Cape Peninsula University of Technology

MICROBIAL POLLUTANTS IN STAGNANT WATER IN RR SECTION, KHAYELITSHA, WESTERN CAPE, SOUTH AFRICA

by

QENEHELO ALICE LEUTA

Dissertation submitted in partial fulfilment of the requirements for the degree

Master of Technology: Environmental Management

in the Faculty of Applied Science

at the Cape Peninsula University of Technology

Supervisor: Dr. A.N. Paulse
Co-supervisor: Prof. J.P. Odendaal

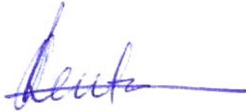
Cape Town

April 2015

CPUT copyright information
The dissertation may not be published either in part (in scholarly, scientific or technical journals), or as a whole (as a monograph), unless permission has been obtained from the University

DECLARATION

I, Qenehelo Alice Leuta, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



Signed **Date**

ABSTRACT

Greywater is domestic wastewater from daily kitchen, laundry, bath, shower, hand washing practices and does not include wastewater from the toilet. Greywater from informal settlement has been identified as important environmental pollution sources. Inadequate sanitation and poor drainage in informal settlements result in greywater being stagnant at the base of communal taps. This water has a potential to cause health problems to those who come in contact with it. Studies of greywater quality in informal settlements in South Africa tend to concentrate on physico-chemical analysis and microbial indicator organisms. In order to adequately manage greywater in informal settlements there is a need to understand the microbial pathogens present in such water. Therefore this study is aimed at determining the level of microbial contamination of stagnant greywater in the RR Section of Khayelitsha, Western Cape. Six sampling sites were identified and sampling of stagnant greywater was conducted twice a month (from January to May 2013) from the base of six communal taps, which served as the sampling sites.

The microbial enumeration techniques employed in this study were the Most Probable Number (MPN) techniques, the Heterotrophic Plate Count (HPC) technique and the Flow Cytometric (FCM) technique. The API 20E and the RapID™ ONE systems were used to identify possible pathogenic Gram-negative microorganisms, while possible pathogenic Gram-positive microorganisms were identified with the BBL Crystal™ Gram Positive (GP) Identification (ID) system. The highest MPN counts were 1.6 x 10^8 microorganisms/100mℓ recorded at Site A (weeks 3 and 5) as well as at Site B (week 5). The corresponding highest faecal coliform count was 4.7 x 10^6 microorganisms/100mℓ obtained at Site B (week 5). The highest *E. coli* count observed was 1.8 x 10^6 microorganisms/100mℓ recorded at Site A (week 5) and Site F (week 5). In comparison, the highest HPC count was 2.9 x10^5 microorganisms/mℓ recorded at Site C in week 4. The results obtained by the MPN and HPC techniques were significantly ($p < 0.05$) higher than the water quality standards by Department of Water Affairs and Forestry (DWAF) (1996a; 1996b) and the SABS (2011). The highest total FCM and viable FCM counts were 3.4 x 10^7 microorganisms/mℓ and 3.1 x 10^7 microorganisms/mℓ, respectively recorded at Site A in week 5. The FCM technique displayed significantly ($p < 0.05$) higher results than both the MPN and HPC techniques, which highlighted its reliability in obtaining more accurate enumeration results.

The RapID™ ONE and the API 20E identification systems mostly identified *Escherichia coli, Klebsiella pneumonia*, *K. oxytoca*, *Acinetobacter baumannii/calcoaceticus and Enterobacter cloacae*, while the organisms more commonly identified by the BBL Crystal™ Gram Positive (GP) Identification (ID) system, were the *Corynebacterium* species,

and *Bacillus cereus*. The presence of these organisms raises health concern to the community of RR Section, as some are known to cause waterborne diseases, while others are known to cause nosocomial infections.

ACKNOWLEDEMENTS

I wish to thank:

- First and foremost my mother, **Makhotso Cecilia Leuta,** for instilling in me the value of education. You gave up everything you had in order for me to acquire my education. I hope to pass everything you have taught me on to my son.
- **Dr. Arnelia Paulse**, my supervisor, to whom I owe my deepest gratitude for the hard work, dedication, guidance and support.
- **Prof. James Odendaal**, my co-supervisor for all the assistance, guidance and provision of laboratory space to conduct my research.
- **Håvard Ovesen,** my husband, and **Odin Thabo Leuta Ovesen**, my son, thank you for being there for me. My husband, for encouraging me to go on even during those moments I wanted to quit. My son, for sacrificing those moments he wanted to be with me in order for me to do my work. Thabo, your inquisitive mind and interest in my work always humbled me and made me creative when explaining what my work is all about.
- **Dustin Kramer**, for introducing me to Social Justice Coalition.
- The **Social Justice Coalition**, for taking the time to assist and accompany me every time I went sampling. Special thanks to **Luthando Tokota** of Social Justice Coalition who always accommodated my sampling needs within his busy schedule. He is one of the most reliable people I know, and his dedication to his work serves as a good example to all of us. Thank you for making me understand the complexities around water and sanitation issues in RR Section.
- **Thando Ndlovu,** for the assistance provided whenever I need one.
- My mother and father in law, **Gerd Karin and Egil Øivind Ovesen**, thank you for your love and constant support.
- **Vincent Zungu**, thank you very much for the time you took to assist me to develop my maps and also for the constant support in my research.
- **All the postgraduate** students at Cape Peninsula University of Technology – Cape Town campus, who became my friends. They provided emotional support and served as unofficial psychologists throughout this tough journey.
- **Cape Peninsula University of Technology** and the financial assistance of the **National Research Foundation** towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

DEDICATION

This dissertation is dedicated to Håvard Ovesen and Odin Thabo Leuta Ovesen, without whom I am nothing.

TABLE OF CONTENTS

DECLARATION……………………………………………………………………….…...ii
ABSTRACT…………………………..……………………………………………...……iii
ACKNOWLEDGEMENTS……………………..………………………………………v
DEDICATION……………………………..…………………………………….……...vi
TABLE OF CONTENTS……………………..…………………………………..……vii
LIST OF TABLES ………………………………...…………………...………………....x
LIST OF FIGURES……………………………………………………...…………..……..xi
APPENDICES……………………………………………………..……………...………xiii
GLOSSARY…………………………………………………………...…………………..…xiv
REFERENCE MAP…………………………………………...………………………...…xv

CHAPTER ONE: LITERATURE REVIEW 1

1.1 INTRODUCTION 1
1.2 BACKGROUND TO THE STUDY AREA 2
1.3 INFORMAL SETTLEMENTS AND THE PROVISION OF WATER AND SANITATION IN SOUTH AFRICA 5
1.4 THE SITUATION OF GREYWATER IN INFORMAL SETTLEMENTS OF SOUTH AFRICA 7
1.4.1 Greywater 7
1.4.2 Characteristics of greywater 8
1.4.3 Microbial quality and health risks of greywater 8
1.4.4 City of Cape Town greywater guidelines 9
1.5 WATER QUALITY 10
1.6 WATERBORNE PATHOGENS 11
1.6.1 Bacteria 13
1.6.1.1 *Salmonella* 13
1.6.1.2 *Helicobacter pylori* 13
1.6.1.3 *Vibrio cholerae* 14
1.6.1.4 *Legionella* 14
1.6.1.5 *Pseudomonas aeruginosa* 14
1.6.1.6 *Campylobacter* 15
1.6.1.7 *Yersinia* 15

1.6.1.8 Indicator organisms 15
1.6.1.8.1 Total coliform bacteria 16
1.6.1.8.2 Faecal coliform bacteria 17
1.6.1.8.3 *Escherichia coli* 17
1.6.1.8.4 Heterotrophic bacteria 18
1.6.1.9 *Clostridium* spp 19
1.6.1.10 *Staphylococcus* spp 19
1.6.1.11 *Bacillus* spp 19
1.6.1.12 *Listeria monocytogenes* 20
1.6.1.13 The Viable-but-non-culturable (VBNC) state of bacteria 20
1.7 DETECTION, ISOLATION, ENUMERATION AND IDENTIFICATION OF WATERBORNE ORGANISMS 21
1.7.1 Heterotrophic Plate Count 21
1.7.2 Most Probable Number (MPN) technique 21
1.7.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe 22
1.7.4 API 20E system 23
1.7.5 RapID™ ONE system 23
1.7.6 BBL Crystal™ Gram-positive Identification system 24
1.8 OBJECTIVES OF RESEARCH 25

CHAPTER TWO: MATERIALS AND METHODS 26

2.1 SAMPLING SITES 26
2.2 SAMPLING 26
2.3 ENUMERATION TECHNIQUES 26
2.3.1 Heterotrophic Plate Count 26
2.3.2 Most Probable Number (MPN) technique 28
2.3.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe 32
2.3.4 Statistical analysis 33
2.4 IDENTIFICATION TECHNIQUES 33
2.4.1 API 20E system 33
2.4.2 RapID™ ONE system 34
2.4.3 BBL Crystal™ Gram-positive Identification system 34

CHAPTER THREE: RESULTS AND DISCUSSION 35

3.1 PHYSICAL PARAMETERS 35
3.2 ENUMERATION OF BACTERIAL CONTAMINANTS IN STAGNANT WATER POOLS 36
3.2.1 Most Probable Number (MPN) technique 36
3.2.2 Heterotrophic Plate Count 38
3.2.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe 42
3.3 IDENTIFICATION OF GRAM-NEGATIVE BACTERIA 51
3.3.1 Identification of isolates by means of the API 20E and RapID™ ONE identification systems 55
3.3.1.1 Health concerns linked to isolated microorganisms 59
3.3.1.2 Comparison of the API 20E and RapID™ ONE systems 64
3.4 IDENTIFICATION OF GRAM-POSITIVE BACTERIA 65
3.4.1 Health concerns linked to isolated microorganisms 68

CHAPTER FOUR: GENERAL CONCLUSIONS AND RECOMMENDATIONS 72

4.1 ENUMERATION OF BACTERIAL CONTAMINANTS IN STAGNANT WATER POOLS 72
4.2 IDENTIFICATION OF GRAM-NEGATIVE BACTERIA 74
4.3 IDENTIFICATION OF GRAM-POSITIVE BACTERIA 75
4.4 THE IMPLICATIONS OF POOR SANITATION IN RR SECTION 76
4.5 RECOMMENDATIONS 78

CHAPTER FIVE: REFERENCES 79

CHAPTER SIX: APPENDICES 106

LIST OF TABLES

Table 1.1	Results of microbial water analysis conducted by Barnes 2010 (adapted from Barnes, 2010)	5
Table 1.2	Results of a preliminary study used to substantiate Barnes' claims on microbial content of water in RR Section	5
Table 1.3	Water Quality Guidelines of microbiological indicators (DWAF, 1996a, DWAF 1996b; SABS, 2011)	10
Table 1.4	Properties of Waterborne pathogens (WHO, 2011b)	12
Table 1.5	Criteria used to determine an indicator organism (WHO, 2011b)	16
Table 3.1	Physical parameters recorded during the sampling period at all sites	35
Table 3.2	The average percentage ratio of the HPC count to the MPN count for all samples analysed over the sampling period	52
Table 3.3	The average percentage ratio of MPN counts to total FCM counts based on flow cytometric analysis for all samples analysed over the sampling period	52
Table 3.4	The average percentage ratio of HPC counts to total FCM counts based on flow cytometric analysis for all samples analysed over the sampling period	53
Table 3.5	The average percentage ratio of HPC counts to viable FCM counts based on flow cytometric analysis for all samples analysed over the sampling period	53
Table 3.6	The average percentage ratio of viable FCM count to total FCM based on FCM analysis for all samples analysed over the sampling period	54
Table 3.7	Gram-negative bacteria isolated form the six sampling sites	57
Table 3.8	Gram-positive bacteria isolated form the six sampling sites	67

LIST OF FIGURES

Figure 1.1	Locational Map of RR adapted from Community Organisation Resource Centre (CORC, 2013)	3
Figure 1.2	View of RR Section, Khayelitsha, Cape Town	3
Figure 2.1	A map of RR Section showing all the six sampling sites adapted from CORC (2013)	27
Figure 2.2	Stagnant water accumulating at bottom of a communal tap – Site E sampling point	28
Figure 2.3a	Inoculation of undiluted and diluted water samples into double and single strength LTB tubes to obtain positive presumptive test results	29
Figure 2.3b	Re-inoculation of positive LTB tubes into corresponding BGBB and TW tubes for further analysis of feacal coliforms and *E. coli*	30
Figure 2.3c	Enumeration of faecal coliforms (from BGBB tubes) and *E. coli* (from TW tubes) in water samples	31
Figure 3.1	Comparison of the Most Probable Number (MPN), demonstrating all possible gas-producing organisms, faecal coliforms (FC), and *E. coli* per 100 mℓ of stagnant water pools sample for all sites	37
Figure 3.2	Average heterotrophic plate counts for Site A to Site C analysed and recorded over the sampling period	39
Figure 3.3	Average heterotrophic plate counts for Site D – Site F analysed and recorded over the sampling period	41
Figure 3.4	Comparison of heterotrophic plate counts (HPC) and the most probable number (MPN), representing all possible gas-producing organisms	43
Figure 3.5	Total cell counts from the stagnant water pool samples using flow cytometric analysis: the upper right and lower right quadrats indicate dead and live cells	44
Figure 3.6	FCM results of total, viable and dead bacterial cells within stagnant water samples at RR Section	45
Figure 3.7	Comparison of MPN (representing all possible gas-producing organisms) results to total FCM results	46
Figure 3.8	Comparison of heterotrophic plate counts (HPC) and total FCM and viable FCM results	48
Figure 3.9	Different colonies cultured on EMB agar from water samples collected from RR Section	55

Figure 3.10	Different colonies cultured on MAC-WS agar from water samples collected from RR Section	56
Figure 3.11	Different colonies cultured on MAC agar from water samples collected from RR Section	56
Figure 3.12	Different colonies cultured on SD agar from water samples collected from RR Section	66
Figure 3.13	Different colonies cultured on BP agar from water samples collected from RR Section	66

APPENDICES

Appendix A	Organisms identified from RR Section greywater samples using the API 20E System	106
Appendix B	Organisms identified from RR Section greywater samples using the RapID™ ONE System	108
Appendix C	Organisms identified from RR Section greywater samples using the BBL Crystal System	110

GLOSSARY

Abbreviation	Definition
AIDS	Acquired immune deficiency syndrome
BD	Becton Dickinson
BGBB	Brilliant Green Bile Broth
BP	Baird Parker
CORC	Community Organisation Resource Centre
DAEC	Diffuse adhering *Escherichia coli*
DWAF	Department of Water Affairs and Forestry
EAEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin Methylene Blue
EPA	Environmental Protection Agency
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FC	Faecal coliforms
FCM	Flow cytometry
FDA	Food and Drug Administration
FSC	Forward scatter
GP	Gram Positive
HDA	Housing Development Agency
HPC	Heterotrophic Plate Count
ID	Identification
LTB	Lauryl Tryptose Broth
MAC	MacConkey
MAC-WS	MacConkey Without Salt
MDR	Multiple drug resistant
MPN	Most Probable Number
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
PI	Propidium Iodine
RMA	Repeated measures ANOVA
SABS	South African Bureau of Standards
SD	Sabouraud Dextrose

SJC	Social Justice Coalition
SSC	Side scatter
TO	Thiazole Orange
TW	Tryptone Water
UN	United Nations
UNDP	United Nations Population Division
UNESCO	United Nation Educational Scientific and Cultural Organization
UNICEF	United Nations Children's Fund
VBNC	Viable-but-non-culturable
WASH	Water, Sanitation and Hygiene
WHO	World Health Organisation

REFERENCE MAP

Map of RR Section in Khayelitsha……………………………………………………………29

Sites A to F: communal standpipes, at more or less evenly distributed sites, each allocated to approximately five families for daily use.

LITERATURE REVIEW

1.1 INTRODUCTION

Access to an adequate freshwater supply for daily use, is a basic human right and requirement for individuals to have a healthy life (Global Water, Sanitation and Hygiene [WASH] Cluster, 2009; United Nations [UN] Special Rapporteur, 2014). In addition, access to improved sanitation is also a fundamental human need as well as a basic human right (Global WASH Cluster, 2009; UN Special Rapporteur, 2014). Approximately 2.5 billion people in the world are without access to basic sanitation, while 748 million people have no access to improved drinking water (WHO [World Health Organisation] & UNICEF [United Nations Children's Fund], 2014). Inadequate provision of water and sanitation affect the livelihoods of people in many ways, especially people in poorer communities who tend to suffer the most (WHO & UNICEF, 2014).

According to Tsinda *et al.* (2013), it is challenging to provide basic sanitation and water to informal settlements due to the high population density in such areas. For this reason, municipalities have installed communal toilets and standpipes, which each serve approximately five or more families (United Nations Development Programme [UNDP], 2006). These communal facilities often have problems such as clogging, resulting from poor maintenance and poor hygienic practices (UNDP, 2006). Informal settlements commonly lack effective drainage systems or facilities, which result in stagnant water pools accumulating at the base of communal taps (Granfone *et al*., 2008). These stagnant water pools tend to mix with raw sewage coming from broken and unmaintained toilets as well as from the washing of night pots [Granfone *et al*., 2008; Social Justice Coalition (SJC), 2011]. The result is a potentially dangerous mixture as the stagnant water pools is a breeding ground for potentially pathogenic microorganisms (Moe & Rheingans, 2006).

The supply of drinking water alone cannot reduce the risk of water related diseases (Esrey, 1996). When it comes to the provision of safe public health, access to improved sanitation is just as important as access to improved drinking water (Fry *et al*., 2008). Many waterborne diseases such as cholera, dysentery and typhoid are a result of a lack of safe drinking water and adequate sanitation (Jabeen *et al*., 2011). Approximately 3.4 million people worldwide die from infections caused by waterborne pathogens each year (Prüss-Üstün *et al*. 2008). Therefore, a combination of adequate water supply and sanitation and hygiene practices can, to a great extent, reduce incidences of diarrhoea (Fry *et al*., 2008; Prüss-Üstün *et al*. 2008).

Detection of pathogenic bacteria requires laboratory-conducted tests (Zamxaka *et al.*, 2004), while the detection and enumeration of indicator organisms is a basic microbiological technique in monitoring the quality of water (Pillai & Rambo, 2014). According to the South African water quality guidelines (Department of Water Affairs and Forestry [DWAF], 1996a), coliforms are the principal indicators of water safety for domestic, industrial and other uses.

1.2 BACKGROUND TO THE STUDY AREA

The informal settlement of RR Section in Khayelitsha is one of approximately 250 informal settlements situated in Cape Town, Western Cape, South Africa. It is located between Mew way Road, Govan Mbeki Road and Bonga Drive (**Figure 1.1**). It consists of approximately 4000 households (Goldberg, 2009). The RR Section is a homogenous area with informal housing constructed from various materials such as corrugated iron, wood, plastic, and other recycled materials (**Figure 1.2**).

In a study conducted within informal settlements in Cape Town (which included RR Section), Goldberg (2009) found that sanitary facilities in these areas do not conform to the standards of basic sanitation. This is due to factors such as high household to toilet ratios, the condition and location of the sanitation infrastructure as well as the overall maintenance of sanitary facilities (Goldberg, 2009). According to the City of Cape Town (2011), the standard rule for the provision of basic water and sanitation in informal settlements is a minimum of one tap for every 25 households within a distance of 200m and one toilet for every five households.

In 2009 the director of Strategy, Support and Coordination for the City of Cape Town reported a total of 569 communal toilets and 61 standpipes in RR Section (City of Cape Town, 2009). Given this high number of households compared to the available toilets and standpipes, it is clear that this target ratio is not being met, leading to the increased use of toilets and taps by more households than intended. This high ratio is further exacerbated by the fact that many toilets and standpipes are either in a poor condition or non-functional (Silber, 2011; Department of Monitoring & Evaluation [DPME] & Department of Human Settlement [DHS], 2012).

Many problems exist in relation to access to basic water and sanitation in RR Section. The broken toilets and taps are a result of the poor quality of the construction or building materials used (DPME & DHS, 2012). At the same time, the inadequate provision of toilets and standpipes lead to an overload of the existing sanitation infrastructure. Coupled with infrequent or inadequate maintenance, this leads to many toilets and standpipes breaking

down (SJC, 2011). With the lack of proper drainage systems in informal settlements, stagnant water pools form at the base of standpipes (SJC, 2011).

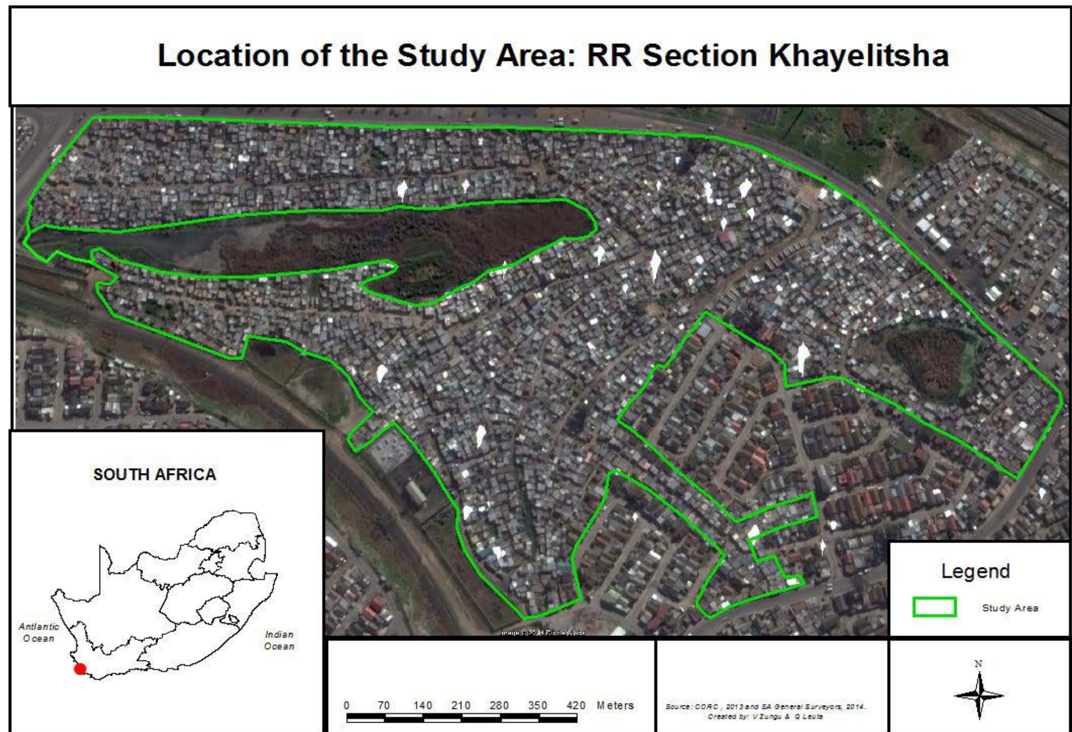


Figure 1.1 Locational Map of RR Section, adapted from Community Organisation Resource Centre (CORC, 2013).

Figure 2.2 View of RR Section, Khayelitsha, Cape Town.

This is a result of inhabitants using the standpipes as a wash-up area for dishes, laundry and food (Granfone *et al.,* 2008). In some instances, raw sewage, i.e. diluted faecal matter and urine, finds its way from broken sanitary facilities to these stagnant pools forming a mixture of unidentified possible pathogenic components (SJC, 2011). This mixture in itself could lead to a public health hazard (SJC, 2011). Poor drainage is a common problem within informal settlements and thus not unique to RR Section. It also has a significant contribution towards the prevalence of illnesses (UN-Habitat, 2003c). As mentioned, a combination of poor drainage systems and inadequate sanitation practices causes raw sewage to mix with surface runoff and as a result possible pathogens are spread throughout the community (Parkinson, 2003).

