliphages

Somatic coliphages were enumerated by means of the Plaque Assay method, in small volumes of water (generally 1.0 mL), with Double Agar Layer (SP-DL) on small petri dishes (90 mm diameter) (ISO, 1995; Grabow et al., 1993; Grabow, 2000).

1 Growth medium for the host culture (Nutrient broth)

Ordinary nutrient broth (Difco® or equivalent) was prepared according to the manufacturer's instructions. The mixture was heated to dissolve the powder, dispensed in convenient containers - i.e. 100 mL quantities in 200-mL medical flats, autoclaved, and stored at about 4°C for not longer than 30 days.

1.1 Phage bottom agar

Bacto agar	14.0 g
Tryptone	13.0 g
NaCl	8.0 g
Glucose	1.5 g
Distilled water	1000 mL

Heat to dissolve agar and autoclave. Pour about 20 mL agar in 90 mm diameter petri dishes. Store prepared plates for a maximum of 10 days at 4°C.

1.2 Phage top agar

Bacto agar	8.0 g
Tryptone	10.0 g
NaCl	8.0 g
Glucose	3.0 g
Na_2CO_3 solution	5.0 mL
$MgCl_2$ solution	1.0 mL
Distilled water	1000 mL

Autoclave and cool media to 55-60°C. Add naladixic acid solution if considered necessary (1.0 mL/100 mL). Distribute 2.5 mL aliquots into test tubes with caps. Store at 4°C for a maximum of 30 days.

1.3 Host culture

E. coli strain C (ATCC 70078)	=	WG4
Naladixic acid resistant mutant of WG4	=	WG5

1.4 Test sample

Water (e.g. wastewater treated effluents and surface river water). Make tenfold dilutions in peptone saline solution as necessary.

1.5 Naladixic acid solution

- Dissolve 0.5 g of naladixic acid in 4 mL of 1M NaOH.
- Add 16 mL of sterile water, mix well.
- Decontaminate by membrane filtration, e.g. syringe filter, 0.22-µm membranes.
- Store at 4°C for a maximum 4 weeks.

1.6 $MgCl_2$ solution

- Prepare 4 M stock solution by dissolving 820 g of $MgCl_2.6H_2O$ crystals in 1 L of water.
- Sterilise by autoclaving. Store at room temperature in the dark.

1.7 $CaCl_2$ solution

- Prepare 1 M stock solution by dissolving 147 g of $CaCl_2.2H_2O$ in 1 L water by gentle heating.
- Decontaminate by membrane filtration, e.g. syringe filter, 0.22-µm membranes.
- Store at 4°C for a maximum 6 months.

1.8 Peptone saline solution

- Dissolve 1 g peptone and 8.5 g sodium chloride in 950 mL water by boiling.
- Adjust pH to 7.0 ± 0.1 using 1 M NaOH or HCl.
- Make up to 1 L with distilled water. Dispense in convenient volumes.
- Autoclave.
- Store at 4°C for a maximum 6 months.

1.9 Test procedure

1. Steam the required number of test tubes with top agar to liquefy agar and adjust to 48°C in a heating block.
2. Add 0.5 mL of the host culture (grown overnight in stored volume of growth medium at 35-37°C) to the top agar.
3. Add 1 mL of the test sample, or an appropriate dilution of the test sample, to the top agar in each test tube.

4. Mix gently and pour the to[illegible]ith minimum delay onto the bottom agar layer in a 90 mm phage agar plate.

5. Repeat the above in tenfold to obtain counts per 10 mL. If tenfold dilutions are required, three plates should preferably be used for each dilution to obtain meaningful results.

6. Incubate inverted plates overnight at 35-37°C and count plaques of somatic coliphages.

1.10 Notes

1 In the case of heavily contaminated test samples (e.g. wastewater), interfering microbial growth may be suppressed by the addition of 1.0 mL of the naladixic acid solution to 100 mL of molten phage top agar. Final concentration of naladixic acid in phage top agar = 250 g/mL. The resistant mutant WG5 must be used as host in these assays.

2 A heating block should be used for tubes with top agar instead of a water bath, if possible, in order to avoid contamination by phages in water bath water.