Another major issue with the sanitary facilities and the cause for its breakdown is the lack of toilet paper. People tend to make use of materials such as stones, leaves, newspapers or plastics instead of toilet paper and then dump these down the toilet. The use of these and other materials could therefore lead to toilet blockages and thus overflows. In addition to breakdowns and the subsequent increased pressure on existing facilities, possible overflow may lead to raw sewage spills seeping into the open spaces (Granfone *et al.*, 2008).

Due to infrequent and inadequate maintenance of toilets and taps in RR Section as well as security concerns, inhabitants either resort to relieving themselves in plastic bags or make use of night pots in which they urinate and defecate at night (Bregman, 2011; Tokota, 2012). These pots are emptied in the morning and cleaned at the standpipes (Tokota, 2012), which may result in the water at the base of the standpipes becoming contaminated with human excreta. In other instances, the excreta are thrown into the ditches or the nearby swamp, but may still make its way to water pools when it rains (Bregman, 2011). Raw sewage often leaks into the streets or walkways where it comes into contact with stagnant water pools (SJC, 2011). Reports of children playing next to these pools as well as reports of children being hospitalised due to gastrointestinal infections and skin diseases, have been documented. Symptoms reported included diarrhoea and skin rashes (SJC, 2011).

In 2010, Barnes conducted preliminary studies on water samples in the RR Section. **Table 1.1** indicates total coliform and *E. coli* counts at three different water sources. According to the Department of Water Affairs and Forestry (DWAF, 1996b) faecal coliforms and *E. coli* may not exceed counts of 130 microorganisms/100mℓ, respectively, in water used for recreational purposes.

Even though counts exceeded the maximum acceptable limit for these microbial types, an increased microbial trend could not be established as water analysis was only performed once in 2010. Another preliminary study (**Table 1.2**, unpublished) was conducted

in 2012 to substantiate whether or not Barnes' claim was valid and whether a follow-up study could be pursued.

Table 1.1 Results of microbial water analysis conducted by Barnes 2010 (adapted from Barnes, 2010).

Sample	Total coliforms (per 100mℓ)	*E. coli* (per 100mℓ)
A (water sample taken from one of the standpipes in RR Section)	624 000 000	2 000 000
B (water sample taken from the canal that runs through RR Section)	63 000 000	1 000 000
C (water sample taken from the run-off of one of the toilets from RR Section)	10 000 000	100 000

The results showed continuously high microbial counts. Seven samples (three potable water samples from standpipes, three stagnant water samples taken from the base of the standpipes, and one sample from the canal running adjacent to the informal settlement) were analysed, and found that none of the potable water samples contained any faecal coliforms or *E. coli*, while samples from the stagnant water and the canal did not meet recreational standards.

Table 1.2 Results of a preliminary study used to substantiate Barnes' claims on microbial content of water in RR Section.

Sample site	Total coliform (per 100mℓ)	Faecal coliform (per 100mℓ)	*E. coli* (per 100mℓ)
A (pool water underneath standpipe near house RR49)	4 600	28 000	2 300
B (pool water underneath standpipe near house RR240)	92 000	54 000	1 700
C (pool water underneath standpipe near house RR129)	1 600 000	1 600 000	140 000
D (Swamp water collected from a culvert in Mew Way road as the water enters RR Section)	1 600 000	1 600 000	1 600 000

1.3 INFORMAL SETTLEMENTS AND THE PROVISION OF WATER AND SANITATION IN SOUTH AFRICA

Due to rapid urbanisation, many people in sub-Saharan Africa live in informal settlements (Morakinyo *et al.*, 2012). In 2003, it was estimated that approximately 32% of the world population live in informal settlements, of which 78% live in urban areas of developing countries (UN-Habitat, 2003b). During the year prior to these statistics being documented,

the United Nation Population Division [UNPD] (2002) projected that the world's urban population would reach two billion new residents before 2030. Furthermore, it is estimated that the urban population will increase by 200 000 people per day between 2010 and 2015, of which 91% will take place in developing countries (UN-Habitat, 2013).

In 2011, it was estimated that 62% of the South African population lived in urban areas (South African Institute of Race Relations, 2013), of which 1.11 million households lived in informal settlements (Housing Development Agency [HDA], 2013a). More recently, it is estimated that approximately 140 000 people are informal shacks dwellers in Cape Town (HDA, 2013b). This number is expected to grow because of the projected population growth in Cape Town. Dorrington (2005) predicted a 17% increase in the population in the City of Cape Town by 2014.

Informal settlements are characterised by insecure tenure and a high population density as well as inadequate access to housing, safe water, sanitation and other infrastructure (Morakinyo *et al.*, 2012; UNICEF, 2012). Informal settlements tend to be situated in hazardous or unstable areas such as floodplains, next to landfills, or in high industrial pollution areas (UN-Habitat, 2003a). In addition, this land is often privately owned. Given these conditions, providing informal settlements with adequate water and proper sanitation facilities proves to be challenging (Mels *et al.*, 2010). While the government has made efforts to provide low income settlements with municipal water, the establishment of such facilities has not been provided with adequate drainage and management strategies (Carden *et al.*, 2007). Consequently, pools of stagnant water formed around these standpipes, provide a breeding ground for potential pathogens such as *Escherichia coli*, *Vibrio cholera* and *Pseudomonas aeruginosa* (Granfone *et al.*, 2008). These pathogens may lead to diseases such as gastroenteritis as well as skin-, eye- and ear- infections (Carden *et al.,* 2007). *Escherichia coli* counts, greater than 1800 counts/100mℓ, were recorded from greywater in many informal settlements throughout South Africa (Carden *et al.*, 2007).

Even though the supply of piped, treated water to all households is very important to public health, the provision of improved sanitation is as equally important (Fry *et al.,* 2008). Esrey (1996) suggested that the benefits of improved sanitation would be higher than improving the benefits of only the quality of drinking water. According to Curtis *et al.* (2000) the safe disposal of human excreta is far more effective than any amount of hand-washing to ensure protection against infectious disease transmission. Therefore good sanitation, availability of good quality water, adequate disposal of human and animal excrement and public education in hygiene practices are major factors that reduce the impacts of diarrhoeal diseases (UNICEF & WHO, 2009).

South Africa is a water scarce country (Friedrich *et al.,* 2009) with an average annual rainfall of 450mm (DWAF, 2013). In addition, water contamination from informal settlements,

lacking an adequate water supply and proper sanitation facilities, is increasingly threatening the quality of South African rivers (Fatoki *et al.*, 2001). According to various studies conducted, several South African rivers do not meet the water quality standards due to faecal pollution from informal settlements. In the Free State Province of South Africa, Griesel and Jagals (2002) conducted a faecal indicator study in the Renoster Spruit system of the Modder-Riet River catchment and found that the water quality was not suitable for domestic-, recreation- and irrigation purposes. In the Western Cape, Ndlovu (2013) recorded microbial counts as high as 9.2×10^6 microorganisms/100mℓ and 1.6×10^7 microorganisms/100mℓ in the Berg and Plankenberg Rivers, respectively, through the use of the Most Probable Number (MPN). In the Umtata River catchment in the Eastern Cape, the highest faecal coliform and total coliform counts recorded were 2.1×10^4 microorganisms/100mℓ and 6.9×10^4 microorganisms/100mℓ (Fatoki *et al.*, 2001), respectively.

1.4 THE SITUATION OF GREYWATER IN INFORMAL SETTLEMENTS OF SOUTH AFRICA

1.4.1 Greywater

Greywater can be defined as wastewater generated from domestic activities (such as laundry, bathing and dishwashing) without any input from toilet wastewater (Birks & Hills, 2007; Rodda *et al.*, 2010). There are three sources of greywater. Kitchen greywater is wastewater contaminated with food particles, fat and oil as well as dishwashing detergent (Rodda *et al.*, 2010). It may contain pathogenic bacteria from washing raw meat and vegetables (Eriksson *et al.*, 2002). Bathroom greywater is wastewater as a result of hand washing, showering and bathing. It may contain pathogenic microorganisms as a result of washing hands after toilet use as well as washing babies and small children after changing their diaper (Eriksson *et al., 2002)*. Laundry greywater is a result of wastewater generated after washing clothes (Rodda *et al.*, 2010). Pathogenic microorganisms may be introduced into laundry greywater through washing of soiled nappies (Eriksson *et al.,* 2002; Rodda *et al.*, 2010). The National Strategy for Managing Water Quality Effects of Settlements (DWAF, 2001a) and the City of Cape Town's Greywater Guidelines (City of Cape Town, 2005) identify stagnant water, accumulating at the base of communal standpipes in informal settlements, as greywater. Therefore, the samples collected for this study are considered as greywater as per the definition by the National Strategy for Managing Water Quality Effects of Settlements (DWAF, 2001a) and the City of Cape Town's Greywater Guidelines (City of Cape Town, 2005).

Carden *et al.* (2006) reported that rapid urbanisation in South Africa can be associated with increased quantities of contaminated runoff from settlements resulting in accelerated degradation of water resources. Greywater pollution is a result of physical problems (when no services are provided or the services are inadequate), institutional problems (when services are not operated or maintained properly), social problems (when people do not use or pay for services properly), or when vandalism occurs (DWAF, 2001a; 2001b). According to DWAF (2001a; 2001b), these problems are interlinked. Wood *et al.* (2001) reported that greywater management is severely limited in many informal settlements with streams of contaminated water being a predominant character arising from the general provision of standpipe water supplies and inability to integrate management of basic services (water, waste, sanitation and stormwater). According to Carden *et al.* (2008), the management of greywater is further complicated by the fact that many informal settlements are temporary in nature and are often fragmented with respect to social structures.

1.4.2 Characteristics of greywater

According to Eriksson *et al.* (2002), the characteristics of greywater depend firstly on the quality of the water supply, secondly on the type of distribution network for both drinking water and grey wastewater and thirdly from the activities in the household. The compounds present in greywater are influenced by various factors such as lifestyles, customs, installations and the use of chemical household products (Eriksson *et al.*, 2002; Carden *et al.*, 2007). Eriksson *et al.* (2002) provided detailed characteristics of greywater from a developed country perspective, while Rodda *et al.* (2010) provided detailed greywater characteristics within the South African context. Both studies characterised greywater into physical parameters (e.g. temperature, colour, turbidity and content of suspended solids), chemical parameters (e.g. pH, biological and chemical oxygen demand and heavy metals) as well as microorganisms, which could contain pathogenic viruses, bacteria, protozoa and helminthes.

1.4.3 Microbial quality and health risk of greywater

In general, greywater is assumed to be safe due to a widespread misconception that its contamination levels are lower compared to blackwater (domestic sewage) (Birks & Hills, 2007). Even though greywater has a lower strength and concentration of health-related microorganisms and nutrients than blackwater (Rodda *et al.,* 2010), it can have a significantly higher microbial load (Birks *et al.*, 2004).

Microbial quality of greywater is primarily measured by the presence of indicator microorganisms (Arnone & Walling, 2007; Rodda *et al*., 2010). When determining the quality of greywater, most studies in South Africa concentrate on the physico-chemical analysis (Carden *et al.,* 2007; Mofokeng, 2008; Muanda, 2009; Mzini, 2013). With regards to the microbial load in greywater, there is little information on bacteriological quality of greywater produced in South Africa as various studies concentrate on assessing microbial indicator organisms (Carden *et al.,* 2007; Mofokeng, 2008; Muanda, 2009; Govender *et al.,* 2011). However, pathogenic microorganisms such as *Pseudomonas aeruginosa* (Khalaphallah & Andres, 2012), *Salmonella, Shigella* and *Vibrio cholerae* (Nganga *et al.,* 2012) have been found in greywater. Consequently, greywater is a health risk to those who come in contact with it (Eriksson, 2002; WHO, 2006).

According to Rodda *et al.* (2010) people living in informal settlements in South Africa rely on a free basic potable water supply of 200-300 litres/day per household or on the communal standpipes situated away from the boundaries of their properties. Consequently, this results in a shortage of water, which in turn causes people to minimise their water use apart from water used for drinking and cooking (Rodda *et al.,* 2010). It is therefore common for people living in informal settlements to reuse their greywater several times before it is disposed (Rodda *et al.,* 2010). For instance, water collected for laundry, may first be used for bathing babies, and children. Adults then use the same water to wash themselves, after which the same water is then used to do the laundry and finally used to do household chores, such as washing of floors, etc. (Rodda *et al.,* 2010). The repeated reuse can result in high microbial concentration loads (Rodda *et al.,* 2010). Even though an increase in the microbial contamination of greywater results in an increased health risk (Salukazana, 2006), the incidence of disease depends not only on the concentration of pathogenic organism but on exposure, health and age of the person affected (WHO, 2011b).

In their study of the Khayelitsha Water and Sanitation Programme, Stern *et al.* (2004) showed linkages between health, sanitation and poverty. It was reported that due to poor toilet facilities, there is a high rate of worm infestation and diarrhoea among children in the Khayelitsha informal settlements. This health problem, according to Carden *et al.* (2007) is further exacerbated by highly contaminated stormwater runoff caused by, among others, greywater and solid waste disposal.

1.4.4 City of Cape Town Greywater Guidelines

The aim of the City of Cape Town Greywater Guidelines (2005) is to provide guidance on how and where greywater from informal settlements within the City of Cape Town should be disposed of. These guidelines make a provision for soakaways to drain greywater as well as

for the regular maintenance of these soakaways. However, these guidelines do not make any reference to the microbial characteristics of greywater. Added to the fact that currently water still accumulates at the base of the standpipes, it can be assumed that the above mentioned guidelines are not adhered to, thereby making a need for this study valid and necessary.

1.5 WATER QUALITY

It is estimated that every year, 2.2 million people die in developing countries from diarrhoeal diseases as a result of poor water quality and inadequate sanitation (Koola & Zwane, 2014). Therefore water quality standards were established to protect aquatic life as well as safeguarding the public against diseases. These quality guidelines are for domestic, agricultural, recreational as well as industrial purposes (DWAF, 1996b). According to the Department of Water Affairs and Forestry (DWAF, 1996a), domestic water refers to water used for drinking, bathing, personal hygiene and other general household purposes. Recreational water on the other hand, is identified as any water that is inland and is used for recreational purposes. The Department further divides recreational water into full-contact (which involves full-body contact with water), intermediate-contact (involves any form of contact with water except full-contact) as well as non-contact (involves non-contact activities with water e.g. picnicking alongside water bodies) (DWAF, 1996b). **Table 1.3** gives a summary of microbial indicators used to determine the quality of water used for domestic and recreational purposes.

Table 1.3 Water quality guidelines of microbiological indicators (DWAF, 1996a, DWAF 1996b; SABS, 2011).

Microorganism	DWAF (microorganisms/100 mℓ)	SABS (microorganisms/100mℓ)
Total coliform	No value indicated for recreational water ~5 (domestic)	≤ 10 (domestic)
Faecal coliforms	~130 (recreational – full contact ~1000 (recreational – intermediate contact) ~0 (domestic)	~0 (domestic)
Escherichia coli	~130(recreational- full contact, no value for intermediate contact)	~0 (domestic)
Enterococci	~230(recreational – intermediate contact)	No value indicated
Heterotrophic plate count (HPC)	~100 (domestic)	≤1000/mℓ (domestic)

According to the DWAF (1996a) and South African Bureau of Standards [SABS], (2011) guidelines, water to be used for domestic purposes, should not contain a total coliform count of more than 5 microorganisms/100mℓ, and 10 microorganisms/100mℓ, respectively. Furthermore, such water should not contain any faecal coliforms (DWAF, 1996a & SABS, 2011) and should not contain heterotrophic bacterial counts of more than 100 microorganisms/100mℓ (DWAF, 1996a). The South African Bureau of Standards (2011) has a lower limit of heterotrophic bacterial counts of 1000 microorganisms/mℓ. Thus, when water containing microbial counts exceeding these limits is consumed, human health may be compromised (SABS, 2011).

The DWAF (1996b) guidelines stipulate that water used for recreational purposes may present a risk of gastrointestinal illness if there is exposure to water with a faecal coliform count of 130 microorganisms/100mℓ (during full contact) or a faecal coliform load of 1000 microorganisms/100mℓ (during intermediate contact). Gastrointestinal illness may also occur where there is full contact with water containing an *E. coli* load of 130 microorganisms/100mℓ. Due to insufficient information regarding the risks associated with intermediate contact with *E. coli,* DWAF (1996b) could not determine the risk. Water with microbial counts exceeding these limits may lead to increased waterborne infection risks.

1.6 WATERBORNE PATHOGENS

Pathogens are disease-causing microorganisms, which are a major concern for managers of water resources (Arnone & Walling, 2007). Once in a water body, pathogens can infect humans through contaminated food (such as fish and shellfish), skin contact or through drinking contaminated water (Arnone & Walling, 2007). Various pathogenic microorganisms may exist and multiply in domestic wastewater sources (Bitton, 2011). According to Bitton (2011) three categories of pathogens are found in the environment. The first group includes waterborne bacterial pathogens, which include either enteric bacteria such as *Salmonella* spp. or indigenous aquatic organisms such as *Aeromonas* spp. Secondly, even though viral pathogens are released into the aquatic environments they can only multiply within host cells. The third group includes the protozoan parasites, which are released into aquatic environments as cysts or oocysts. Cysts and oocysts are very resistant in aquatic environments as well as many common water treatment procedures, disinfectants and antiseptics (Bitton, 2011). Most waterborne pathogens come from faeces of infected humans and animals (Arnone & Walling, 2007).

According to WHO (2011b) waterborne pathogens have several characteristics (**Table 1.4**), which distinguish them from other contaminants. They can be transmitted

through the faecal-oral route such as ingestion of contaminated drinking water, inhalation of water droplets, contaminated food, hands, utensils, clothing or skin contact particularly in areas of inadequate sanitation and poor hygiene. Thus to prevent faecal-oral transmission of pathogens, it is important to have adequate water and sanitation, and good hygiene practice. Infection depends on factors such as age, immunity, sex and health of an individual affected (WHO, 2011b).

Table 1.4 Properties of waterborne pathogens (WHO, 2011b).

- They should be able to cause acute and chronic illnesses
- They should have the ability to grow in the environment
- They should be distinctive
- Pathogens are often aggregated or adherent to suspended solids in water, and pathogen concentrations vary in time, so that the likelihood of acquiring an infective dose cannot be predicted from their average concentration in water
- For the pathogen to cause disease, it will depend upon the dose, invasiveness and virulence of the pathogen as well as the immune status of the individual
- Pathogen can only multiply in their host once the infection has been established
- Some waterborne pathogens are also capable of multiplying in food, beverages or warm water systems increasing the likelihood of infection
- Compared to chemical agents pathogens do not show signs of a cumulative effect

According to Mihelcic *et al.* (2009) aquatic pathogens occur in four classes of water-related diseases: waterborne, water-washed, water-based, and water-related insect vectors. Of the four the first three are directly related to water quality, while water-related insect vectors are spread by insects that breed or feed near contaminated water. Waterborne diseases are caused by drinking water contaminated with pathogens transmitted from human and animal excreta. The diseases include cholera, typhoid, amoebic and bacillary dysentery. Water-washed diseases are those diseases caused by poor personal hygiene as well as contact with contaminated water. The diseases include trachoma, typhus, and diarrheal diseases. Water-based diseases are caused by parasites that live in intermediate organisms or require water for part of their life cycle. Diseases caused by these pathogens are dracunculiasis, schistosomiasis, and other helminths. These diseases are contracted by drinking or coming in contact with contaminated water.

Provision of safe drinking water and sanitation plays a critical role in the health of a population (Fry *et al.*, 2008). Millions of people die every year due to drinking water contaminated with pathogenic microorganisms and children below five years, the elderly and the immunocompromised are the most affected (Environmental Protection Agency [EPA], 2013). Globally, many children under the age of five die from diarrhoea, which kills around 760 000 children per year (WHO, 2013). Diarrhoea has been identified as the second leading cause of death among children below the age of five (WHO, 2013).

1.6.1 Bacteria

1.6.1.1 *Salmonella*

Salmonellae are gram-negative, motile, facultative anaerobic enteric bacilli (WHO, 2011b). These organisms are widely distributed in the environment and include more than 2500 serotypes (Callaway *et al*., 2008; Bitton, 2011). In humans, the bacterium is transmitted via the faecal-oral route through contaminated drinking water or food (Da Silva *et al*., 2013). It is estimated that 131.6 million cases of gastroenteritis are reported each year of which 155 000 result in death (Majowicz *et al*., 2010). *Salmonella* spp. cause typhoid fever and gastroenteritis (Raffatellu *et al*., 2008) and patients may show symptoms that range from nausea, vomiting, abdominal cramps, diarrhoea, headache, fever and loss of appetite (Food and Drug Administration [FDA], 2012). Salmonellosis can be contracted by all age groups even though it may be more severe in the elderly, infants and immunosuppressed, e.g. acquired immune deficiency syndrome (AIDS) patients are estimated to suffer from salmonellosis 20 times more than the general population and may suffer from recurrent episodes. *Salmonella typhi* (causing typhoid fever) and *S. paratyphi* (causing paratyphoid fever) are usually found in humans (FDA, 2012). However, on rare occasions *S. paratyphi* can also be found in domesticated animals (Rusin *et al.,* 2000). Improved sanitation and health care can reduce the incidence of typhoid fever (Raffatellu *et al*., 2008).

1.6.1.2 *Helicobacter pylori*

Helicobacter pylori is a gram-negative, highly motile, spiral-shaped bacterium first identified in human intestinal biopsies in 1982 (Madigan & Martinko, 2006). It is a bacterial agent that is strongly linked to peptic ulcers, gastritis, lymphoma and gastric cancer (Madigan & Martinko, 2006; Bitton, 2011). According to a study conducted by Malaty (2006), socio-economic status played a major role in *H. pylori* infection. Children with poor living conditions such as inadequate sanitation are at high risk of contracting *H. pylori* (Ahmed *et al*., 2007). Of all the reservoirs of *H. pylori*, humans are the principal hosts (WHO, 2011b). *Helicobacter pylori* is primarily transmitted through the faecal-oral route (Malaty, 2006). According to Gião (2008) it is difficult to isolate *H. pylori* from water sources using standard plate methods, yet it has been successfully isolated using the Polymerase Chain Reaction (PCR) method.

1.6.1.3 *Vibrio cholera*

The *Vibrio* genus has more than one species that is pathogenic to humans. The most well-known is the *Vibrio cholerae* (Cabral, 2010), which is a gram-negative, curved rod (Bitton, 2011). According to Madigan & Martinko (2006) the organism is frequently found in areas where there is a lack of or inadequate sanitary facilities and is endemic to Africa, Southeast Asia, the Indian subcontinent and Central- and South America. *Vibrio cholerae* releases enterotoxins that cause mild to profuse diarrhoea, vomiting and rapid fluid loss, which can be fatal to immunocompromised people (Bitton, 2011). *Vibrio cholerae* was found in 55% of sampled greywater from an informal settlement in Nairobi, Kenya (Nganga *et al.* 2012). Inadequate sanitation and access to potable water have been linked to cholera outbreaks in South Africa, where 1 144 cholera cases were reported between 15th November 2008 and 30th November 2009, of which 8% were cases from the Western Cape (Archer *et al.*, 2009).

1.6.1.4 *Legionella*

Legionella is a gram-negative, aerobic, rod that causes Legionnaire's disease and Pontiac fever (Bitton, 2011). At least 50 species with approximately 70 serotypes of *Legionella* are known to exist (WHO, 2011b). Over 28 species of *Legionella* cause disease, but the prominent pathogenic species is *L. pneumophila*. Symptoms of infection are characterised by influenza-like fever, nausea, vomiting, painful muscles, coughing and headache (WHO, 2011b). Symptoms can show after 2 to 14 days after exposure (Center for Disease Control and Prevention [CDC], 2013). Birks *et al.* (2004) isolated *Legionella* from greywater.

1.6.1.5 *Pseudomonas aeruginosa*

Pseudomonads are aerobic, gram-negative, non-spore-forming bacilli, oxidase-and catalase-positive and motile by the use of polar flagella (Da Silva *et al.*, 2013). There are 100 species of Pseudomonads of which *Pseudomonas aeruginosa* is an important medical strain (Percival *et al.*, 2000). *Pseudomonas aeruginosa* is able to grow at 41°C and produce O and H antigens used for serogrouping (Percival *et al.*, 2000). Of these serogroups, possibly nine groups of *P. aeruginosa* are known to be pathogenic to humans (Percival *et al.*, 2000). *Pseudomonas aeruginosa* can be found in faeces, soil, water and sewage (WHO, 2011b). It has the ability to multiply in water as well as suitable organic matter, which is in contact with water. This organism has the ability to be persistent as it can survive in nutrient poor environments as well as tolerate diverse physical conditions (Lister *et al.*, 2009). It

predominantly cause infections in patients with burns, surgical wounds, underlying respiratory tract diseases and physically damaged eyes where it can then lead to destructive lesions or septicaemia and meningitis (WHO, 2011b). Winward *et al.* (2008) tested greywater and found *P. aeruginosa* in all tested greywater samples.

1.6.1.6 *Campylobacter*

Campylobacter spp. are gram-negative, slender-curved microaerophilic, motile rods (WHO, 2008). It has also been characterised as thermotolerant bacterial pathogens that infect both humans and animals (domestic and wild) (Bitton, 2011). The most important natural reservoir for *Campylobacter* is birds, although environmental water is a significant source of human contamination (Lemarchand *et al.*, 2004). Globally, they are considered to be one of the most significant causes of acute gastroenteritis, with *Campylobacter jejuni* as the most frequent species to be isolated from patients with acute diarrhoeal diseases (WHO, 2008). After ingestion, this organism multiplies in the small intestine, invades the epithelium and causes inflammation, resulting in disease (Madigan & Martinko, 2006). Patients show symptoms of headache, malaise, nausea, abdominal cramps, and profuse diarrhoea, often with bloody stools (WHO, 2011b; Bitton, 2011).

1.6.1.7 *Yersinia*

Yersinia enterocolitica and *Y. pseudotuberculosis* are small rod-shaped, gram-negative bacteria that are often isolated from warm-blooded animals such as birds, cats and dogs, with pigs being the major animal reservoir (Bitton, 2011). Of the two species, *Y. enterocolitica* has been isolated from environmental and food sources such as meat (Lambertz *et al.,* 2008). After 24 to 48 hours of ingestion of contaminated food or drink, patients frequently show symptoms of gastroenteritis with diarrhoea, fever and vomiting (Lambertz *et al.,* 2008). Infections caused by *Yersinia* mimic appendicitis (Antonopoulos *et al.,* 2008), but these bacteria may also cause infection of other sites such as joints and the urinary tract (Gutierrez, 2005). Poor and improper sanitation techniques of food handlers contribute to contamination of food and drink by *Yersinia* (Okwori *et al.,* 2009).

1.6.1.8 Indicator organisms

Indicator organisms are microorganisms whose detection in water indicates possible presence of pathogenic microorganisms (Haller *et al.*, 2009). They are microbial organisms

found in high numbers in faecal matter and their presence in water usually indicates faecal contamination and treatment efficiency (WHO, 2011b). For an organism to be considered an ideal indicator, it has to achieve the criteria as outlined in **Table 1.5**. It should be noted that even though an organism might meet this criteria, they are designed to determine whether or not a human faecal contamination has occurred (Teplitski & Butler, 2008). Indicator microorganisms commonly used to assess water quality include total coliforms, *E. coli*, faecal coliforms and enterococci (UNICEF, 2008; Rodda *et al.*, 2010). However, their ability to predict pathogen presence and health risks are limited (Arnone & Walling, 2007) as these organisms are not pathogenic themselves but may indicate possible presence of pathogenic microorganisms (Rodda *et al.*, 2010). Thus, water detected with indicator organisms should be considered unsafe prior to consumption and further testing should be employed to determine the presence of possible pathogenic microbial organisms.

Table 1.5 Criteria used to determine an ideal indicator organism (WHO, 2011b).

Indicator organism should:
• be commonly present in faecal matter of humans and animals in high numbers • not multiply in natural water • survive in water as long as faecal pathogen • be present in numbers higher than those of faecal pathogens; • respond to treatment processes in same way as faecal pathogens • be readily detectable by simple and cost-effective techniques

1.6.1.8.1 Total coliform bacteria

According to Pindi *et al.* (2013) coliforms are members of the *Enterobacteriaceae* family and are commonly used as bacterial indicators of hygienic quality of water and food. They are ambiguous in soil, vegetation and in the gastrointestinal tract of warm blooded animals, including humans. Although many strains of coliforms are harmless they include pathogenic bacteria belonging to genera such as *E. coli, Klebsiella, Pantoea, Serratia, Citrobacter* and *Enterobacter.* Total coliform bacteria are gram-negative, non-spore-forming, facultative anaerobic, rod-shaped bacteria that ferment lactose and produce gas, and are able to grow at high concentrations of bile salts. They ferment lactose to produce acid and gas within 24 hours at 35-37°C (WHO, 2011b). According to WHO (2011b), they are heterogeneous and include lactose-fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii.* Total coliform bacteria are more robust than pathogenic microorganisms and may survive for longer periods in water or food sources. Their absence may therefore indicate that water sources are free of pathogens and thus bacteriologically safe (WHO, 2011b). According to DWAF (1996b), total coliform bacteria are primarily used to indicate the overall microbial quality of water and are routinely used to monitor the quality of drinking water. However,

WHO (2011b) emphasize that total coliforms cannot be exclusively used as indicators of faecal contamination as these group of organisms include both environmental and faecal species. They, nonetheless, indicate the effectiveness of water treatment and possible presence of biofilms.

1.6.1.8.2 Faecal coliform bacteria

Faecal coliforms are those coliform bacteria that produce typical blue colonies on m-FC agar within 20 – 24 hours at 44.5°C (WHO, 2011b). Faecal coliforms are members of total coliforms and thier themotolerant characteristis distinguishes them from the other coliforms (Knechtges, 2012). They constitued about 93% to 99% of coliform bacteria in fecal matter (DWAF, 1996b) and include species such as *E. coli*, *Salmonella* and *Vibrio* (Myers *et al.* 2014). These microorganisms are considered as indicators of faecal contamination of water due to their close association with faecal pollution rather than any of the total coliforms (Ji, 2008). These organisms are used for the assessment of faecal pollution of wastewater, raw water supplies and natural water sources used for recreational purposes (DWAF, 1996b).

1.6.1.8.3 *Escherichia coli*

Escherichia coli are gram-negative, non-spore-forming, rod-shaped bacteria, which inhabit the intestinal tract of humans and other animals (Madigan & Martinko, 2006). They represent more than 90% of the faecal coliform group and are useful indicators of water quality (Lemarchand *et al.,* 2004). *Escherichia coli* are considered a highly specific faecal pollution indicator as it forms part of the natural gut flora of humans and warm-blooded animals (WHO, 2011b) and restricts the influence of microbial organisms of environmental origin (Luyt *et al.*, 2012). Although *E. coli* is used as an indicator organism, it is also regarded as an opportunistic pathogen. *Escherichia coli* has many strains most of which are non-pathogenic. Nevertheless, some can cause diseases such as diarrhoea, urinary tract infections, respiratory illnesses and pneumonia. Pathogenic *Escherichia coli* strains are classified into heterogeneous groups based on their virulence factors: Enterotoxigenic *Escherichia coli* (ETEC), Enteroaggregative *Escherichia coli* (EAEC), Enteroinvasive *Escherichia coli* (EIEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteropathogenic *Escherichia coli* (EPEC) and diffuse-adhering *Escherichia coli* (DAEC) (O' Sullivan *et al.* 2007). Of these six EHEC, EIEC and ETEC are exceptionally important and can be contracted through contaminated water (Scheutz & Strockbine, 2005).

According to WHO (2011a), EHEC has been isolated from water bodies, even though its primary reservoir is cattle. It can cause severe foodborne disease in young children and the elderly, whereby it can cause haemolytic uraemic syndrome (WHO, 2011a). According to Ramamurthy and Albert (2012), EIEC causes a shigellosis-like syndrome with high fever and prolific diarrhoea. Transmission of EIEC is through contaminated food or water and symptoms occur within 12 to 72 hours after ingestion (FDA, 2012). Symptoms include mild dysentery, abdominal cramps, diarrhoea, vomiting, fever, chills, general malaise as well as stools containing blood and mucus (FDA, 2012). Enterotoxigenic *E. coli* cause diarrhoea in infants and children in developing countries (Madigan & Martinko, 2006; McKenzie *et al.*, 2007). It is also known as a leading cause of enteric infection that causes diarrhoea in visitors to the developing world (McKenzie *et al.*, 2007). It is acquired through consumption of contaminated food and beverages and every year, it affects approximately 27 million travellers and 210 million children (McKenzie *et al.*, 2007). Patients will show symptoms of abdominal cramps, low grade fever, nausea, malaise and sudden watery diarrhoea, which has no blood or mucus but rarely have high fever or vomit (FDA, 2012). In a study conducted by Keddy (2013) *E. coli* was found to be a leading cause of diarrhoea in South Africa with children below the age of five affected the most. In addition, DAEC, EAEC, EHEC, EPEC and EIEC were amongst the *E. coli* strains isolated in the Western Cape (Keddy, 2013).

1.6.1.8.4 Heterotrophic bacteria

Heterotrophic bacteria are organisms that use organic nutrients for growth and are present in all types of water, food, soil, vegetation and air sources (WHO, 2011b). These organisms include bacteria, yeasts and moulds (WHO, 2011b). Heterotrophic bacteria are regarded as a small percentage of microorganisms culturable on organic growth media due to the fact that a lot of microorganisms cannot be cultured on organic growth media. Furthermore, microorganisms that have abeen recovered through Heterotrophic Plate Count (HPC) tests generally include those that are part of the natural microbiota of water, which are typically non-hazardous. In some cases however, HPC organisms may be derived from diverse pollutant sources (Bartram *et al.*, 2004). Heterotrophic bacteria are not a direct indication of faecal contamination but may indicate a variation in water quality (WHO, 2011b) and a potential for pathogen survival and re-growth (Rusin *et al.,* 2000). In their study of greywater, Winward *et al.* (2008) found heterotrophic bacteria in concentration of 7.8×10^{10} microorganisms/mℓ.

1.6.1.9 *Clostridium* spp.

Clostridium spp. are gram-positive, endospore-forming anaerobic fermentative rod-shaped organisms (Madigan & Martinko, 2006; Johnson, 2009). There are numerous *Clostridia* that cause disease in humans. *Clostridium botulinum* causes botulism, while *C. tetani* and *C. perfringens* cause tetanus and gas gangrene, respectively (Madigan & Martinko, 2006). According to WHO (2011b), *C. perfringens* is a common member of the intestinal tract flora of humans and animals. It produces spores that are heat resistant, persist for a long time in the environment, and are extremely resistant to disinfectants (WHO, 2011b). Not only is *C. perfringens* unable to multiply in aquatic environments, it is an extremely specific indicator of faecal contamination (WHO, 2011b).

1.6.1.10 *Staphylococcus* spp.

Staphylococcus is a gram-positive bacterium that is arranged in grape-like clusters of cocci (Madigan & Martinko, 2006; Da Silva, 2013). This facultative anaerobe is a catalyse-positive, oxidase-negative, non-motile and non-spore forming microorganism (Madigan & Martinko, 2006; Da Silva, 2013). The organism can either be commensal or parasitic on humans and animals, occasionally causing serious infections (Madigan & Martinko, 2006). *Staphylococcus epidermidis, S. aureus* and *S. saprophyticus* are linked to diseases in humans with *S. aureus* being the most pathogenic of the *Staphylococcus* spp. (Madigan & Martinko, 2006). *Staphylococcus aureus* can cause diseases such as skin infections, respiratory infections, toxic shock syndrome, bacteraemia, meningitis endocarditis and food poisoning (Bien *et al.,* 2011). According to WHO (2011b), faecal matter is not a source for *Staphylococcus aureus*, but it can be introduced into water when humans come in contact with water contaminated with it. Hand contact is the most common transmission routes and poor hygiene practices can result in the contamination of food by *S. aureus* (WHO, 2011b). *Staphylococcus aureus* has previously been found in 25% of greywater samples tested by Winward *et al.* (2008).

1.6.1.11 *Bacillus* spp.

Bacillus spp. are gram-positive, endospore-forming, facultative anaerobic, rod-shaped organisms (Madigan & Martinko, 2006). Many species of the *Bacillus* genus are able to demonstrate a wide range of physiologic abilities that enables them to survive in any natural environment (WHO, 2011b). *Bacillus cereus* is an opportunistic pathogen to patients who

are immunocompromised or critically ill (Senesi & Ghelardi, 2010). It causes food poison with symptoms such as diarrhoea and vomiting (FDA, 2012).

1.6.1.12 *Listeria monocytogenes*

Listeria monocytogenes is an opportunistic food-borne, gram-positive, aerobic or facultative anaerobic, non-spore-forming, rod-shaped bacterium (Cossart & Toledo-Arana, 2008). It causes meningitis, meningo-encephalitis, matemo-fetal and perinatal infections as well as febrile gastroenteritis (Cossart & Toledo-Arana, 2008). Those at greater risks are immunocompromised individuals, the elderly, pregnant women, and newborn babies (Bowie *et al.*, 2004). Paillard *et al.* (2005) conducted a one year study and found *Listeria* spp. to be present in 84.4% of treated water and 89.2% of raw sludge of the six French urban wastewater treatment plants and one composting facility. In South Arica, Odjadjare *et al.* (2010) also conducted a one-year study on wastewater and found *Listeria* spp. to be present in all the wastewater samples.

1.6.1.13 The viable-but-non-culturable (VBNC) state of bacteria

The viable-but-non-culturable (VBNC) state of bacteria is a stage in which enteric bacteria starve and/or are injured, rendering them unable to grow on routine culture media plates (Oliver 2005), thereby resulting in underestimating the number of viable cells (Fakruddin *et al.*, 2013) in a test sample. According to Epstein (2009), *Shigella* can become VBNC in water, but may become pathogenic when it enters a human host. There are many situations where a cell loses its culturability, yet remains viable and potentially able to grow. This situation can be due to bacterial populations in the environment being frequently exposed to stress caused by limiting factors such as changes in nutrient availability, and temperature (Epstein, 2009). According to Barcina and Arana (2009) less than 1% of planktonic and soil microorganisms are culturable on culture media. When flow cytometry is used in conjunction with the *Bac*Light™ Viability probe, it can provide more accurate total cell counts in environmental samples, as flow cytometry has the ability to distinguish between viable and dead microbial cells (Berney *et al.*, 2007).

1.7 DETECTION, ISOLATION, ENUMERATION AND IDENTIFICATION OF WATERBORNE ORGANISMS

1.7.1 Heterotrophic Plate Count

There is no universal measurement for Heterotrophic Plate Count (HPC) although there are formalised standardised methods that include a range of qualitative and quantitative results (Bartram *et al.,* 2004). The type of genera of bacteria that can be recovered on HPC test methods depend on factors such as type of media used, temperature (20°C – 40°C), incubation time, type of sample (river water, surface water reservoir, treated and disinfected drinking water, etc.), season, sample age and the same sampling location over time (WHO, 2011b). The routine HPC methods include the pour plate, spread-plate and membrane filtration method (Allen *et al.,* 2004). Venter (2010) used the HPC technique to identify pathogenic microorganisms such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. megaterium* and *Kocuria rosea.* Osamwonyi *et al.* (2013) were able to identify pathogenic organisms such as *Acinetobacter sp., Bacillus sp., Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Staphylococcus epidermidis* and *Micrococcus luteus*.

1.7.2 Most Probable Number (MPN) technique

The Most Probable Number (MPN) technique or the Multiple Tube Fermentation technique has for many years been routinely used to monitor water quality by enumerating coliforms (Rompre *et al*., 2002). This method provides a statistical estimate of bacterial density within a sample (UNICEF, 2008). This method is easy to conduct with little requirement for specialised equipment and is best in enumerating cells in highly turbid or contaminated samples (UNICEF, 2008). Paulse *et al*. (2007) and Ndlovu (2013) used MPN to enumerate microorganisms in water samples obtained along the Berg- and Plankenberg Rivers, in the Western Cape, South Africa. For the Berg River, Paulse *et al*. (2007) and Ndlovu (2013) recorded MPN counts of 1.7×10^7 microorganisms/100mℓ and 9.2×10^6 microorganisms/100mℓ respectively, while MPN counts of 1.1×10^5 microorganisms/100mℓ (Paulse *et al*., 2007) and 1.6×10^7 microorganisms/100mℓ (Ndlovu, 2013) were recorded in the Plankenberg River, respectively.

1.7.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe

Flow cytometry (FCM) is an optically-based method for analysing individual cells in complex matrices (Castaño-Boldú & Comas-Riu, 2012). The technique identifies different cell types within a heterogenous population (Macey, 2007). As microorganisms suspended in a liquid sample pass a beam of laser light, the light is both scattered and absorbed by the microorganisms (Castaño-Boldú & Comas-Riu, 2012). The extent and nature of scattering, which is an intrinsic property of microorganisms, may be analysed by the spread of the scattered light using a system of lenses and photocells. This is done to estimate the number, size, and shape of microorganisms. Due to its rapid and single cell analysis ability, flow cytometry has become a powerful tool in microbiology (Berney *et al.*, 2007). On its own, the FCM technique lacks the ability to differentiate between dead and viable bacterial cells and therefore should be used in conjunction with the LIVE/DEAD® *Bac*Light™ Viability Probe (Berney *et al.*, 2007). According to Berney *et al.* (2007), the LIVE/DEAD® *Bac*Light™ Viability Probe consists of two components, Propidium Iodide (PI) and SYTO9 or Thiazole Orange (TO), which stain nucleic acids of bacterial cells. Propidium Iodide is a red fluorescing dye, which only enters cells with damaged cytoplasmic membranes, while SYTO9 or TO is a green fluorescing dye which enters all cells.

According to Díaz *et al.* (2010), when FCM is compared to HPC, FCM is found to be an authoritative technique that is able to determine a wide range of cell parameters at a single cell level and capable of obtaining information regarding their distribution within cell populations. Furthermore it is able to detect viable and dead cells (Berney *et al.*, 2007) with the LIVE/DEAD® *Bac*Light™ Viability Probe. Thus, this ability makes the FCM technique superior in assessing the total microbial activity in water compared to the HPC technique (Hammes *et al.*, 2010), which enumerates less than 1% of the total planktonic microbial activity (Siebel *et al.*, 2008). This is due to the fact that the HPC technique is only capable of enumerating culturable microorganisms and visible colonies (Burtscher *et al.*, 2009), while the FCM technique can analyse a large number of events through its computer software (Díaz *et al.*, 2010). Additionally, results obtained by the HPC technique have a relatively higher standard error of greater than 30% compared to the results obtained by the FCM technique with a standard error of less than 5% (Hammes & Egli, 2005).

1.7.4 API 20E system

The API 20E system is a standardised, miniaturised version of conventional procedures for rapid identification of *Enterobacteriaceae* and other gram-negative bacteria through the use of 20 miniaturised biochemical tests (or cupules) (Hill *et al*., 2005). When comparing the API 20E system with *invA* PCR and 16S rRNA sequencing, Nucera *et al*. (2006) found the API 20E system to be more cost-effective if it is used in small laboratories with limited equipment and low sample number. The *invA* PCR method involves the amplification of the virulence chromosomal gene (*inv*A) in *Salmonella* (Jamshidi *et al*., 2009). The *invA* gene of *Salmonella* contains sequences unique to this genus and has been proved as a suitable PCR target region, with potential diagnostic applications (Rahn *et al*., 1992). The system was found to be 100% sensitive and also had 96% specificity as compared to the 16S rRNA sequencing in the identification of *Salmonella enterica* (Nucera *et al*., 2006). Sabae & Rabeh (2007) used the API 20E system to isolate and identify 100 pathogenic bacteria representing 11 genera from water samples collected from the Nile River in Egypt. During the study *Klebsiella pneumoniae* (14%), *Pseudomonas aeruginosa* (12%), *Pseudomonas fluorescens* (4%), *Salmonella colerasuis* (11%), *Shigella* sp. (9%), *Serratia liquefaciens* (8%), *Proteus vulgaris* (8%), *Acinetobacter* sp. (7%), *Brenneria nigrifluens* (5%), *Flavimonas oryzihabitans* (3%) and *Chryseomonas lutecla* (3%) were isolated and identified. Ndlovu (2013) was able to identify 87% and 85% of colonies isolated from the Berg- and Plankenburg Rivers, respectively, using the API 20 E system.

1.7.5 RapID™ ONE system

The RapID™ ONE System uses both the conventional and chromogenic substrates for the identification of *Enterobacteriaceae* and other oxidase-negative and gram-negative bacteria (O'Hara, 2005). The system consists of a test strip with 18 wells, which will give 19 test results (O'Hara, 2005). This is due to the fact that the last well is bifunctional after addition of the Innova spot indole reagent (O'Hara, 2005). Results from the RapID™ ONE System are obtained within approximatly after five hours of incubation while the results from the API 20E System are obtained after 18 -24 hours of incubation. According to Kitch *et al.* (1994), the system is easy to set up and the results can easily be interpreted. Ndlovu (2013) however, found the interpretation of results from the RapID™ ONE System more challenging as compared to the API 20E System. The system can identify *Enterobacteriaceae* without additional tests and results can accurately be acquired on the day of incubation. Kitch *et al*. (1994) were able to evaluate 379 strains of *Enterobacteriaceae* and correctly identified

95.8% without additional tests. Kitch *et al.* (1994) also compared obtained results with results of other published reports and found that not only did the RapID™ ONE System appear to perform better than other commercial biochemical tests (such as the API 20E), but it also identified members of the family of *Enterobacteriaceae* including taxa that is not included in other commercially available systems that are difficult to identify by conventional methodologies. However, compared to the RapID™ ONE system, Ndlovu (2013) found the API 20E system to be more sensitive than the RapID™ ONE system. In addition, on comparison with other commercially available kits, the RapID™ ONE system uses one reagent for the detection of indole production (Kitch *et al.*, 1994). Escalante *et al.* (2009) used the RapID™ ONE and the RapID™ NF Plus Systems to isolate and identify 49 bacterial strains from sediment samples collected from the Camarones River in Northern Chile. *Enterobacter* and *Pseudomonas* were amongst the majority of genera that were recovered. Ndlovu (2013) was able to identify 82% and 75% of colonies isolated from the Berg- and Plankenburg Rivers, respectively.