Appendix D
ANALYTICAL QUALITY CONTROL

1 GENERAL QUALITY CONTROL PROCEDURES

A quality assurance programme was established to ensure accuracy of results obtained during the study. According to Standard Methods (1998), it is especially important that laboratories performing only a limited number of microbiological testing, exercise strict quality control procedures. The guidelines proposed for minimal quality control programmes, recommended by Standard Methods (1998), were followed during the study.

- For membrane filter tests, the sterility of the media, filters, dilution and rinse water, glassware and equipment was checked with sterile water as a sample during each sample series analyses.
- The medium used in the study was checked by testing for known positive and negative control cultures of the indicator organisms group.

1.1 Control cultures for the microbiological tests

1.1.1 *E. coli*

Stock cultures of *E. coli* (positive control – culture acquired from SABS), *Enterobacter aerogenes* and *Citrobacter freundii* (negative control – culture acquired from SABS) were made up (Standard Methods, 1998; Merck Corporation, 1996; bioMérieux, 1998).

1.1.2 *C. perfringens*

Stock cultures of *C. perfringens* (positive control – culture acquired from SABS) and *C. bifermentans* (negative control – culture acquired from SABS) were made up (Oxoid Corporation, 1990; bioMérieux, 1994).

1.2 Procedures for medium check

Volume units of 1 mL of the positive and negative stock culture solutions were filtered through membranes. The membranes were placed on petri dishes containing the various selective growth media. Stock culture analyses were done at least once a month for the duration of the project to check a specific medium.

The specific colony colour identification and distinction was standardised by the analyst group (making sure everyone see and understand the same colour – including the various nuances / shades) and used to identify the various indicator organisms tested for on the various media.

2 METHOD PRECISION

Precision was calculated in duplicate for the different water types. The test laboratory is also continually involved in surface and drinking water quality testing and has a set precision criterion based on $3.27R$ (Standard Methods, 1998). This criterion was updated every 3 months, using the 15 most recent sets of duplicate results.

Duplicate testing of at least 10% of all samples is a monthly routine and includes duplication for each analyst involved. Results are log transformed and if either of a set of duplicate results was < 1, both values were added with 1 before calculating the logarithms. The range (R) for each pair of transformed duplicates was calculated as the mean (R) of these ranges.

Results from a series that showed excessive variability were not accepted. The analytical problem were identified and resolved.

3 COLONY VERIFICATION

The actual selectivity / specificity of the various selective growth media have been found to be inconsistent. One of the most common reasons is the vast array of species and sub-species often to be found in a single indicator organisms group or species as well as in the multitude of non-indicator organisms groups. Amongst these variants one will inevitably find non-indicator organisms that find the selectivity of a specific medium accommodating and may even manifest in the colours prescribed to the analyst for identification.

To establish the reliability of detected indicator numbers, as well as the selectivity of the various media for detecting the selected indicators, a verification programme was followed according to Standard Methods (1998).

Standard Methods (1998) recommends that at least 10 colonies per month be picked randomly from the membranes of known positive samples and verified. Only colonies counted as indicators on the various selective growth media were selected.

Selections were made from the plates where the particular dilution yielded growth of between 20 and 80 colonies. Between 12% and 40% of all the colonies cultured on the various selective media were randomly selected. Before verification began with multi-test identification system galleries such as the API® and RAPID ID® by bioMérieux, the coloured selected colonies were first stripped of the colouration caused by the selective substrates of the growth medium (discussed in 4.1 and 5.1 below). The colour stripping was done to eliminate all possible interference with the functions of the Identification System Galleries.

The various identification systems (confirmation galleries) consist of strips with a characteristic number of micro-t ehydrated substrates. These substrates support specific enzymatic activity or fermentation of sugars. Each micro-tube is inoculated with a dense bacterial suspension made up of the original selected colony, which at the same time reconstitutes the substrates. Metabolic end products are produced during incubation, which produces spontaneous colour changes or revealed colours afterwards by the addition of reagents. The various reactions are then coded and read into a Reading Table. The identification is obtained from an Identification Table or a computerised Analytical Profile Index.