1.7.6 BBL Crystal™ Gram-Positive Identification system

The BBL Crystal™ Gram-Positive (GD) Identification (ID) System is an 18 hour miniaturised identification method that uses modified conventional, fluorogenic and chromogenic substrates. It is intended for identification of both Gram-positive cocci and bacilli (Bullock & Aslanzadeh, 2013). The system panels consist of 29 dehydrated substrates and a fluorescence control on tips of plastic points (Becton Dickinson [BD], 2012). Venter (2010) found the BBL Crystal™ GP ID System to be reliable and successfully identified 80% of isolates subjected for identification, which included *Bacillus* spp. such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. megaterium* and *Kocuri rosea.* Preliminary identification of *Enterococcus durans* and *Helcococcus kunzii* with the BBL Crystal™ GP ID System requires further confirmation tests in order to eliminate the possibility of the species being *E. faecium.* This is due the fact that Vancomycin-Resistant *E. faecium* produce a substrate reaction, which can lead to *E. faecium* being misidentified as *E. durans* or *H. kunzii* (BD, 2012). However, Stovcik *et al.* (2008) successfully used the BBL Crystal™ GP ID System to identify *E. faecium.*

1.8 OBJECTIVES OF THE RESEARCH

The main aim of this study was to determine the microbial contamination level of stagnant greywater in the RR Section of Khayelitsha, Western Cape. The objectives included the following:

- To identify, select and sample six water points (stagnant water underneath standpipes) twice on a monthly basis for a period of five months;
- To determine the level of bacterial contamination in the stagnant pools by means of the Most Probable Number (MPN) techniques;
- To determine the total bacterial counts in the stagnant water by means of the Heterotrophic Plate Count technique and the Flow Cytometric Analysis using liquid counting beads and the LIVE/DEAD® *Bac*Light™ Probe;
- To isolate and identify Gram-negative bacteria present in stagnant pools by means of the API 20E and the RapID™ ONE systems;
- To isolate and identify Gram-positive bacteria present in stagnant pools by means of the BBL Crystal™ Gram Positive (GP) Identification system.

MATERIALS AND METHODS

2.1 SAMPLING SITES

Given that RR section is a homogeneous area with informal housing and communal taps for domestic use, six sampling points (**Figure 2.1**) were selected for routine monitoring and evaluation of the greywater at the base of communal standpipes in the area. Sampling points were recognised as the stagnant water accumulated at the bottom of communal standpipes (**Figure 2.2**).

2.2 SAMPLING

Two hundred and fifty millilitres water samples were collected in sterile Schott bottles as per the South African Bureau of Standards (SABS) water quality sampling standards (SABS, 2008). The temperature and pH of the water samples were measured with a handheld mercury thermometer and portable pH meter, respectively. Water samples were collected by immersing a sterile Schott bottle into the stagnant pools. The samples were stored, and transported in an ice-filled cooling container to maintain a low temperature and taken directly to the laboratory for Most Probable Number analysis (Paulse *et al.*, 2007; 2009). Most Probable Number analysis was performed within six hours after sample collection (Paulse *et al.*, 2007; 2009). Samples where then stored at 4°C for further analysis. Sampling was done twice a month for a period of five months.

2.3 ENUMERATION TECHNIQUES

2.3.1 Heterotrophic Plate Count

The Heterotrophic Plate Count (HPC) method was used according to the method as described by Paulse *et al.* (2007; 2009). A serial dilution of 10^{-1} to 10^{-5} of each sample was performed and spread-plated (in triplicate) onto Nutrient Agar (NA) (Biolab Diagnostics) plates and incubated for 1 to 2 days at 37°C. Microorganisms per millilitre (microorganisms/mℓ) were counted and recorded.

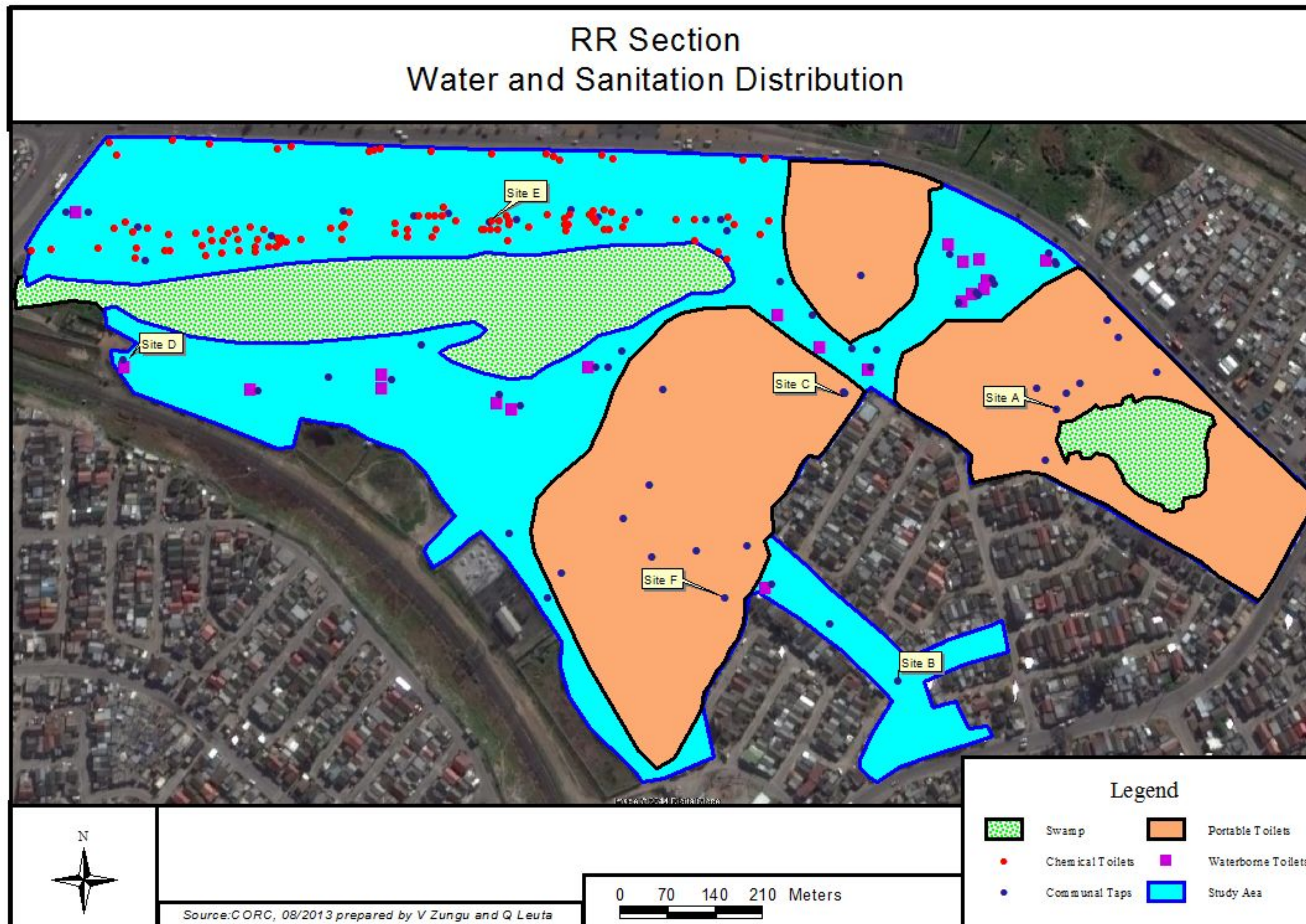


Figure 2.1 A Map of RR Section showing all the six sampling sites adapted from CORC (2013).

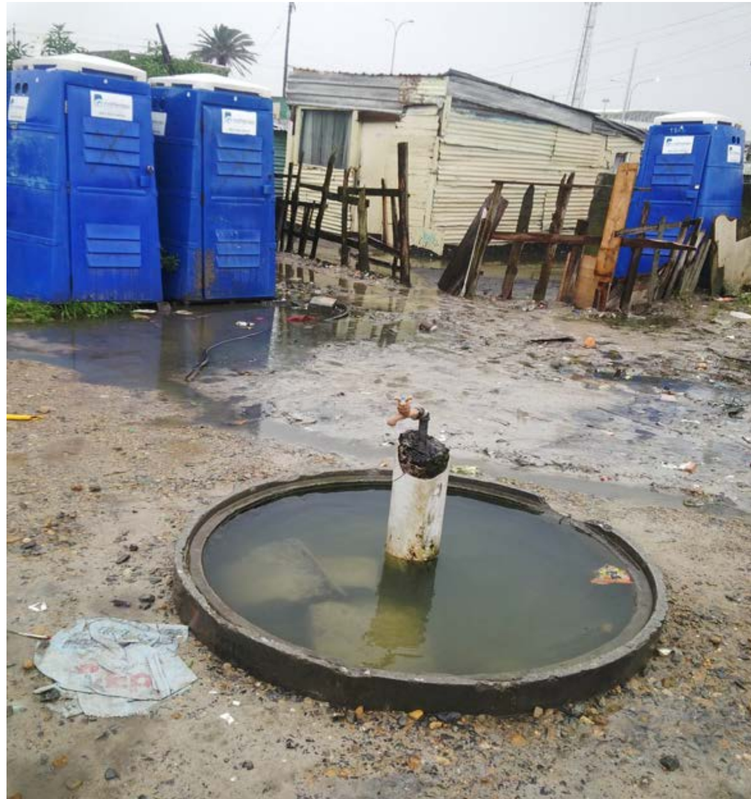


Figure 2.2 Stagnant water accumulating at the bottom of a communal tap – Site E sampling point.

2.3.2 Most Probable Number (MPN) technique

The Most Probable Number technique or Multiple Tube Fermentation technique was used according to the method described by Paulse *et al.* (2007; 2009) (**Figure 2.3a, b, c**). A serial dilution 10^{-1} to 10^{-5} of the water samples was performed before inoculation into 1mℓ of each diluted sample into Lauryl Tryptose Broth (LTB) tubes containing Durham tubes. Durham tubes provide a visual indication of gas production, which was regarded as a positive presumptive test. Five millilitres undiluted sample was inoculated into five individual tubes containing 5mℓ double strength LTB. In addition, 1mℓ undiluted sample and 1mℓ of each diluted (10^{-1} to 10^{-5}) sample were inoculated into five individual tubes containing 10mℓ single strength LTB. This was followed by incubation for 48 hours at 37°C. All tubes demonstrating gas production were regarded as a positive presumptive test and the counts read off the De Mans tables obtained for this purpose. For each positive presumptive LTB tube, a 10mℓ Brilliant Green Bile Broth (BGBB) tube and 10mℓ Tryptone Water (TW) tube was prepared. One hundred microlitres of the sample from each positive LTB tube was re-inoculated into a 10mℓ BGBB tube and a corresponding TW tube, respectively. The tubes were incubated in a 44°C waterbath for 24 hours. With each positive gas producing BGBB tube (indicating the presence of faecal coliforms), a few drops of Ehrlich's reagent was added to the samples' corresponding TW tube.

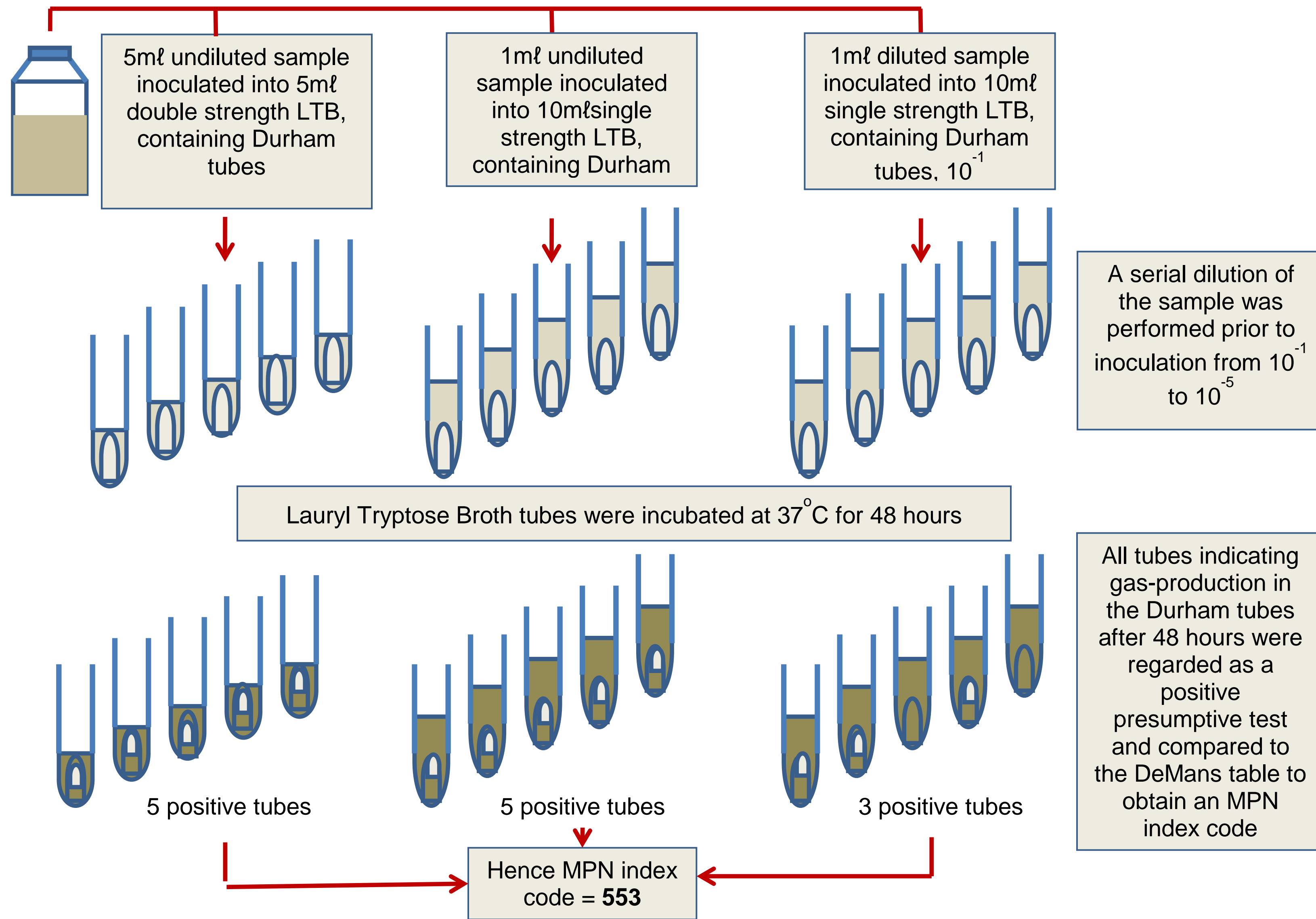


Figure 2.3a Inoculation of undiluted and diluted water samples into double and single strength LTB tubes to obtain positive presumptive test results.

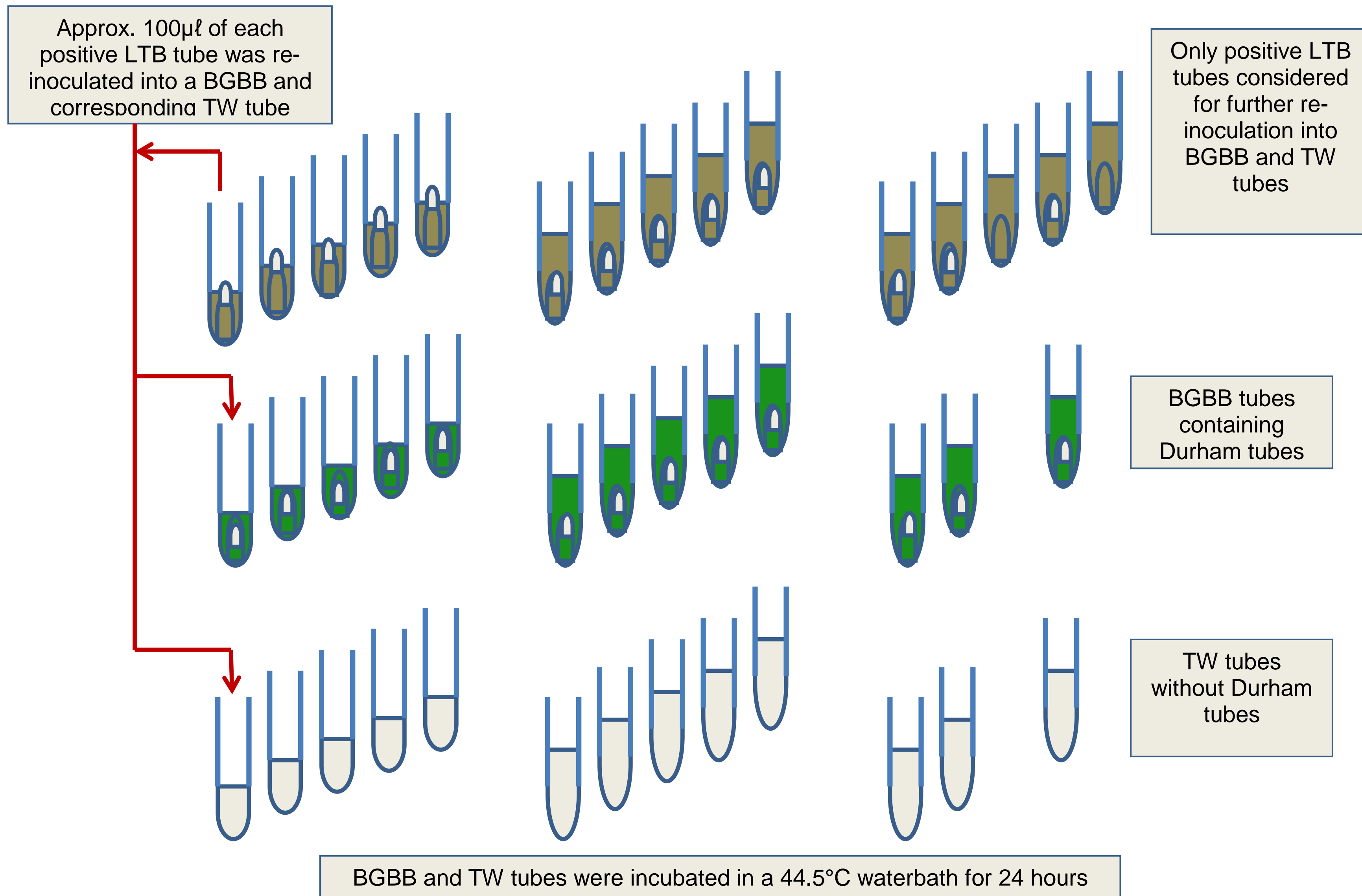


Figure 2.3b Re-inoculation of positive LTB tubes into corresponding BGBB and TW tubes for further analysis of feacal coliforms and E. coli.

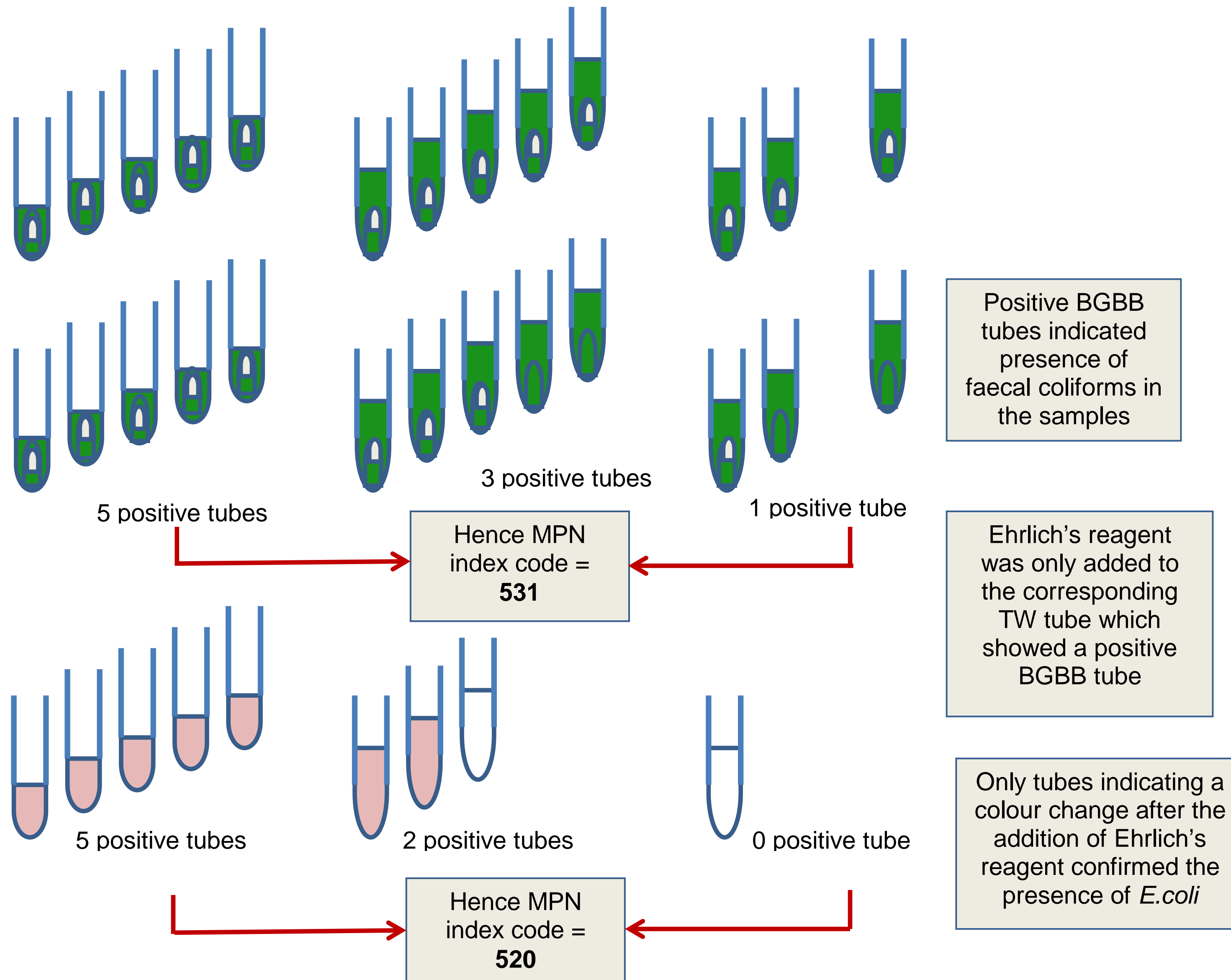


Figure 2.3c Enumeration of faecal coliforms (from BGBB tubes) and E. coli (from TW tubes) in water samples.

A colour change of clear to pink resulting from the addition of Ehrlich reagent indicated the presence of *E. coli* in that particular sample. The positive BGBB and TW tubes were once again compared to the corresponding De Mans codes to obtain the respective faecal coliform and *E. coli* counts.

2.3.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe

Individual samples were analysed with a Becton Dickinson FACSCalibur, which has a 15mW, 488 nm argon-iron laser (Paulse *et al.*, 2007). Bacterial cells and debris were differentiated by a combination of doublet discrimination modules (which uses pulse width and area to eliminate cell clumping i.e. doublets and triplets) and the LIVE/DEAD® *Bac*Light™ Viability Probe. Light scattering, light excitation and emission of fluorochrome molecules were used to generate data from particles or cells in the size range of 0.5 µm in diameter. Fluorescent beads were added in order to make it possible to calculate absolute or total cell counts in the samples. Comparison of cellular events with the bead events measured by the flow cytometer, determined the absolute number (cells/µℓ) of positive cells in a sample. Bacterial population was identified and gated on a forward scatter (FSC) versus side scatter (SSC). A logarithmic amplification scale was used to measure all parameters. A threshold of 52 FSC channels was set for the removal of sample debris. Only bacterial cells satisfying both gates were considered for further analysis.

Equal volumes of 4µℓ of propidium iodine (PI) and 4µℓ of Thiazole Orange (TO) 9 in *Bac*Light™, were dissolved in one millilitre of sterile distilled water. One millilitre of the water sample was then stained with 200µℓ *Bac*Light™ and then kept in the dark for 15 minutes in order for the stain to react or attach to bacterial cells within the samples. Fifty microlitres of liquid counting beads (BD™ Cell Viability Kit, BD™ Liquid Counting Beads) were then added to the stained sample. The sample was analysed with the FACSCalibur and the concentrations of total cell populations were determined by using the following equation:

Equation 1:

$$\frac{\text{Number of events in cell region}}{\text{Number of events in bead region}} \times \frac{\text{Number of beads/test}}{\text{Test volume}} \times \text{Dilution factor}$$

Note: Bead concentration recorded at 1043µℓ for BD Liquid Containing Beads obtained from BD™.

To avoid excessive compensation of fluorescence overlap, TO9 green emittance was measured in fluorescence channel 1 (FL-1) at 530/30 nm, while the PI was measured in fluorescence channel 3 (FL-3) at 670/LP nm. Each water sample was examined by the FACSCalibur until 250 counting beads were detected. An *E. coli* laboratory strain was used as a positive control.

2.3.4 Statistical analysis

Repeated measures ANOVA (RMA) was performed on all data as outlined by Dunn and Clark (1987) using Statistica™. In each RMA, the residuals were analysed to determine their normal distribution. In all hypothesis tests, a significant level of 5% was used as standard.

2.4 IDENTIFICATION TECHNIQUES

2.4.1 API 20E system

A serial dilution of 10^{-1} and 10^{-3} were performed on the obtained samples. The diluted sample was directly spread plated (in duplicate) onto selective media, which included MacConkey (MAC)-, MacConkey Without Salt (MAC-WS)- and Eosin Methylene Blue (EMB) agars and incubated at 37°C for 18 – 24 hours as per manufacturers' instructions. The isolated colonies were then re-streaked onto Nutrient Agar (NA) plates and incubated for 18 – 24 hours as per manufacturer's instructions. Prior to identification, the Gram stain and oxidase test were performed on each colony and results were recorded. Only Gram-negative and oxidase-negative colonies were considered for further analysis. The API 20E strips were prepared according to manufacturer's instructions (BioMérieux, South Africa). The strip was prepared by adding 5mℓ distilled water into the combed wells of the tray. The strain reference was recorded on the elongated flap of the tray. A single colony was suspended in 5mℓ sterile saline and thoroughly emulsified to achieve a homogeneous bacterial suspension. The suspension was immediately added to each tube of the strip. The strip was then incubated for 18 – 24 hours at 37°C. Thereafter, the strip was read and recorded as per the manufacturer's instructions. The Apiweb™ identification software (BioMérieux, South Africa) was used to identify the organisms. An *E. coli* laboratory strain was used as a positive control.

2.4.2 RapID™ ONE system

Bacterial suspensions were prepared in the same manner as for the API 20E test kits. As with the API 20E system, only Gram-negative and oxidase-negative colonies were considered for further analysis. The RapID™ ONE System (Innovative Diagnostic Systems, Inc.) procedure was followed according to the manufacturer's instructions. Colonies from the NA plates were suspended into 2mℓ of the RapID™ Inoculation Fluid. After achieving a turbidity corresponding to the McFarland No. 2 Standard, the bacterial suspension was inoculated into the panels of the RapID™ ONE kits and incubated at 37°C for four hours. Thereafter, two drops of spot indole were added to well 18, whereby colour development was not allowed for more than two minutes. Colour changes were interpreted and recorded in an appropriate box of the report form according to manufacturer's instructions. Specific isolated organisms were identified by the Electronic RapID™ Compendium on the manufacturer's website. An *E. coli* laboratory strain was used as a positive control.

2.4.3 BBL Crystal ™ Gram Positive ID Identification System

A serial dilution of 10^{-1} and 10^{-3} were performed on the obtained samples. The diluted samples were directly spread-plated (in duplicate) onto selective media, which included Sabouraud Dextrose (SD) and Baird Parker (BP) agars. The isolated colonies were then re-streaked onto NA plates and incubated at 37°C for 18 – 24 hours as per manufacturers' instructions. Prior to identification, a Gram stain was performed on each isolated and selected colony and the results recorded. Only Gram-positive colonies were considered for further analysis. The BBL Crystal™ Gram Positive (GP) Identification (ID) System (Becton Dickinson Microbiology System) procedure was followed according to the manufacturer's instructions. Pure colonies were suspended into the BBL inoculum fluid (2.3 ± 0.15mℓ) tubes to achieve a visual turbidity equivalent to a 0.5 McFarland Standard. A sterile loop was used to recover a small drop from the inoculum fluid and spread-plated onto nutrient agar to obtain a purity plate. The entire contents of the inoculum fluid tube were then poured into the base of a test strip. The lid was snap shut in place. The inoculated panels were incubated face down in a 40 – 60% humidity incubator at 37°C for 18 - 24 hours. This was followed by reading all panels using the BBL Crystal Panel Viewer to interpret the reactions. The reaction results were recorded on the results pad to get a possible isolate profile number. This number as well as the cell morphology was then entered into BBL Crystal MIND software to identify the respective Gram-positive isolates. A *B. cereus* laboratory strain was used as a positive control.

RESULTS AND DISCUSSION

3.1 PHYSICAL PARAMETERS

The water temperature and pH values of the samples were measured and recorded at the sites during the sampling period as illustrated in **Table 3.1**. The lowest temperature recorded for all the sites were 8°C (week 8, Site C) during the winter months, while the highest temperature recorded for all the sites were 25°C (week 3, Sites A and F as well as week 4, Site F) during the summer months. The lowest pH reading recorded for all the sites was 4.8 (Site D, week 5), while the highest pH reading recorded was 7.7 (Site E, week 6). The pH and seasonal variation in water temperature may have a significant impact on the life cycle and growth rate, abundance and distribution of planktonic microbial organisms (Khalil *et al.*, 2008).

Table 3.1 Physical parameters recorded during the sampling period at all sites.

Sampling week	Site A		Site B		Site C		Site D		Site E		Site F	
	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	pH
1	24	6.2	17.5	6.4	18	6.2	20	6.4	19.5	6.4	21	6.7
2	22	6.2	19	5.4	18	5.8	22	6.7	19	6.4	19	6.7
3	25	6	21	6	20	6	22	5	22	6	25	6.5
4	24	5.8	21	5.8	20	6.2	23	6.7	22	6.2	25	6.4
5	20	6.4	18	6.7	19	6.2	19	4.8	19	5.8	18	6.2
6	17	7.2	16	7.5	15	7.2	16	7.4	16	7.7	11	7.2
7	12	6.3	13	7.2	13	7.4	13	7.4	15	7.2	17	6.9
8	11	6.4	10	6.7	8	6.5	9	6.4	10	6.6	12	6.6
9	12	7.1	12	7.1	11	7	12	6.4	11	6.7	13	6.2

3.2 ENUMERATION OF BACTERIAL CONTAMINANTS IN STAGNANT WATER POOLS

As previously mentioned, the Heterotrophic Plate Count (for total culturble microorganisms), the Most Probable Number (for total coliform, faecal coliform and *E. coli*) and Flow Cytometric techniques were performed on water samples collected from stagnant water pools at the base of the communal taps, which marked the 6 sampling points in RR Section in Khayelitsha, Western Cape, South Africa. In order to determine acceptable levels of indicator organisms in drinking and recreational water, respectively, the Department of Water Affairs and Forestry (DWAF, 1996a; DWAF, 1996b) and the South African Bureau of Standards (SABS, 2011) have developed guidelines for drinking and recreational water, which stipulates acceptable levels of indicator organisms.

According to the DWAF (1996a) and SABS (2011) guidelines for domestic water, water to be used for domestic purposes, should not contain total coliform of more than 5 microorganisms/100mℓ and 10 microorganisms/100mℓ, respectively. Both guidelines stipulate that domestic water should not contain any faecal coliforms. These institutions' guidelines further indicate that domestic water should be absolutely free of any *E. coli*. For recreational purposes, the DWAF (1996b) guidelines stipulate that water used for recreational purposes should not exceed an *E. coli* count of 130 microorganisms/100mℓ. Furthermore, the Recreational Water Quality Guideline stipulates that recreational water should not contain faecal coliforms of more than 1000 microorganisms/100mℓ. According to DWAF (1996a) water with microbial counts exceeding these limits may lead to increased waterborne infections.

3.2.1 Most Probable Number (MPN) technique

The Most Probable Number (MPN) method was used to determine the levels of planktonic organisms associated with faecal pollution. This technique enabled the measurement of total coliforms, faecal coliforms (FC) as well as *E. coli* organisms within water samples (**Figure 3.1**). All of the MPN and FC counts recorded exceeded the acceptable limits (DWAF, 1996a, DWAF, 1996b; SABS, 2011). Most Probable Number counts ranged between 4.5×10^4 microorganisms/100mℓ (lowest) recorded at Site E (week 4) and 1.6×10^8 microorganisms/100mℓ (highest) detected at Site A (weeks 3 and 5) as well as at Site B (week 5). The lowest FC counts were 1.3×10^3 microorganisms/100mℓ and 1.4×10^4 microorganisms/100mℓ obtained at Site E in week 4 and Site F in week 7, respectively.

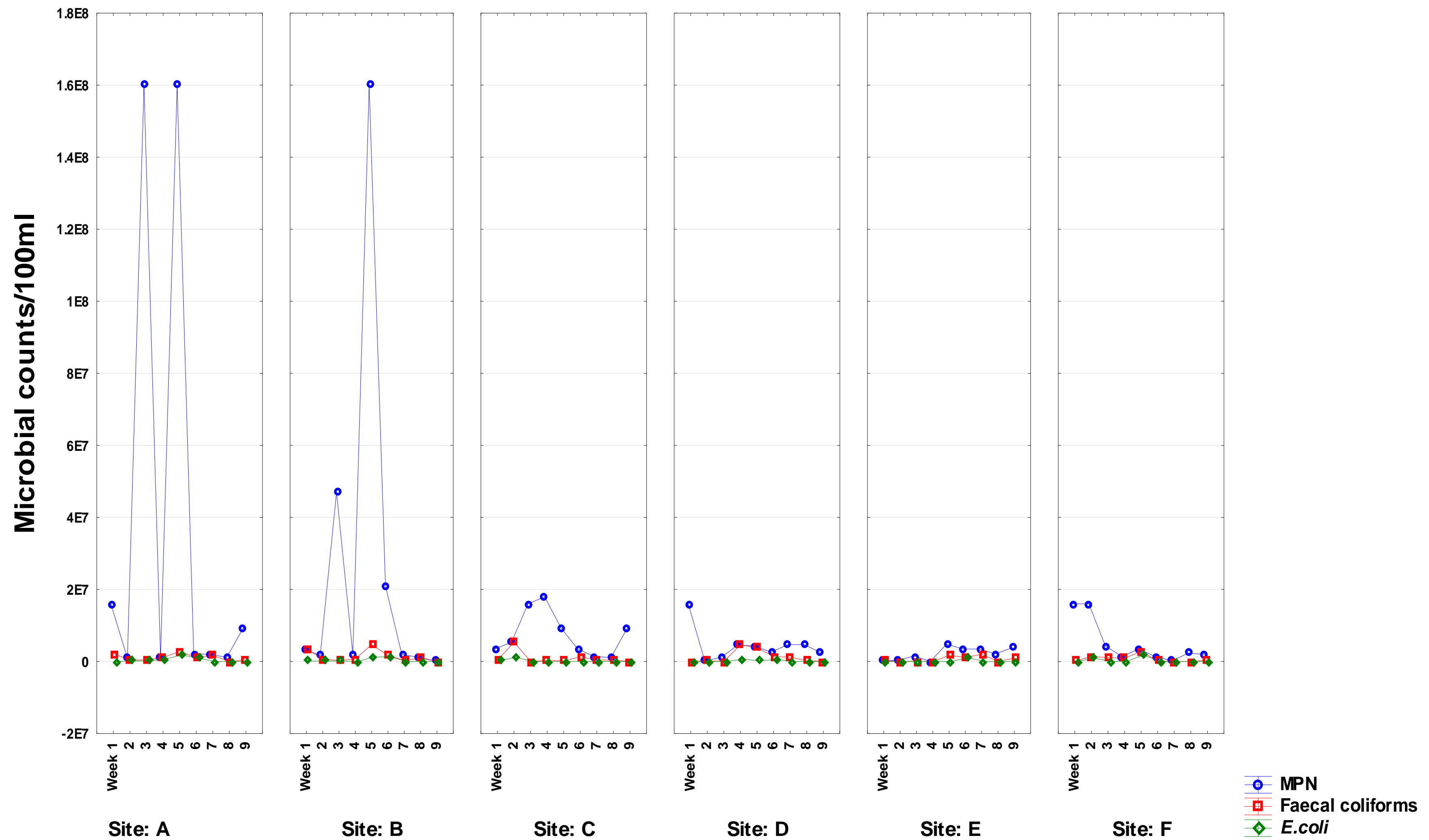


Figure 3.1 Comparison of the Most Probable Number (MPN), demonstrating all possible gas-producing organisms, faecal coliforms (FC), and E. coli per 100 mℓ of stagnant water pools sample for all sites.

The corresponding highest faecal coliform (FC) counts were 4.7×10^6 microorganisms/100mℓ and 4.6×10^6 microorganisms/100mℓ recorded at Site B (week 5) and Site D (week 4), respectively. The highest counts recorded at Site B were also the highest counts recorded throughout the entire sampling period. The *E. coli* counts recorded during the sampling period ranged from zero counts recorded at Site F (week 9) to 1.8×10^6 microorganisms/100mℓ recorded at Site A (week 5) and Site F (week 5). The lowest *E. coli* counts were 70 microorganisms/100mℓ, 200 microorganisms/100mℓ and zero counts all observed at Site F in weeks 7, 8 and 9, respectively. In comparison, the highest *E. coli* counts were 1.3×10^6 microorganisms/100mℓ recorded at Site A (weeks 5 and 6) and 1.8×10^6 microorganisms/100mℓ recorded at Site F (week 5).

The results show that the level of faecal contamination is significantly ($p < 0.05$) higher than the guidelines set by DWAF (1996a; 1996b) and the SABS (2011). In addition, the majority of the *E. coli* counts exceeded the stipulated acceptable limits (DWAF, 1996b; SABS, 2011) with the exception of two occasions. No *E. coli* were observed at Site F in week 9. A low count was also recorded at Site F in week 7 (70 microorganisms/100mℓ), which met the DWAF (1996b) recreational allowable limit, but not the SABS (2011) drinking water standard.

The high MPN, FC and *E. coli* counts observed during this study seem to show constant high counts compared to previous studies conducted in RR Section. Carden *et al.* (2007) conducted a study in RR Section and recorded *E. coli* counts as high as 1.8×10^4 microorganisms/100mℓ. As previously mentioned, Barnes (2010) on the other hand, found counts of 6.2×10^7 microorganisms/100mℓ for total coliforms and 2×10^6 microorganisms/100mℓ for *E. coli*. Both studies by Carden *et al.* (2007) and Barnes (2010), however, do not show a significant trend in the microbial contamination in RR Section over a period of time as sampling was done only once. The current study could however, serve as a continuation to both studies as microbial counts still proved to be significantly higher ($p < 0.05$) than the guidelines by DWAF (1996a; 1996b) and SABS (2011).

3.2.2 Heterotrophic Plate Count (HPC)

The total heterotrophic plate counts for Sites A to C obtained throughout the sampling period are shown in **Figure 3.2**. The highest HPC obtained were 9.3×10^4 microorganisms/mℓ, 1.2×10^5 microorganisms/mℓ, and 2.9×10^5 microorganisms/mℓ at Site A (week 2), Site B (week 6) and Site C (week 4), respectively. The lowest counts were 1.7×10^3 microorganisms/mℓ, 1.4×10^3 microorganisms/mℓ and 1.4×10^3 microorganisms/mℓ obtained at Site A (week 7), Site B (week 4) and Site C (week 8), respectively.

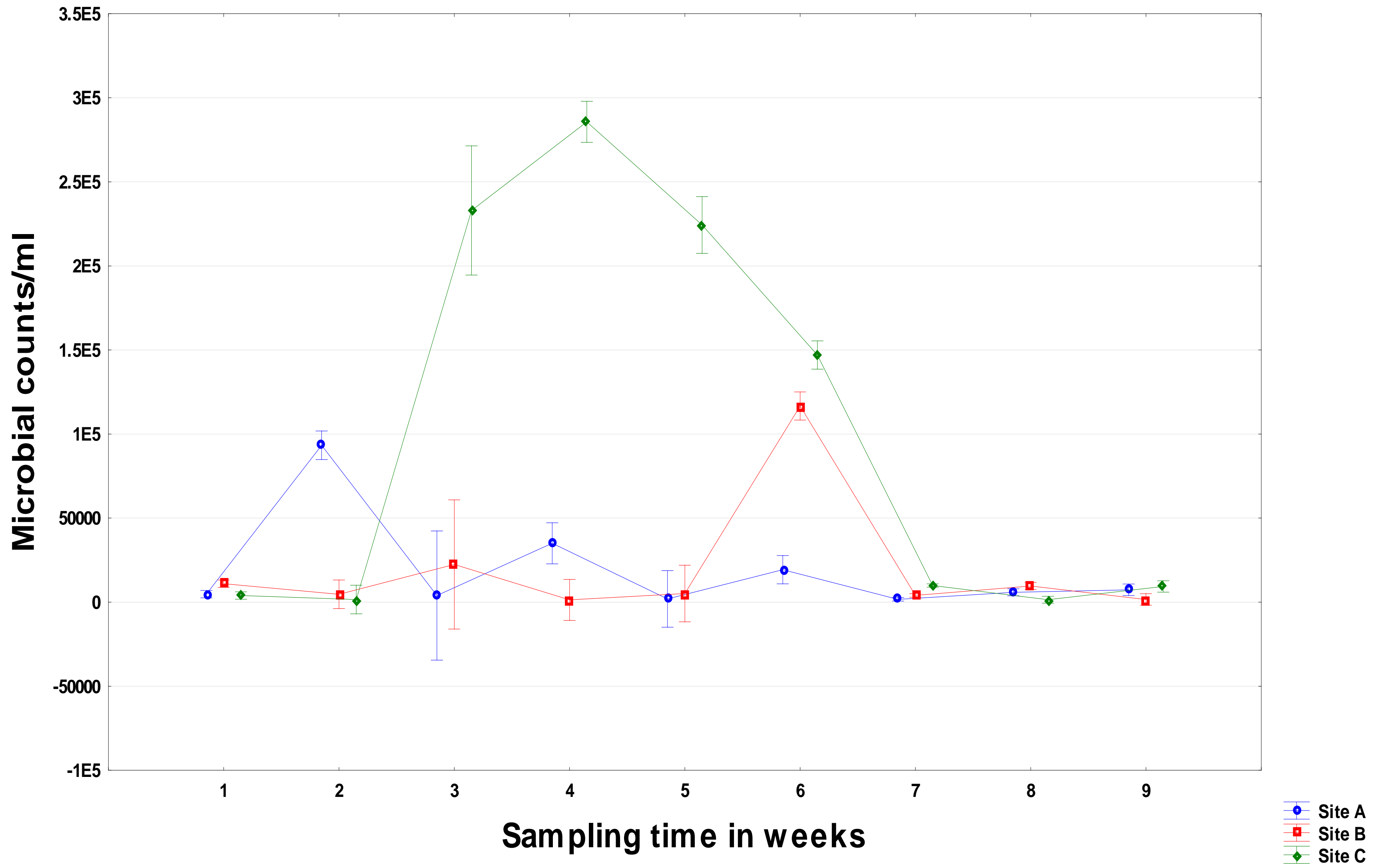


Figure 3.2 Average heterotrophic plate counts for Site A to Site C analysed and recorded over the sampling period.

Figure 3.3 shows the HPC counts for Sites D to F. The highest counts observed were 1.6×10^5 microorganisms/mℓ (Site D; week 5), 2.8×10^4 microorganisms/mℓ (Site E; week 5) and 2.3×10^5 microorganisms/mℓ (Site F; week 1). The lowest counts were observed at Sites D (week 8), E (week 7) and F (week 3) where counts of 1.1×10^3 microorganisms/mℓ, 1.1×10^3 microorganisms/mℓ and 6.8×10^3 microorganisms/mℓ were measured. As mentioned, the highest HPC recorded throughout the sampling period was 2.9×10^5 microorganisms/mℓ at Site C (week 4), while the lowest count observed was 1.1×10^3 microorganisms/mℓ at Site D (week 8) and Site E (week 7).

According to the SABS (2011) and DWAF (1996a) guidelines, water with HPC counts higher than 1000 microorganisms/mℓ and 100 microorganisms/mℓ, respectively, may pose an increased health risk where such water is used for domestic purposes. All heterotrophic plate counts recorded throughout the sampling period were significantly ($p < 0.05$) higher than the SABS (2011) [1000 microorganisms/mℓ] and DWAF (1996a) [100 microorganisms/mℓ] guidelines. Even though sampled water in this study is considered to be greywater by the Department of Water Affairs (DWAF, 2001a), it was observed that this water was used for hand washing by some of the community members instead of the installed standpipes. In addition, children make use of the area around the stagnant pools as a playground. With the high microbial counts recorded, it is evident that the stagnant pools are not suitable for domestic purposes.

According to WHO (2011b), the 100 microorganisms/mℓ guideline is not directly associated with health risk, but it simply reflects the efficiency of water disinfection and water treatment processes as well as determining the aesthetic quality of water. According to DWAF's (1996b) Water Quality Guidelines for Recreational Use, contact with microbially contaminated water may cause skin and ear infections as well as gastroenteric diseases. The severity of these diseases depends on the nature of the microbial contaminants as well as their mode of contamination (DWAF, 1996b). Venter (2010) indicated that there should be a health concern whenever high HPC bacterial counts are present in domestic water, as health related issues may be associated with high HPC bacterial counts. Although HPC has been proven to only represent less than 1% of the total microbial organisms (Hammes *et al.,* 2008; Siebel *et al.*, 2008; Hammes *et al.*, 2010), it is still a commonly used and accepted method for indicating water quality (Allen *et al.*, 2004; Burtscher *et al.*, 2009). Various studies have used this method to indicate microbial quality of water (Paulse *et al.,* 2007; Paulse *et al.,* 2009; Hammes *et al.,* 2008).