4 CONFIRMATION PROCEDURE *E. coli*

4.1 Chromocult Coliform® Agar:

Deep blue-to-violet colonies (*E. coli* on Chromocult Coliform® Agar) that the analyst would count as the coloured *E. coli* colonies on a given specific media were selected. The colony morphology was carefully noted and included colour, size, shape, composition, and edge appearance. A note was also made of the number of colonies counted from every particular plate (membrane) as well as the number taken for verification on the API® 20E identification system

Membrane-grown colonies to detect *E. coli* on the Chromocult Coliform® medium were only partially picked up. The remaining colony was used for intermediate *E. coli* verification with KOVACS' indole reagent according to the user manual (Merck Corporation, 1996). This was necessary because it was feared that the indole reaction might influence further refinement of the selected colony. The partially recovered material generally proved enough to produce strong single colonies during the next round of streaking-out.

In order to confirm *E. coli* detection directly on the membranes, the dark blue to violet coloured colonies were coated with a drop of KOVACS' indole reagent. A cherry-red colouring after some seconds confirmed a positive indole formation and consequently the presence of *E. coli*. This method proved to be very useful for positive identification of *E. coli* when plates inoculated with heavily polluted waters were used. However, KOVACS' indole reagent on plates containing weaker colonies of *E. coli* actually lessened the efficiency of the reagent because a drop of the reagent tended to colour the whole membrane cherry-red, making the positive identification of especially small *E. coli* colonies very difficult.

To obtain pure and strong single *E. coli* colonies, the colonies were picked up from the membranes with inoculum needles and streaked out on the same selective medium and incubated at the prescribed temperature. Single colonies on the selective media were then

5 CONFIRMATION OF

5.1 Perfringens (OPSP) Agar

Colonies exhibiting dark brown to black colour (*C. perfringens* on Perfringens (OPSP) from Oxoid Agar) were selected. The colony morphology was carefully noted and included colour, size, shape, composition, and edge appearance. These would be colonies that the analyst will count as the various coloured *C. perfringens* colonies on the various growth media.

A note was also made of the number of colonies counted from every particular plate as well as the numbers taken for verification by the Rapid ID® 32A-identification system.

The colonies were purified as described in section 3 above. After this process, the colonies were emulsified according to prescription and the emulsion flooded onto Columbia sheep blood agar (Oxoid®) plates. The plates were incubated anaerobically at 37°C for 24-48 hours.

5.2 Rapid ID® 32A Multi-test galleries (bioMérieux, 1994)

Rapid ID® 32A is a standardised identification system combining 29 biochemical tests that offers a multitude of capabilities for identifying anaerobes.

5.3 Preparation of the inoculum

Homogenous bacterial suspensions of the harvested colonies from the blood plates were made according to the prescriptions contained in the manual provided with the commercial identification kit (bioMérieux, 1994).

5.4 Inoculation of the strips

The micro-tubes on the prepared strips were filled according to prescription and incubated for anaerobically for 4 hours at 37°C.

5.5 Reading the strips

After the incubation time, the spontaneous colour reactions from each strip were recorded. Reagents were added to the prescribed tubes and the colour reaction recorded. All these recording were done on the result sheets provided with the kit.

5.6 Identification

The pattern of each of the reactions was hand-coded, on the result sheets into, a complex numerical profile. These numerical profiles then read into the ANALYTICAL PROFILE INDEX as a number. The Index provides the name of the species matching the code.

5.7 Quality control (QC)

Several QC tests were done on the various batches of strips acquired. The stock cultures, *C. histolyticum*, were obtained from local medical commercial pathological laboratories.

Appendix E
STATISTICAL APPROACHES

1. CHARACTERISTICS OF WATER RESOURCE DATA

Microbiological water resources data generally have substantial variations, which causes the data not to be normally distributed around the mean for the particular data set (Standard Methods, 1998). According to Helsel and Hirsch (1995), data analysed by water resource scientists often have the following characteristics:

1) A lower bound of zero – no negative values are possible.

2) Presence of "outliers". These are observations considerably higher or lower than most of the data. This occurs infrequently but regularly. Outliers on the high side are more common in water resource data.

3) Positive skewness, due to items 1 and 2. Skewness can be expected when outlying values occur only in one direction. For positive skewness, the mean exceeds more than 50 percent of the data.

4) Non-normal distribution of data, due to items 1 – 3 above. While many statistical tests assume that data follow a normal distribution, water resource data often do not.

5) Data reported only below or above some threshold (censored data).