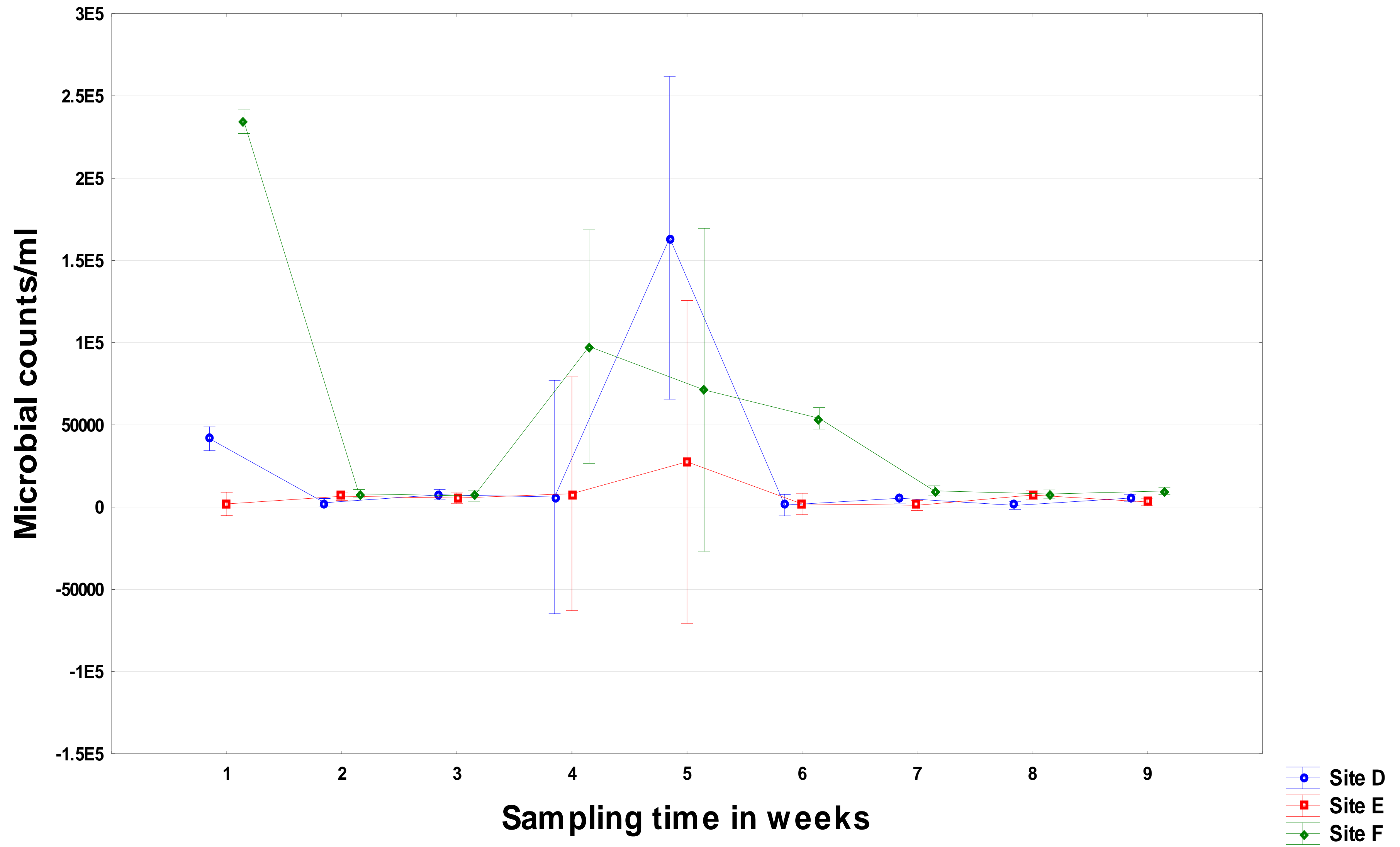


Figure 3.3 Average heterotrophic plate counts for Site D to Site F analysed and recorded over the sampling period.

As indicated earlier, the high microbial counts observed in this study, is a possible health hazard to the RR Section community particularly the children who use the stagnant water pools as a playground. These children are at risk of contracting skin and ear infections as well as other infectious diseases from contact with possible opportunistic pathogenic organisms. Therefore, the Water Quality Guidelines should be revised to meaningfully address the HPC guidelines.

Comparisons of MPN counts to HPC counts are depicted in **Figure 3.4**. The highest MPN results were observed in weeks 3 and 5 at Site A as well as in week 5 at Site B. On all three occasions, the counts recorded were 1.6 x 10^8 microorganisms/100mℓ. In comparison, the HPC at these sites were 4 x 10^5 microorganisms/100mℓ (Site A, week 5), 2 x 10^5 microorganisms/100mℓ (Site A, week 5) and 5.1 x 10^5 microorganisms/100mℓ (Site B, week 5). When considering that the MPN technique only accounts for the gas-producing microorganisms in the water samples, which in itself is a fraction of the total microbial population which the HPC represents, it is clear that the HPC technique is not a true reflection of the total possible count of all microorganisms in the test water sample. Even though the MPN counts did not exceed the HPC counts at all the sampling sites throughout the sampling period, results show that the MPN counts exceeded the HPC counts 70% of the time throughout the sampling period. However, irrespective of which technique yielded the better result, both the HPC and MPN techniques displayed significantly ($p < 0.05$) higher counts than the SABS (2011) and DWAF (1996a; 1996b) guidelines for domestic and recreational water, which in itself may pose a major health risk to the inhabitants of the informal settlement.

3.2.3 Flow Cytometric Analysis (FCM) and LIVE/DEAD® *Bac*Light™ Viability Probe

The LIVE/DEAD® *Bac*Light™ Viability Probe enables the distinction between the live and dead cells through fluorescence intensities (**Figure 3.5**), i.e. either red or green fluorescence. The Propidium Iodide (PI) is a red fluorescing dye, which enters dead bacteria when their cell membranes are compromised, while Thiazole Orange 9 (TO9) is a green fluorescing dye, which enters all cells (Berney *et al.*, 2007). Propidium Iodide therefore differentiates the dead cells from the viable cells. The total FCM counts recorded throughout the sampling period are represented in **Figure 3.6**.

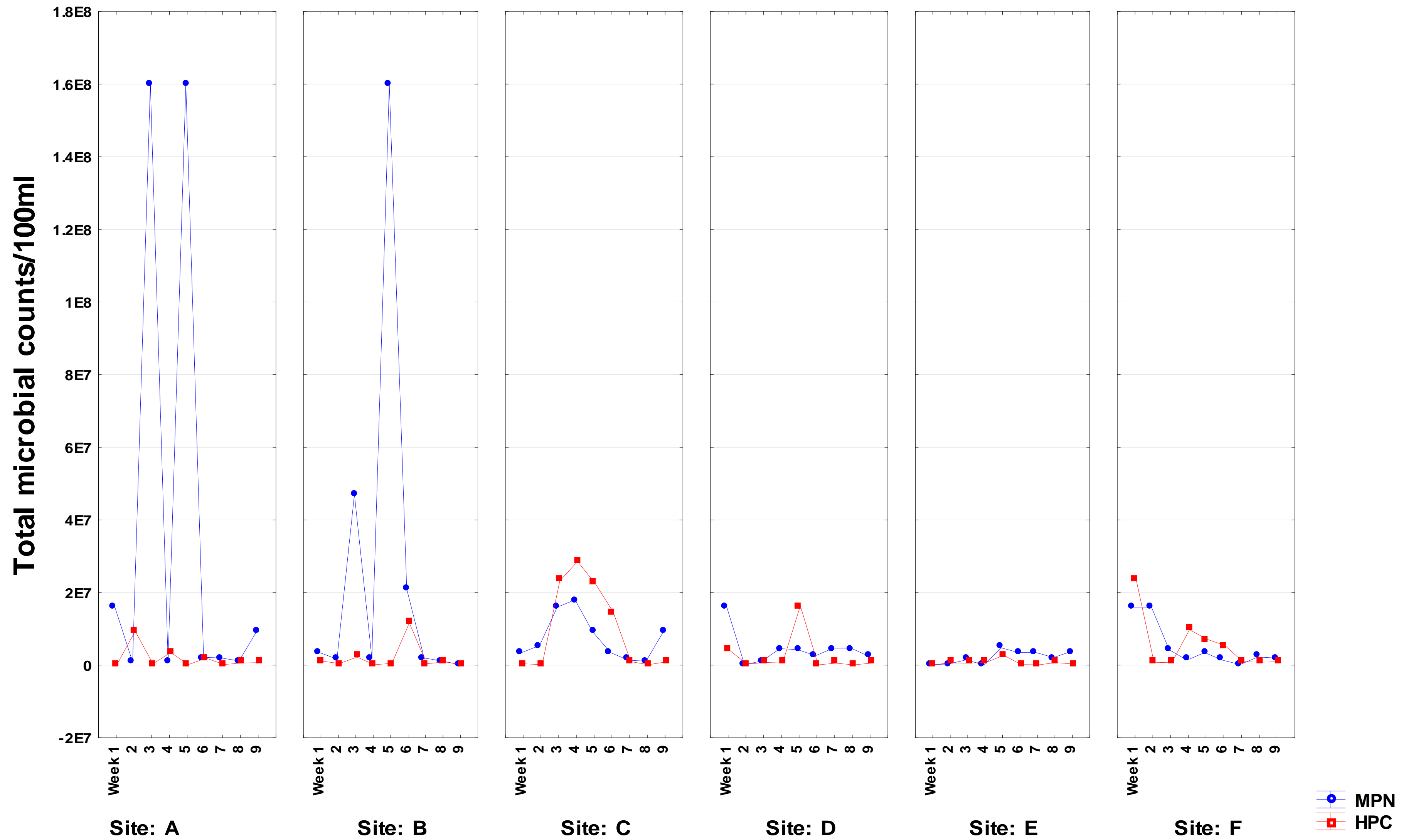


Figure 3.4 Comparison of heterotrophic plate counts (HPC) and the most probable number (MPN), representing all possible gas-producing organisms.

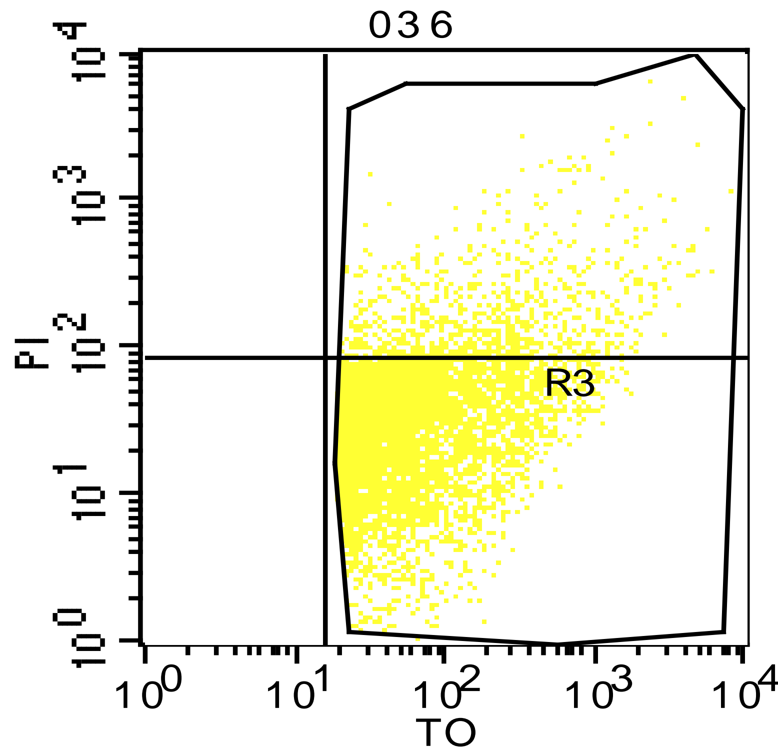


Figure 3.5 Total cell counts from the stagnant water pool samples using flow cytometric analysis: the upper right and lower right quadrats indicate dead and live cells.

The total FCM counts ranged from the lowest, 4×10^4 microorganisms/mℓ at Site E in week 1 to the highest, 3.4×10^7 microorganisms/mℓ at Site A in week 5. The highest total FCM counts recorded at each of the sampling sites throughout the entire sampling period were 3.4×10^7 microorganisms/mℓ (Site A, week 5), 8.9×10^6 microorganisms/mℓ (Site B, week 5), 2.4×10^6 microorganisms/mℓ (Site C, week 6), 9.6×10^6 microorganisms/mℓ (Site D, week 6), 2×10^6 microorganisms/mℓ (Site E, week 3) and 1.1×10^7 microorganisms/mℓ (Site F, week 5). More or less the same daily activities were observed at the various sampling sites during week 5. It is thus unknown as to why Site A had significantly higher counts compared to the rest of the sites.

In comparison, the total FCM counts were significantly ($p < 0.05$) higher than the total MPN counts. **Figure 3.7** illustrates the comparison of MPN results to the total FCM results. When compared to the highest total FCM counts, as mentioned above, the total MPN counts were 1.6×10^7 microorganisms/100mℓ (Site A, week 5), 1.6×10^8 microorganisms/100mℓ (Site B, week 5), 3.5×10^6 microorganisms/100mℓ (Site C, week 6), 2.6×10^6 microorganisms/100mℓ (Site D, week 6), 1.5×10^6 microorganisms/100mℓ (Site E, week 3) and 3.5×10^6 microorganisms/100mℓ (Site F, week 5). This proves to be an obvious expectation as the MPN technique only accounts for the gas-producing microorganisms in

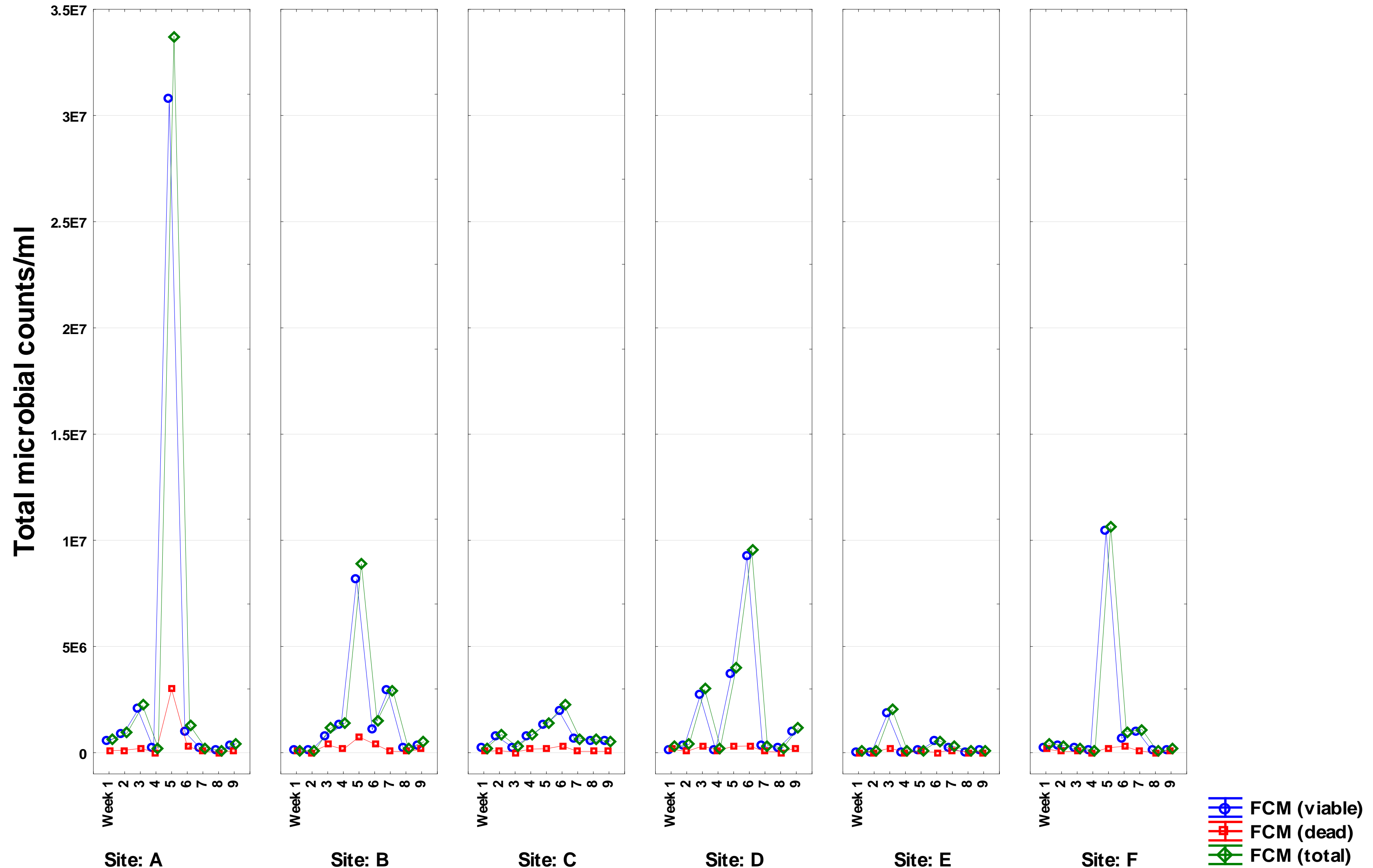


Figure 3.6 FCM results of total, viable and dead bacterial cells within stagnant water samples at RR Section.

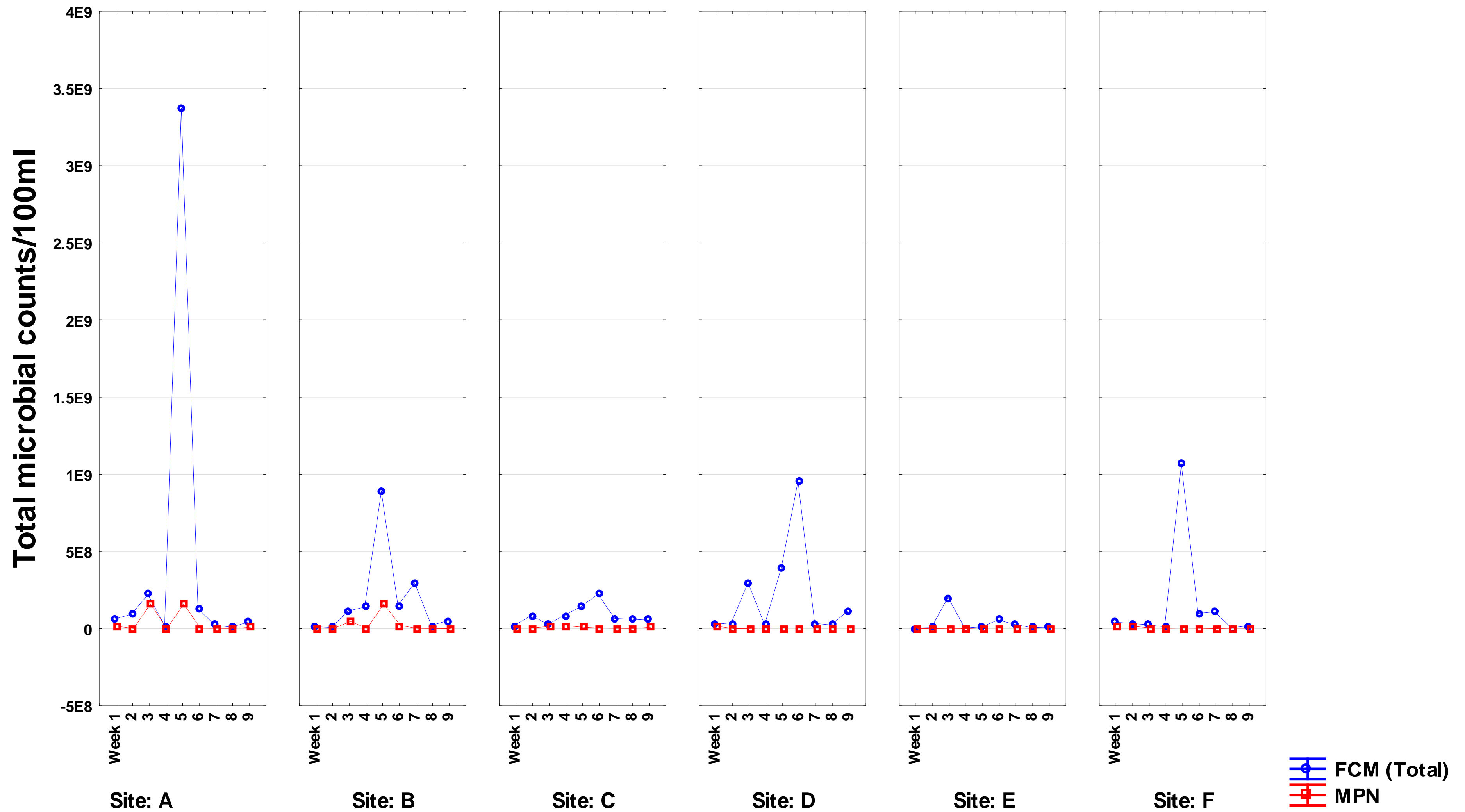


Figure 3.7 Comparison of MPN (representing all possible gas-producing organisms) results to total FCM results.

the water samples, thus illustrating a fraction of the total microbial count as indicated by the FCM technique.The high total FCM counts observed at these sites during the sampling period is clearly a result of microorganisms other than the gas-producers.

When comparing the total FCM to the HPC counts (**Figure 3.8**), the total FCM counts were significantly ($p < 0.05$) higher than the total HPC counts. As mentioned the highest FCM counts were 3.4×10^7 microorganisms/mℓ (Site A, week 5), 8.9×10^6 microorganisms/mℓ (Site B, week 5), 2.4×10^6 microorganisms/mℓ (Site C, week 6), 9.6×10^6 microorganisms/mℓ (Site D, week 6), 2×10^6 microorganisms/mℓ (Site E, week 3) and 1.1×10^7 microorganisms/mℓ (Site F, week 5). In comparison, the HPC counts recorded at these sites were 2×10^4 microorganisms/mℓ, 5.1×10^4 microorganisms/mℓ, 1.5×10^5 microorganisms/mℓ, 1.2×10^4 microorganisms/mℓ, 5.5×10^3 microorganisms/mℓ and 7.1×10^4 microorganisms/mℓ, respectively. It is evident that the total FCM counts were significantly higher ($p < 0.005$) than the total HPC counts.

In addition, when comparing the counts obtained from the HPC (**Figure 3.8**) technique (which only indicates the viable culturable microorganisms/mℓ) to the highest viable FCM count, the viable FCM counts yielded significantly ($p < 0.05$) higher counts. The highest viable FCM counts observed at the same sites were 3.1×10^7 microorganisms/mℓ (Site A, week 5), 8.2×10^6 microorganisms/mℓ (Site B, week 5), 1.9×10^6 microorganisms/mℓ (Site C week 6), 9.2×10^6 microorganisms/mℓ (Site D, week 6), 1.8×10^6 microorganisms/mℓ (Site E, week 3) and 1×10^7 microorganisms/mℓ (Site F, week 5). Whereas the highest HPC result of 2.87×10^5 microorganisms/mℓ was significantly ($p < 0.05$) higher than the allowable limit as set out by the DWAF (1996a) and the SABS (2011), it accounted for only 32.43% (when compared to total FCM) and 40.24% (when compared to viable FCM) counts recorded by the FCM technique, which highlights the inaccuracy of the HPC technique. The lower counts obtained by the HPC technique compared to the FCM technique were observed throughout the entire sampling period.