6) Seasonal patterns. Values tend to be high, or lower in certain seasons of the year.

7) Autocorrelation. Consecutive observations under similar circumstances tend to be strongly correlated with each other. An example of the most common kind of autocorrelation in water resources is that high numbers of microbiological indicator organisms will tend to follow high numbers of microbiological indicator organisms in circumstances such as intermittent high volumes of intensive rainfall.

8) Dependence on other uncontrolled variables.

2. CENTRAL TENDENCY (MEASURE OF LOCATION)

Most used measures of location are usually the mean or median. Central tendencies in this study are expressed as follows:

2.1 Classical measure – the mean

The mean is computed as the sum of all the data values (X_i), divided by the sample size (n). Arithmetic mean values of the colony counts on each membrane, per sample, were calculated because of the predominantly syn ons of the colonies per triplicate set (the formula in Appendix A). The mean is however influenced by outlying data and could therefore not be used as a measure of central value for the data obtained at the sampling sites.

2.2 The geometric mean

The geometric mean is the mean of the logarithms, transformed back to the original unit. This format of central location was used in most of the discussion areas. The geometric mean is the preferred best estimate of central tendencies of the transformed microbiological data (Standard Methods, 1998). Transformations involved power functions of the form $y = x^{\theta}$, where x is the untransformed data, y the transformed data, and $^{\theta}$the power exponent 10 as used in this study.

2.3 The median

The median is a measure of the central value of the distribution when the data are ranked in order of magnitude. The median is the 50th percentile ($P_{.50}$) of the ranked data set. The median is only minimally affected by the magnitude of a single observation such as an outlier (Helsel and Hirsch, 1995). The median will therefore be resistant to the effect of outliers, which had been kept in the data sets throughout this study. For positively skewed data, the geometric mean was usually quite close to the median.

3. MEASURE OF SPREAD

Summary statistics that measure the amount of variation in a data set are called measures of spread. Variability is quantified by measures of spread.

3.1 The 95% confidence interval (CI)

The 95% confidence interval is used when the analyst wants to make certain predictions about the data. The 95% CI is an estimate of the range of values within which the sample parameter could, with some confidence, be expected to lie (Helsel and Hirsch, 1995).

4. OUTLIERS

Outliers are observations whose values are quite different from other values in the data set (Helsel and Hirsch, 1995). While it is often found that analysts would discard outliers, this procedure was not followed in this study. Outliers, usually characterised as the high indicator organism counts, were associated with specific events such as rain events and were kept in the data sets for further investigation.

Outliers generally have one of three causes:

- A measurement or recording error.

- An observation from a popu ɔ most of the data.
- A rare event from a single population that is quite skewed.

When outliers occurred during the study, the following where investigated and actions taken:

- Recording errors such as erroneous entering into calculation programmes.
- Copying, decimal points or other obvious errors.
- Comparing the outlying tendency with the other indicators enumerated from the same sample to see if a similar event occurred.

Where no errors were detected, the outliers where kept in the sets as they presented real events in the sampling and analysis routines such as higher pollution in the particular water type at the particular time of the season. (Outliers were usually associated with the higher microbiological indicator organism numbers found in water samples after rain events.)

5. HYPOTHESIS FORMULATION

Scientists often have prior ideas of how the systems they investigate might behave. These are called hypotheses (Helsel and Hirsch, 1995). Statistical tests are quantitative methods to determine whether hypothesis can be substantiated or whether they must be modified or rejected outright.

Hypotheses for the various sections contained in the Chapter 3: Results and Discussion were formulated within each section according to the objectives for the section. The significance level (∝- value) is the probability of incorrectly rejecting the null hypothesis. The significance value for this study is set at default 5%. The 0.05 for the significant value is a statistical tradition (Helsel and Hirsch, 1995) that was also followed for this study.

The null hypothesis (H_0)

The H_0 is what is assumed to be true about the system under study prior to data collection, until indicated otherwise (Helsel and Hirsch, 1995).

The alternative hypothesis (H_1)

The (alternative) hypothesis is the situation anticipated to be true if the data showed that the null hypothesis is unlikely (Helsel and Hirsch, 1995).