In addition, it has to be noted that the HPC technique is a formalised and standardised technique that can only recover or cultivate different types of genera depending on factors such as type of media used, temperature, incubation time, type of sample (river water, surface water reservoir, treated and disinfected drinking water, etc.), season, sample age and the same sampling location over time (WHO, 2011b). Various stress conditions can cause planktonic microorganisms to enter a viable-but-non-culturable (VBNC) state (Ganesan *et al.*, 2007), which could render the HPC technique inadequate for the representation of the total viable microbial population within a water sample. Bacteria that have entered a VBNC stage can remain viable and maintain virulence (Dusserre *et al.*, 2008; Fakruddin *et al.*, 2013). According to Epstein (2009), *Shigella* can become VBNC in water

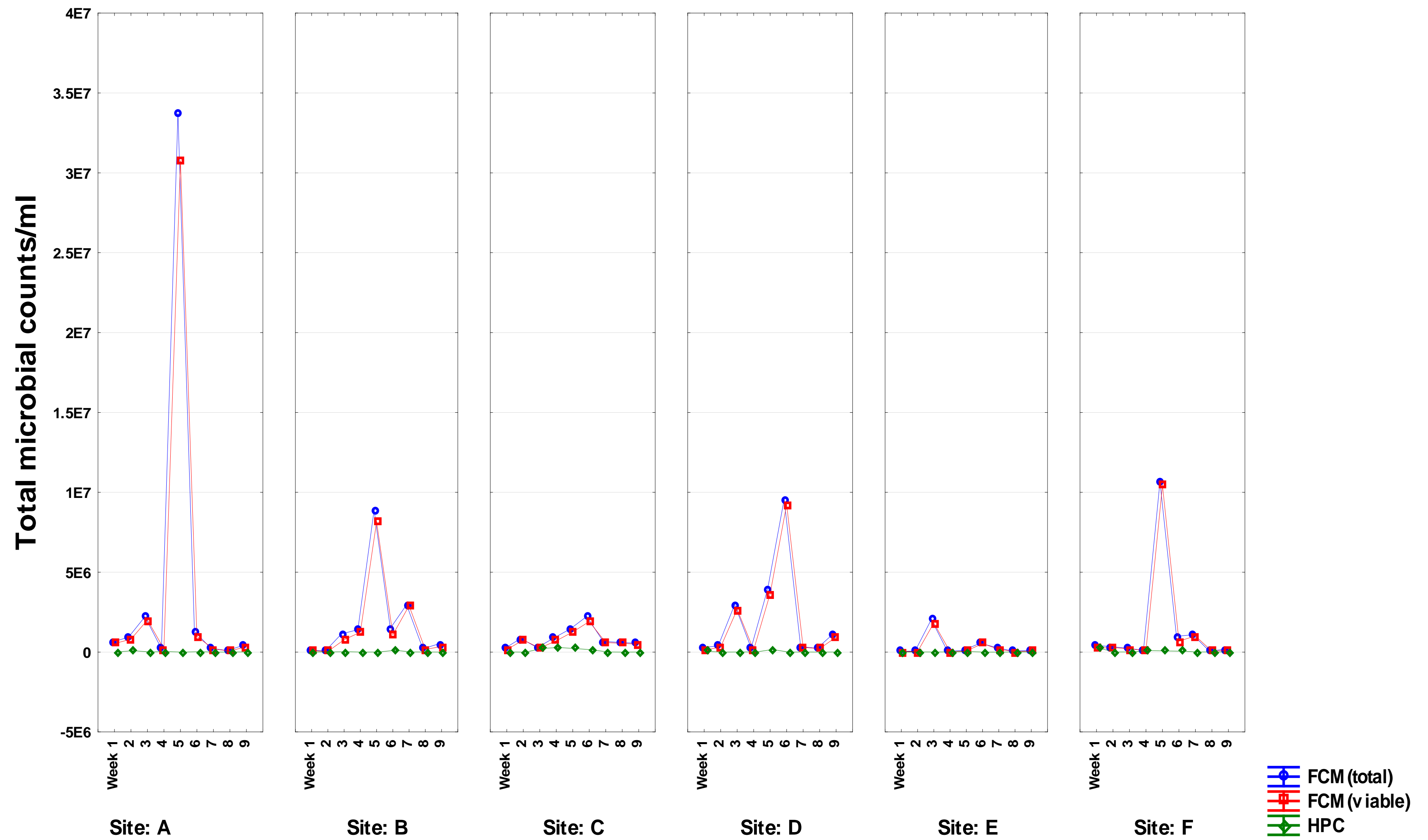


Figure 3.8 Comparison of heterotrophic plate counts (HPC) and total FCM and viable FCM results.

and become pathogenic when it enters a human host. Due to the fact that the HPC is only able to enumerate culturable microorganisms (Burtscher *et al.*, 2009), it can underestimate the number of viable cells (Fakruddin *et al.*, 2013) in a test sample. The HPC method clearly displays limitations with regards to providing a reliable account of the microbial numbers in a test sample. Various municipalities still employ this technique to enumerate total microbial counts as it is a farely simple and cost-effective technique. However, in this study it was used in comparison to the FCM technique in order to promote or motivate the use of more reliable enumeration techniques in municipalities.

Temperature and pH readings recorded at these sites were 20°C and 6.4 (Site A, week 5), 18°C and 6.7 (Site B, week 5), 15°C and 7.2 (Site C week 6), 16°C and 7.4 (Site D, week 6), 22°C and 6 (Site E, week 3) and 18°C and 6.2 (Site F, week 5). Even though temperature and pH values have a major influence on microbial activity and numbers (Khalil *et al.,* 2008), a direct link between increased temperature and increased microbial counts could not be established as higher temperatures corresponding to lower counts and vice versa, could be observed. The highest FCM counts were observed in week 5 and week 6 when temperatures ranged between 18 and 20°C, not the highest temperatures recorded during the sampling period (**Table 3.1**). Apart from temperature fluctuations, increased microbial counts could also be ascribed to various human activities and infrastructural problems observed throughout the sampling period. The general availability of functioning taps for certain sections of the community were lacking. The fact that several taps were broken, forced families to use taps and wash-up areas further away from their homes, putting more strain on certain water facilities (SJC, 2011). Furthermore, infrequent and inadequate maintenance of toilets and taps in RR Section as well as security concerns caused the inhabitants to either resort to urinating and defecating in plastic bags or in night pots (Bregman, 2011; Tokota, 2012). The night pots are emptied in the morning and cleaned at the standpipes (Granfone *et al.,* 2008; SJC, 2011), thus introducing microorganisms into the stagnant pools. Poor drainage could also have contributed to the high microbial counts. Raw sewage from broken toilets may combine with rainwater where it finds its way to the stagnant pools at the base of the taps (SJC, 2011).

Also, the intestines of slaughtered animals were seen being washed at several taps throughout the course of the sampling period. As a lack of proper drainage is a major concern, the bloody, intestinal water (which also contain faecal matter from the intestines) runoff provides an ideal substrate for possible increased microbial growth. A major health concern arose where inhabitants were seen washing their hands in the stagnant water rather than using water from the installed standpipes. Children were also seen playing with the stagnant water pools on various occasions at various sites throughout the sampling period.

In contrast, the FCM technique is able to distinguish between viable and dead microbial cells when used in conjunction with the LIVE/DEAD® *Bac*Light™ Viability Probe and thus provide more accurate total cell counts in environmental samples (Berney *et al*., 2007). Furthermore, the FCM is an authoritative technique that is able to determine a wide range of cell parameters at a single cell level and capable of obtaining information regarding their distribution within cell populations (Díaz *et al.,* 2010). Thus the results obtained in this study clearly demonstrate that the number of microbial organisms that are capable of forming colonies on HPC are significantly less than the actual number of viable microbial organisms in the sampled water as obtained by the FCM technique. .

Tables 3.2 – **3.6** present relative values in percentage ratios of the MPN to HPC, MPN to FCM, HPC to viable FCM, HPC to total FCM, and viable to total FCM, respectively. Generally, the HPC technique represents all culturable (gas-producing and non-gas-producing) microorganisms, while the MPN technique (indicating only gas producers) should thus only account for a fraction of the HPC results. The results indicate that the HPC obtained a small fraction of 0.25%, 0.12% and 0.32% (**Table 3.2**), respectively, of the highest MPN results recorded at Site A (weeks 3 and 5) and Site B (week 5). On the other hand, the highest MPN results accounted for 72.33%, 4.74% and 17.99% (**Table 3.3**), respectively, of the total FCM results during the same sampling period and sampling times. When comparing the MPN results to the highest total FCM counts, MPN results accounted for 4.74% (Site A, week 5), 17.99% (Site B, week 5), 1.57% (Site C, week 6), 0.27% (Site D, week 6), 0.74% (Site E, week 3) and 0.33% (Site F, week 5). These results indicate that even though faecal contamination is a significant contributor to the microbial pollution of the water in the stagnant pools at some of the sampling points, various other non-gas-producing microorganisms are also major contributors to the microbial load.

The highest HPC count enumerated at Site C (week 4) accounted for 32.43% (**Table 3.4**) of the total FCM count and 40.24% (**Table 3.5**) of the viable FCM count. Comparatively, the viable FCM accounted for 80.58% (**Table 6**) of the total FCM count at the same site during the same sampling week. When HPC results were compared to the highest total and viable FCM results, HPC only represented much lower ratios. compared to the total FCM and viable FCM the majority of the results obtained by the HPC were less than 1% of the counts obtained by the FCM technique. Therefore, this study is in agreement with previous studies where the HPC technique was found to record significantly lower overall counts than the FCM technique. In a study conducted by Paulse *et al*. (2007), HPC was found to be 3.65% of the total FCM count and 6.06% of the viable FCM count. In studies where the HPC technique was compared to the FCM technique, Hammes *et al. (*2008; 2010) compared the FCM to the HPC technique and found the HPC technique to be able to enumerate only a fraction (1%) of the total bacteria enumerated by FCM.

The FCM technique proved to be a more reliable technique in obtaining higher and more accurate microbial counts from the sampled water. The reliability of FCM has been tested by various authors and was found to yield more accurate results than with many other enumeration techniques (Paulse *et al.*, 2007; Hammes *et al.,* 2008; Hammes *et al.*, 2010; Van der Wienlen & Kooij, 2010; Wang *et al.*, 2010; De Roy *et al.,* 2012; Liu *et al.*, 2013). This could be due to the FCM technique having the ability to detect organisms that have entered a VBNC state (Berney *et al.*, 2007; Wang *et al.*, 2010) as well as giving an indication of dead cells. The HPC technique enumerated lower counts due to the fact that it can only obtain culturable microorganisms (Burtscher *et al.*, 2009), while MPN only enumerates gas-producing microorganisms.

Even though the HPC and MPN techniques are labour intensive and time consuming, it is more cost-effective in the routine analysis and monitoring of environmental samples (Wang *et al.*, 2010; De Roy *et al.,* 2012). However, according to Paulse *et al.* (2007) it is essential to use accurate methods to determine the actual level of planktonic microbial pollutants when measuring the level of microbial activity in aquatic samples. Even though the FCM technique is fast, and yields more accurate results, it is a challenge in routine laboratory analyses as it is more expensive to operate.

3.3 IDENTIFICATION OF GRAM NEGATIVE BACTERIA

Both the MAC (Allen, 2013) and the EMB (Madigan & Martinko, 2006) agars are commonly used to select Gram-negative bacilli as well as differentiating lactose-fermenting Gram-negative bacilli from non-lactose fermenting Gram-negative bacilli. Lactose-fermenting organisms appear pink-red in the centre on MAC agar (Allen, 2013; Cowan & Tolaro, 2009), while they are coloured (dark purple for vigorous lactose-fermenting organisms and brown-pink for slow fermenting organisms) on EMB agar (Lal & Cheeptham, 2007). Non-lactose-fermenting organisms appear transparent or off-white with no dye reaction on MAC (Cowan & Tolaro, 2009) agar and colourless or light lavender on EMB (Lal & Cheeptham, 2007) agar. Ndlovu (2013) found it impossible to differentiate *E. coli* to other coliforms when using the MAC agar. According to Becton Dickinson [BD] (2013), the MacConkey without Salt are used to isolate and differentiate Gram-negative bacilli, Staphylococi and Enterococci based on their ability to ferment lactose. Lactose-fermenting organisms form pink-red colonies, while non-lactose-fermenting organisms form transparent colonies on MAC-WS agar. Isolates were randomly selected based on their morphological properties and then re-streaked onto Nutrient Agar (NA) plates. **Figures 3.9** – **3.11** show the different isolates on the selective media.

Table 3.2 The average percentage ratio of the HPC count to the MPN count for all samples analysed over the sampling period.

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
A	2.98	1014.49	0.25	318.18	0.12	87.73	8.42	48.89	7.84
B	31.33	27.65	4.77	6.75	0.32	55.56	20.00	80.00	66.81
C	11.33	2.84	145.63	158.70	243.84	420.00	70.71	13.06	9.93
D	26.04	168.75	63.06	13.33	380.62	4.74	11.63	2.24	22.13
E	140.48	190.48	36.67	1814.81	57.36	5.67	3.18	35.24	8.33
F	146.46	5.08	15.81	697.62	203.81	385.71	496.67	34.64	49.33

Table 3.3 The average percentage ratio of MPN counts to total FCM counts based on flow cytometric analysis for all samples analysed over the sampling period.

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
A	25.51	0.99	72.33	5.11	4.74	1.69	8.34	12.70	21.78
B	27.02	22.83	40.99	1.43	17.99	14.07	0.67	5.67	0.46
C	16.34	6.32	57.58	20.43	6.36	1.57	2.11	1.81	16.99
D	56.90	0.42	0.40	18.03	1.09	0.27	13.51	19.85	2.15
E	3.50	5.33	0.74	1.11	41.16	6.22	12.27	33.22	38.35
F	37.65	46.29	17.56	11.86	0.33	1.42	0.18	43.41	10.50

Table 3.4 The average percentage ratio of HPC counts to total FCM counts based on flow cytometric analysis for all samples analysed over the sampling period.

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
A	0.76	10.04	0.18	16.26	0.01	1.48	0.70	6.21	1.71
B	8.47	6.31	1.96	0.10	0.06	7.82	0.13	4.53	0.31
C	1.85	0.18	83.86	32.43	15.50	6.60	1.49	0.24	1.69
D	14.82	0.70	0.25	2.40	4.14	0.01	1.57	0.44	0.48
E	4.91	10.15	0.27	20.18	23.61	0.35	0.39	11.71	3.20
F	55.15	2.35	2.78	82.72	0.67	5.48	0.91	15.04	5.18

Table 3.5 The average percentage ratio of HPC counts to viable FCM counts based on flow cytometric analysis for all samples analysed over the sampling period.

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
A	0.89	11.55	0.20	18.08	0.01	1.96	0.86	9.63	2.13
B	12.34	11.38	2.89	0.11	0.06	10.71	0.14	6.01	0.45
C	2.58	0.20	93.08	40.24	17.81	7.71	1.65	0.26	1.91
D	36.85	0.85	0.28	4.36	4.48	0.01	1.85	0.50	0.59
E	7.48	19.73	0.30	34.34	41.25	0.37	0.65	24.30	4.66
F	97.43	2.83	3.52	90.04	0.68	7.93	0.98	16.46	8.60

Table 3.6 The average percentage ratio of viable FCM count to total FCM based on FCM analysis for all samples analysed over the sampling period.

Site	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
A	85.67	86.98	90.92	89.92	91.17	75.65	81.64	64.46	80.14
B	68.60	55.46	67.66	88.71	91.68	72.99	97.70	75.47	67.97
C	71.67	88.86	90.09	80.58	87.07	85.56	90.38	89.49	88.47
D	40.21	83.20	89.53	55.19	92.42	96.66	85.13	88.37	80.86
E	65.63	51.43	89.43	58.76	57.25	95.51	59.87	48.18	68.63
F	56.60	83.10	78.96	91.87	97.81	69.19	92.61	91.34	60.24

3.3.1 Identification of isolates by means of the API 20E and RapID™ ONE identification systems

The API 20E and RapID™ ONE identification kits have been used to successfully identify *Enterobacteriaceae* in various studies (Sabae & Rabeh, 2007; Escalante *et al*., 2009; Ackermann, 2010; Hoffman *et al*., 2010; Ndlovu, 2013). These kits are specific for identification of Gram-negative/oxidase-negative microorganisms, hence the Gram stain and oxidase tests were performed prior to subjecting the isolates to the abovementioned techniques (Sabae & Rabeh, 2007; Ndlovu, 2013). Based on their Gram-negative/oxidase-negative reactions, presumptive positive colonies were identified for further identification using the API 20E and the RapID™ One systems. Fifty one morphologically different colonies grown on the three selective media were randomly isolated. However, only 49 and36 could be subjected for identification by the API 20E and RapID™ ONE systems, as they respectively met the manufacturer's requirements. Organisms identified mostly by the two systems belong to the *Enterobacteriaceae* (**Table 3.7**), which include the coliform bacteria (Pindi *et al*., 2013).

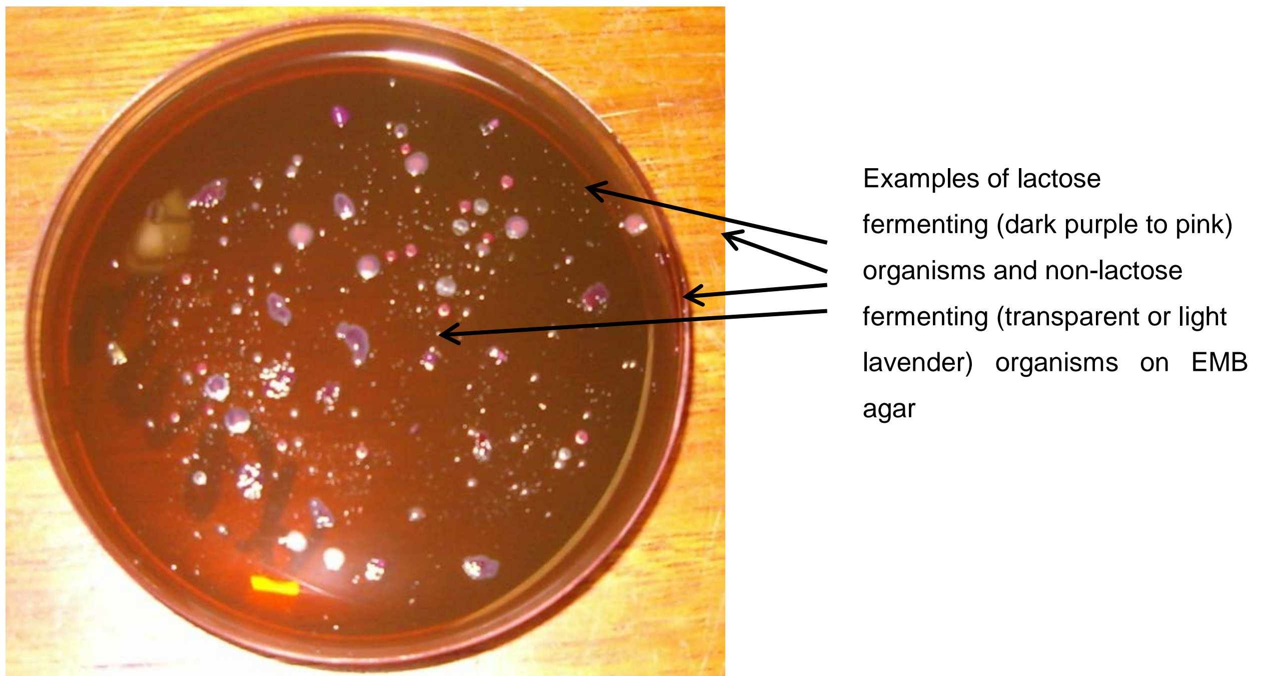


Figure 3.9 Different colonies cultured on EMB agar from water samples collected from RR Section.

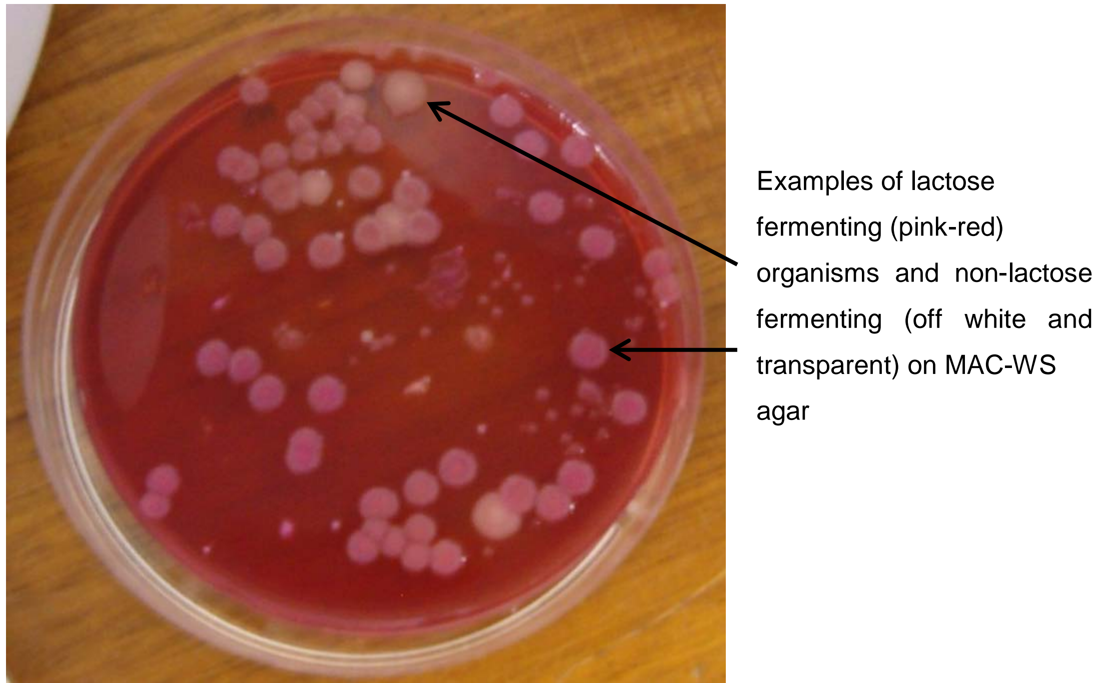


Figure 3.10 Different colonies cultured on MAC-WS agar from water samples collected from RR Section.

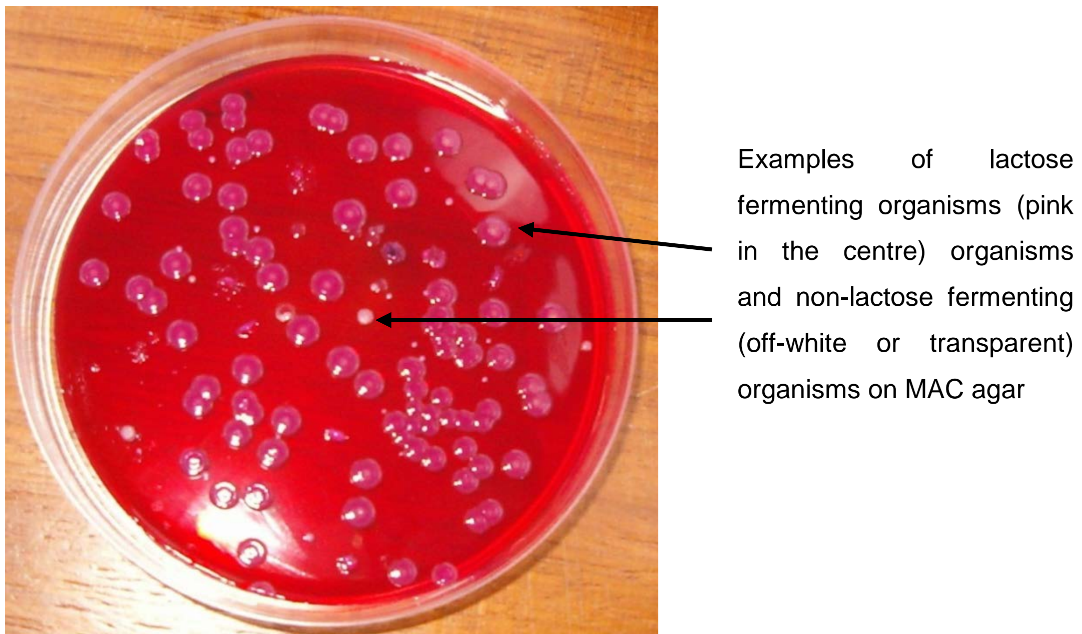


Figure 3.11 Different colonies cultured on MAC agar from water samples collected from RR Section.