6. MINIMUM SAMPLE SIZE

The data about which a statement or summary is to be made, are called the population or sometimes the target population (Helsel and Hirsch, 1995). It is known that it may be physically or financially impossible to collect all the data of concern and therefore a subset of the data, called the sample, is extended to the entire population.

The minimum sample sizes for st[illegible] differences were determined before each series of experiments commenced at the various levels and approaches of this study.

One should determine approximately how big the sample size has to be – crude or not – in order to detect an impact or statistical difference at a specified level. The larger the sample size, the greater the power of the relevant test applied (SigmaStat® 2, 1997; Helsel and Hirsch, 1995). The statistical programme SigmaStat® 2 (1997) was used to calculate the sample size needed for statistical significance.

6.1 Sample size and ANNOVA testing

For this study, crude initial estimates of 15 samples for each microorganisms group used for each water category were made based on the minimum number of samples prescribed by Standard Methods (1998) for an intra-laboratory proficiency programme.

After assessing the 1st 15 samples, the mean differences of each (n = 15) data set was used to estimate the final minimum sample size and to confirm whether the initial sample sizes were big enough.

ANOVA testing procedures (parametric or non-parametric) depend on whether the comparative data is normally distributed with equal variance. However, to determine the minimum sample size, the normality of the data is generally ignored and the sample size determined (Helsel and Hirsch, 1995; SigmaStat® 2, 1997).

The sample size for an intended experiment is determined by the power, alpha, the size of the difference, and the population variability.

1) The size of the minimum expected differences in the group means is entered. Based on typical null-hypothesis or data reliability theory, no differences should be encountered between the means of the data groups. However, standard statistical packages used to calculate the estimated sample sizes do not accept a zero entry as this is seen as statistically unrealistic (SigmaStat® 2, 1997). Literature is also not very clear on how to approach the selection of minimum expected differences in the group means. The size of the minimum differences in the group means for the data was, therefore, calculated for each comparison group individually based on the mean differences encountered after using data from the initial 15 samples.

2) The size of the standard deviation of the data is entered. The size of the standard deviation could be the size expected (an estimate) or can be derived from previous experiments. Again, literature was unclear about what could be expected. The initial data from this study was used to calculate an overall mean standard deviation for each

microorganisms group used . These were entered as the "expected" standard deviation and the calculated sample size suggested by the programme were then used as a minimum sample size.

3) According to Helsel and Hirsch (1995), desired power (sensitivities) in water resources testing is traditionally set to achieve a power of 0.08, which means that there is an 80% chance of detecting a difference / an association / a central value estimate with 1-$\propto$ confidence (i.e. 95% confidence when $\propto = 0.05$). The closer the power is to 1, the more sensitive the test.

4) The desired alpha ($\propto$) level is the acceptable probability of incorrectly concluding that there is an association. This indicates that a 1 in 20 chance of being wrong is acceptable (willing to conclude that there is a difference / an association / a central value estimate when $\propto \leq 0.05$).

7. NORMALITY OF DATA

Application of statistical techniques in the field of water resource management generally requires the assumption that data sets have symmetrical distributions such as the normal curve. Serious problems can occur when statistical procedures are employed assuming symmetry or linearity (Helsel and Hirsch, 1995). In most water quality related chemical analyses, the distribution of analytical results follows the Gaussian (normal) curve, which has symmetrical distribution of values about the mean. However, microbiological distributions are often not symmetrical.

Organism counts often have a skewed distribution because of more low/or high counts than most of the counts in a given monitoring set (Standard Methods, 1998). Incorrect statements about the data could then be made when the analyst uses statistical test procedures that assume symmetry or linearity for the microbiological data (Helsel and Hirsch, 1995).

8. ANALYSIS OF VARIANCE (ANOVA)

Equal variance test results display whether or not the data passed or failed the test of the assumption that the samples were drawn from populations with the same variance (SigmaStat® 2, 1997). The classic technique for this comparison of data is analyses of variance (ANOVA) (Helsel and Hirsch, 1995). ANOVA includes a series of parametric tests done under the assumption that the data concerned are normally distributed around the mean with similar variance. For this study, the instances where data did not pass normality, was by far in the majority. Non-parametric testing for variance was employed throughout the study.

8.1 Non-parametric tests

Where parametric test methods loose considerable power to detect differences in non-normal data, non-parametric testing display considerable power in non-normal as well as normal data testing and display (Helsel and Hirsch, 1995). The following non-parametric **ANOVA** tests were used:

- **Rank-sum tests** (Helsel and Hirsch, 1995). A rank-sum test is a non-parametric test for whether data in one group tends to differ from data in another group by being larger, smaller or larger and / or smaller. To test the hypotheses, the non-parametric **Mann-Whitney Rank-sum test** was used. The test was selected because:
  - ≠ No assumptions about the normality or variance (shape) of the data are needed.
  - ≠ It can determine whether data from each of the two groups come from the same population.

- **Kruskal-Wallis** ANOVA on Ranks (based on rank transformation) (Helsel and Hirsch, 1995). This test was selected when three or more groups were compared (Helsel and Hirsch, 1995; Glantz, 1997; SigmaStat®, 1997). It compares results from several different experimental groups that may be affected by a single factor. Rank transformation of data implies that the original data are replaced by ranks, which omits substantial variance and error from the multiple comparison procedure (Helsel and Hirsch, 1995).
  - ≠ The *P value* is the probability of being wrong in concluding that there is a true difference in the groups. This implies falsely rejecting the zero hypotheses. The smaller the *P value* ($P < 0.05$), the greater the probability that the results from the samples in the selected data sets are significantly different.

- Multiple comparison tests (MCT's). When more than two sample points are compared, the interest is not only whether the indicator organism numbers determined at each point differed, but also which differed from the others. Therefore, multiple comparison tests (MCT's) were applied where significant differences where encountered. MCT's compare all possible pairs of group medians involved in the comparison. However, MCT's are only applied after the H_0 had been rejected (Helsel and Hirsch, 1995). The following MCT's were used:
  - ≠ The Dunn multiple comparison test uses the following parameters:
    - o *P* value indicates the difference in the ranks of the group means (median

values) being compared. Group means are ranked in order from the highest to the lowest and P is the number of means spanned in the comparisons.

- o Q test statistic indicates the number of means spanned in the comparison p. The larger the values of Q, the more acceptable the conclusion that the difference of 2 groups being compared is statistically significant.
- o If the P value is greater than 0.05, it cannot be confidently concluded that there is a statistically significant difference between the means of the two groups compared.

9. DATA PRESENTATION

Graphs provide visual summaries of the data and describe essential information more quickly and completely than do tables. Graphs are essential for two purposes (Helsel and Hirsch, 1995):

- To provide insight for the analyst into the data under scrutiny.
- To illustrate important concepts when presenting the results.

Numbers of indicator organisms detected (log Y-axis) at the various sampling points (X-axis) were illustrated on the following graph types:

9.1 Boxplots for summarising data

Boxplots are used because of their design ability to graph representing certain statistical values (SigmaPlot® 6, 2000). According to Helsel and Hirsch (1995), boxplots provide the clearest visual summaries of the following:

- The centre of the data – [the geometric mean, the yellow line, is the preferred measure of central tendency for the data in the various Results Sections (Chapter 2). The median, black centre line in the box, is also resistant to the effects of the outliers, and would therefore tend to indicate the more realistic central point in the data.]
- The variation or spread (interquartile range (IQR) – the box height indicates the spread of data between the 25th to the 75th percentile). The closer the data are clustered to the median within the IQR, the less variation (more stable) the data have.
- The skewness. This is also referred to as the quartile skew and is represented by the relative size of the box halves. The smaller the upper quartile skews, the more positive the data are skewed – a characteristic of water resources data (Helsel and Hirsch, 1995).
- The presence (or absence) of unusual values. The whiskers on the lines protruding

above and below the box indicate the 90^{th} and the 10^{th} percentiles. The dot symbols beyond the last percentiles indicate outliers beyond the 90^{th} and 10^{th} percentiles.

9.2 Spatial appraisals

The spatial appraisals were of the differences in the microbiological water quality of the Renoster Spruit during the first and second phase of the study period.

- The centre of the data – [the geometric mean, the yellow line, is the preferred measure of central tendency for the data in the various Results Sections (Chapter 2).
- The presence (or absence) of unusual values. The whiskers on the lines protruding above and below the circle indicate the 95^{th} and the 5^{th} percentiles.