Of the 49 isolates subjected to identification by the API 20 E system, only 80% (39 isolates) were successfully identified while the remaining 12 could not be identified as they required further biochemical analysis. The API 20E system was able to identify 14 species of

Enterobacteriaceae. The *Enterobacteriaceae* identified most often by this system included *E. coli* (21%), *Klebsiella pneumonia* (13%), *K. oxytoca* (13%), and *Acinetobacter baumannii/calcoaceticus* (10%). The detailed list of microbial organisms identified by the API 20E system in all the sampling sites at RR Section is presented in Appendix A. *Escherichia coli* was the most common organism isolated from all the sites throughout the sampling period. *Klebsiella pneumonia* was isolated and identified from Sites A (week 3), B (week 5), C (week 4), D (week 1) and F (week 1). *Klebsiella oxytoca* was identified at Site B (week 3), Site C (week 2 and week 4) and Site F (week 5). *Pantoea* was identified at Site A (week 2), Site C (week 5), and Site F (week 1).

Table 3.7 Gram-negative bacteria isolated form the six sampling sites.

API 20E	RapID™ ONE
Acinetobacter baumannii/calcoaceticus	*Acinetobacter calcoaceticus*
Enterobacter aerogenes	*Citrobacter freundii*
Enterobacter asburiae	*Enterobacter aerogenes*
Enterobacter cloacae	*Enterobacter asburiae (EG 17)*
Enterobacter sakazakii	*Enterobacter cloacae*
Escherichia coli 1	*Enterobacter sakazakii*
Klebsiella oxytoca	*Escherichia coli*
Klebsiella pneumonia ssp pneumoniae	*Klebsiella pneumonia*
Leclercia adecarboxylata	*Providencia alcalifaciens*
Pantoea spp 2	*Salmonella 1 (Most)*
Raoultella terrrigena	*Salmonella gallinarum*
Salmonella ser. paratyphi A	*Salmonella paratyphi A*
Salmonella spp	*Serratia marcescens*
Serratia marcescens	

Acinetobacter baumannii/calcoaceticus was isolated at Sites A, E and F in weeks 3, 1 and 4, respectively. Even though the API 20E system identified *Enterobacter asburiae* and *Pseudomonas oryzihabitans,* 100% identification was inconclusive and thus required further

biochemical analysis. For this reason, these isolates were not considered as a positive identification. Site A recorded the most diverse group of *Enterobacteriaceae* spp., which included an *Acinetobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Pantoea* sp., *Serratia* sp. and *Salmonella* sp. On the other hand, *Acinetobacter, Enterobacter, Escherichia, Klebsiella, Raoultella* and *Leclercia* were also observed at Site E. Various species of *Enterobacter* spp. were also identified at this site and included *E. cloacae* and *E. aerogenes*. The API 20E system could also identify various *Enterobacteriaceae* species at Site B (*E. asburiae, E. coli, K. oxytoca, K. pneumonia* and *S. ser. paratyphi A*), Site E (*A. baumannii/calcoaceticus, E. coli, Enterobacter aerogenes, E. cloacae, K. oxytoca, Raoultella terrrigena* and *Leclercia adecarboxylata*), Site F (*A. baumannii/calcoaceticus, E. coli, K. oxytoca, K. pneumonia* and *Pantoea* spp.) and Site D (*E. aerogenes, E. coli* and *Klebsiella pneumonia*).

Of the 36 isolates subjected for identification by the RapID™ ONE system, only 72% (26 isolates) were positively identified, while the remaining 28% required further biochemical analysis. The RapID™ ONE system identified 13 species of *Enterobacteriaceae* (**Table 3.7**). The *Enterobacteriaceae* mostly identified by the RapID™ ONE system, which included the coliform bacteria (Pindi *et al*., 2013) were *K. pneumonia* (19%), *Acinetobacter calcoaceticus* (12%), *E. coli* (12%) and *E. cloacae* (12%). The detailed list of microbial organisms identified by the RapID™ ONE system is presented in Appendix B. *Klebsiella pneumonia* was isolated at Site A (week 3), Site B (week 5), Site C (week 4), Site D (week 1) and Site F (week 1). *Escherichia coli* was identified at Site A (week 1), Site B (week 2) and Site C (week 2). *Acinetobacter calcoaceticus* was only identified at Sites B, C and E in weeks 1, 2 and 5, respectively. Another organism of concern, *Enterobacter cloacae* was identified at Site B (week 2) and Site E (weeks 2 and 3). The sites which had the most diverse *Enterobacteriaceae* genera isolated were Site A and Site B. *Enterobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Serratia* sp. and *Salmonella* sp. were identified from Site A, while *Acinetobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Klebsiella* sp. and *Salmonella* sp. were isolated from Site B. On the other hand, *Enterobacteriaceae* genera isolated from Site C included *Acinetobacter sp., Escherichia* sp., *Klebsiella* sp. and *Serratia* sp., while *Citrobacter* sp., *Klebsiella* sp., and *Providencia* sp. were identified at Site D. The sites with the least *Enterobacteriaceae* genera identified were Sites E and F, which included *Citrobacter* sp., *Enterobacter* sp. (identified at Site E), *Acinetobacter sp.,* and *Klebsiella sp.* (identified at Site F).

3.3.1.1 Health concerns linked to isolated microorganisms

Most of the isolated microorganisms belong to the coliforms bacteria, which are commonly used as bacterial indicators of the sanitary quality of food and water. *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Pantoea*, *Leclercia* and *Serratia* are some of the genera belonging to the coliform group (Pindi *et al.*, 2013). The significance of the presence of these coliform bacteria therefore suggests the possible presence of pathogenic microorganism in the obtained water samples in the RR Section. The coliform bacteria, however, are unable to predict the level of pathogen occurrence (Payment & Locas, 2011). It is assumed that these coliform bacteria were introduced into the stagnant water pools as a result of inhabitants washing night pots (Granfone *et al.*, 2008; SJC, 2011), and the intestines of slaughtered animals at the base of the communal taps. These practices were observed throughout the sampling period. Testing for *Enterobacteriaceae* does not only indicate faecal contaminants, but rather identify for the entire family which may include pathogenic organisms (Tortorello, 2003). Therefore, the presence of the *Enterobacteriaceae* group throughout the sampling period is a cause for concern as it is an indication of the possible presence of highly pathogenic bacteria (Gemmell & Schmidt, 2010). Furthermore, their presence could have detrimental effects on public health (Ackermann, 2010).

Klebsiella spp. are members of the coliform group and are common and widespread in many aquatic environments with the ability to flourish in water with high levels of nutrients. These organisms have been found in the excreta of warm blooded animals and have also been isolated from sewage contaminated water (WHO, 2011b). As indicated earlier *K. pneumonia* was isolated at all sites (except Site E). *Klebsiella oxytoca* was detected at Site B, Site C and Site F. Since *K. pneumonia* and *K. oxytoca* are part of the normal gut flora of the large intestine, both are used as indicators of faecal contamination (Prescott *et al.,* 2005). However, *Klebsiella* spp. are regarded as opportunistic pathogens that may pose a threat to children, the elderly, patients with burns or excessive wounds as well as the immunosuppressed (WHO, 2011b). *Klebsiella pneumonia* frequently leads to nosocomial infections (Bojer, 2010), while *K. oxytoca* may cause sepsis and haemorrhagic colitis in humans (Högenauer *et al.*, 2006).

Escherichia coli was also isolated at all sites as mentioned earlier. It is a member of the *Enterobacteriaceae* and forms part of the normal intestinal flora of humans and warm blooded animals (WHO, 2011b). It is an opportunistic organism that can cause serious illnesses such as urinary tract infections, bacteraemia, pneumonia, various intra-abdominal infections (Paterson, 2006) and meningitis (Kaper *et al.*, 2004). Even though most strains of the *E. coli* group are non-pathogenic, some pathogenic strains have been identified, which include Enteroaggregative *Escherichia coli*, Enterohaermorrhagic *Escherichia coli*,

Enteropathogenic *Escherichia coli*, *Enteroinvasive Escherichia coli* and Diffuse-adhering *Escherichia coli* (O'Sullivan *et al*., 2007). Enteropathogenic *E. coli* was found to be the most common cause of diarrhoea amongst children below five years in South Africa (Keddy, 2013). According to WHO (2011b), transmission of pathogenic *E. coli* in recreational water has been well documented. Even though both the API 20E and the RapID™ ONE identification systems do not specify the pathogenicity of the *E. coli* strain isolated from the water samples collected throughout the sampling period, the presence of *E. coli* however, do raise concerns as the standpipes and surrounding areas serve as a playground for infants and toddlers.

Only one isolate was identified at Site A as *Salmonella* (to genus level) by both the API 20E and the RapID™ ONE systems. *Salmonella* spp. are common and prevalent in the environment, however some species are host specific (WHO, 2011b) and are well known to cause food poisoning (Da Silva *et al*., 2013). Human beings are known as the natural host and reservoir for *S. typhi* and *S. paratyphi A* (Whitaker *et al*., 2009; Crump & Mintz, 2010). *Salmonella paratyphi A* is a serotype of *Salmonella enterica* and causes enteric fever (Fangtham & Wilde, 2008). *Salmonella paratyphi A* can cause illness and death especially among infants, children and adolescents, particularly those living in informal settlements without adequate sanitation and access to safe food and water (Whitaker *et al*., 2009). The organism was isolated at Site B by both the API 20E and the RapID™ ONE identification systems. *Salmonella gallinarum,* which like *S. paratyphi A,* is a serotype of *Salmonella enterica* (Rocha-e-Silva *et al*., 2013). This organism was detected at Site A through the RapID™ ONE system. It is a natural pathogen of birds (Rocha-e-Silva *et al*., 2013) and seldom infects humans (Braden, 2006). The presence of *S. typhi* and *S. paratyphi A* in water present a health risk as these organisms have been associated with waterborne disease (especially typhoid and paratyphoid) outbreaks in developing countries (Levantesi *et al*., 2012). People living in crowded and impoverished areas without adequate sanitation and water quality are at risk of contracting febrile disease caused by both *S. typhi* and *S. paratyphi A* (Whitaker *et al*., 2009). Water is important in the spreading of multiple-drug-resistant (MDR) *Salmonella* strains (Levantesi *et al*., 2012). In many African countries MDR non-typhoidal *Salmonella* spp. has been an increasing health risk to children and immunocompromised adults (Kariuki *et al*., 2006; Gordon, 2008; Morpeth *et al*., 2009; Feasey *et al*., 2010). According to Birks and Hills (2007) *Salmonella* is usually introduced to greywater when an infected person washes themselves or when contaminated food is washed.

Acinetobacter is a member of the *Enterobacteriaceae* and it is widespread in soil, water and sewage environments (Talbot *et al*., 2006; WHO, 2011b). *Acinetobacter baumannii-calcoaceticus* is an emerging complex problematic pathogen that is multidrug-

resistant, nosocomial and community-acquired (Talbot *et al.*, 2006). It was isolated at Sites A, E and F. Its natural reservoirs are soil and water sources (Blossom & Srinivasan, 2008). It is also found in healthy humans as normal commensal microflora in the oral cavity, groin as well as respiratory and gastrointestinal tracks (Percival & Williams, 2014). It is an opportunistic bacterium that can cause pneumonia, urinary tract infections, surgical wound infections, bloodstream infections, secondary meningitis and wound infections (Blossom & Srinivasan, 2008; WHO, 2011b; Percival & Williams, 2014). People at risk of acquiring infections are alcoholics, smokers, people with lung disease or invasive operative procedures (Talbot *et al.*, 2006) and patients that are critically ill and immunocompromised. The presence of *Acinetobacter* spp. in water therefore may present a health risk of non-gastrointestinal infections to immunocompromised people (WHO, 2011b; Percival & Williams, 2014).

Citrobacter fruendii is an opportunistic member of the *Enterobacteriaceae* widely found in water, soil, food and in the intestines of animals and humans (Whalen *et al.*, 2007). It was only detected at Site D and Site E. This organism is associated with a wide range of nosocomial infections such as urinary tract, lower respiratory tract, gastrointestinal, central nervous system, skin and soft tissue infections (Choi, 2007). Thus, the presence of this organism may pose a health risk to the community of RR Section.

Another organism of interest is *Leclercia adecarboxylata,* which was isolated at Site E. *Leclercia adecarboxylata* is a member of the *Enterobacteriaceae* family (Davenport & Land, 2007; Correa *et al.*, 2012), which forms part of the intestinal microflora of animals, but has also been isolated from water, food and the environment (Davenport & Land, 2007). *Leclercia adecarboxylata* is an opportunistic pathogen resembling *E. coli* phenotypically and is associated with immunocompromised (such as cancer and chronically ill) patients (Hess *et al.*, 2008). Even though it is commonly found in immunocompromised patients, it has also been isolated from healthy patients (Davenport & Land, 2007; Hess *et al.*, 2008). Contact with water contaminated with *L. adecarboxylata* is a potential health risk as Davenport & Land (2007) isolated the organism from a healthy patient who had a small cut prior to swimming.

Providencia alcalifaciens was identified at Site D. It is an opportunistic member of the *Enterobacteriaceae* family generally considered to be commensal flora in the gastrointestinal tract of humans (Yoh *et al.*, 2005). It has been implicated in traveller's diarrhoea, urinary tract infections, ocular infections, meningitis and gastroenteritis (Ovchinnikova *et al.*, 2012). In their study of a foodborne outbreak caused by *P. alcalifaciens,* Murata *et al.* (2001) was able to establish that nursery school children were at a higher risk of contracting gastroenteritis compared to the older children as well as the teachers. *Providencia alcalifaciens* was also one of the organisms that were isolated by Diab *et al.* (2008) in

hospital wastewater, thus indicating its health risk to people who come in contact with water contaminated with *P. alcalifaciens*. As observed during the sampling period, infants and toddlers were playing in the vicinity of these water sources, which may have been contaminated with this organism.

According to Engelkirk & Duben-Engelkirk (2008) *Pantoea* spp. are common in nature. The organism is also found in human and animal faecal matter, soil, sewages as well as a variety of food products such as meat, fish and vegetables. It causes diseases such as urinary tract infections, respiratory tract infections, cardiovascular infections as well as wound, ear, nose and throat infections. This opportunistic bacterium was isolated from Site A, Site C and Site F. According to Pindi (2013), people who drink water contaminated with *Pantoea* spp. are at an increased risk of contracting infectious diseases.

Serratia spp. are members of the *Enterobacteriaceae* with 10 species of which *S. marcescens* and *S. liquefaciens* are linked to human infections (Hart, 2006). *Serratia marcescens* are widely distributed in the environment and found in the intestinal tract of humans and rats (Hart, 2006) and may also be found in sewage and natural waters (Sadowsky & Whitman, 2011). Even though *S. marcescens* is regarded as an abundant environmental organism, it was not detected throughout the entire sampling period, but was only isolated from Sites A and C. It could be that the organism has the ability to enter a viable-but-non-culturable (VBNC) state, when exposed to environmental conditions (Adams *et al*., 2003).

Raoutella terrigina was previously known as *Klebsiella terrigina* (Shaikh & Morgan, 2011). It is a rarely isolated Gram-negative bacterium, which has been mainly reported as a water and soil organism (Murray *et al*., 2005), but rarely isolated from humans (Shaikh & Morgan, 2011). It can cause sepsis (Shaikh & Morgan, 2011) and bacteraemia (Cleveland *et al*., 2014). This organism was detected at Site E in week 5. *Raoutella terrigina* has been reported as difficult to identify using the API 20E as it was misidentified as *Klebsiella pneumonia* (Monnet *et al*., 1991). However, it has been successfully identified by the API 20E system by Shaikh and Morgan (2011) and Ndlovu (2013).

Various waterborne pathogens, which are commonly isolated from environmental waters were not detected using the API 20E and RapID™ ONE identification techniques. According to a study conducted by Ndlovu (2013) common waterborne organisms such as *Pseudomonas aeruginosa*, *Shigella* species and *Vibrio fluvialis* were successfully identified using the API 20E and RapID™ ONE identification techniques. These organisms were however, not detected during this study by these identification techniques. The possibility that these microorganisms can enter a VBNC state could be regarded as one of the reasons why these organisms were not detected. *Pseudomonas aeruginosa* has been known to enter a VBNC state in aquatic environments (Oliver, 2005). The fact that *P. aeruginosa* has

previously been isolated from greywater (Gross *et al*., 2007) and its ability to enter into a VBNC state, highlights its possible presence in the water samples from the RR Section and thus, should not be disregarded.

Similarly, *Helicobacter pylori* has also been identified as an organism entering into a VBNC state, when exposed to adverse environmental conditions, making it viable in aquatic environments (Adams *et al*., 2003). When ingested by a suitable host, the organism has the ability to cause infection (Oliver, 2005). Oliver (2005) also presented a detailed list of organisms that may enter into a VBNC state when exposed to environmental stress. Amongst these organisms are *Legionella* spp., *L. pneumophila*, *Campylobacter* spp. and *Vibrio cholerae*. Thus, the possibility that these organisms may still be present in the RR Section water samples should still be considered and thus the water should not be regarded as being safe. Organisms that have entered into a VBNC state are not able to form colonies on most selected media, resulting in the underestimation of the contaminants or pathogens (Toranzos *et al*., 2002). The VBNC state is of particular importance when it comes to pathogens, since they can remain in VBNC state for long periods in aquatic environments and also remain virulent and cause infection to humans once resuscitated (Bitton, 2011).

All organisms detected by the API 20E and RapID™ ONE systems in this study belong to the *Enterobacteriaceae* family. Many of the organisms identified are generally used to indicate faecal pollution in environmental samples as they form a commensal relationship with warm blooded animals (Prescott *et al*., 2005). They indicate the presence of infectious microorganisms, which can be released into water through faecal matter. People who are most at risk are children, the immunocompromised and the elderly (Obasohan *et al*., 2010). *Enterobacteriaceae* could have been introduced via many routes. Raw sewage that leaks into the streets or walkways where it comes into contact with the stagnant water pools (SJC, 2011), washing of night pots (which are used by the residents to relieve themselves at night) at the standpipes (Granfone *et al.,* 2008; SJC, 2011; Tokota, 2012) as well as washing of intestines of slaughtered animals at the communal taps. Furthermore, the soaking and washing of clothes and utensils for a prolonged period of time increases the bacterial load of greywater (Nganga *et al*., 2012). In their study, Nganga *et al*. (2012) found greywater containing food particles had a higher bacterial load. Thus food particles present in the stagnant water pools (as observed when collecting samples) could have also added to a high microbial content in the samples. Contact, therefore, with this type of water may lead to humans being infected with possible waterborne related diseases.

3.3.1.2 Comparison of the API 20E and RapID™ ONE systems

Both the API 20E and RapID™ ONE systems identified organisms belonging to the *Enterobacteriaceae* group. Both the identification systems were able to identify different species of *Enterobacter*, which included *E. aerogenes*, *E. asburiae, E. cloacae* and *E. sakazakii*. Furthermore, both systems identified *Enterobacter* as the genus with the most species diversity. In addition to these organisms, *Acinetobacter calcoaceticus, Citrobacter freundii, Providencia alcalifaciens* and *Salmonella gallinarum* were also identified by the RapID™ ONE system whereas the API 20E system could not. Similarly, the API 20E system could identify *K. oxytoca, Pantoea* and *Raoultella terrrigena,* whereas the RapID™ ONE system could not. In a previous study by Ndlovu (2013), *Pantoea* spp. and *Raoultella terrrigena* could only be identified by the API 20E system, while the RapID™ ONE system was not able to. Even though *Serratia marcescens* is abundant in the environment, sewage and natural waters (Sadowsky & Whitman, 2011), they were only identified by both systems in week 5 at Site A and Site E. Isolates identified as *Acinetobacter baumannii/calcoaceticus* by the API 20E were identified as *Acinetobacter calcoaceticus* by the RapID™ ONE system. *Leclercia adecarboxylata* and *K. oxytoca* could only be identified with the API 20E system. The RapID™ ONE system was able to identify more of the *Salmonella* isolates to the species level (*S. paratyphi* type A, *S. gallinarum*), while the API 20E was only able to identify one isolate to the species level (*S. ser. Paratyphi* type A). This study correlated with a study conducted by Ndlovu (2013) where more species of *Salmonella* could be identified with the RapID™ ONE system, while the API 20E system only identified *Salmonella* up to the genus level. This study proofs that the API 20E system is able to identify organisms of more genera diversity compared to the RapID™ ONE system. Furthermore, this study has proven that the RapID™ ONE is more sensitive in identifying *Salmonella* to species level than the API 20E system (**Table 3.7**).

Compared to the API 20E system, the RapID™ ONE system was more user friendly with regards to the interpretation of results and also requires one reagent (to detect indole production) to be added during the process (Kitch *et al.*, 1994). The API 20E system was more challenging in the interpretation of the results and requires the addition of more reagents in order to obtain final results.

For both identification systems, it took approximately 48 hours from initial cultivation to purification into single isolates before samples could be incubated into their respective biochemical wells. The results from the RapID™ ONE System were obtained on the same day (approximately after 5 hours), while the API 20E System results were obtained after 18 – 24 hours. This coincided with previous studies where both systems were compared for identification of *Enterobacteriaceae* from clinical samples (Kitch *et al.*, 1994) and water

samples (Ndlovu, 2013). Compared to the RapID™ ONE system, the API 20E system was found to be more expensive and thus would not be recommended for routine analysis (Ndlovu, 2013). Apart from the *Enterobacter asburiae* and *Pseudomonas oryzihabitans* mentioned previously, both systems could not identify some isolates, which could be due to errors encountered when reading the colour reactions on the strips. Furthermore, the databases of the respective systems do not have certain microorganisms in their list (Popovic *et al*., 2007). Although both systems have been previously successfully used in the identification of *Enterobacteriaceae* (Kitch *et al*., 1994; Nucera *et al*., 2006; Sabae & Rabeh, 2007; Escalante *et al*., 2009; Hoffman *et al*., 2010; Ndlovu, 2013), Ndlovu (2013) further compared both systems with the Polymer Chain Reaction (PCR) technique and found PCR to be more sensitive, cheaper and requires less time for identifying organisms than the API 20E and RapID™ ONE systems. Thus both these systems should be used for preliminary identification and final identification should be used with a more sensitive system like the PCR and16S rRNA gene sequencing.

3.4 IDENTIFICATION OF GRAM-POSITIVE BACTERIA

As previously mentioned, a serial dilution of 10^{-1} to 10^{-3} were performed on the obtained samples and the diluted samples were directly spread-plated (in duplicate) onto Sabouraud Dextrose- (SD) and Baird Parker (BP) agars and incubated at 37°C for 18 – 24 hours as per manufacturers' instructions (**Figures 3.12** – **3.13**). Isolates were randomly selected based on their morphological differences and then re-streaked onto Nutrient Agar plates. Sabouraud Dextrose Agar is commonly used as a selective media of fungi and yeast (Hare, 2008). Even though other organisms may also grow on Baird Parker agar, it is commonly used as a selective and differentiation medium of *Staphylococcus aureus* from other *staphylococci* (BD, 2011). *Staphylococcus aureus* colonies appear as dark grey to black colonies, while other organisms will appear as brown to grey or colourless colonies (BD, 2011).

Sixty eight morphologically different isolates were isolated. However, only 38 isolates could be subjected to identification by the BBL Crystal™ GP ID based on their Gram-positive reaction. Of these 38 isolates subjected to the BBL Crystal™ GP ID system, only 71 % (27 isolates) were successfully identified. According to the manufacturer's instructions, only isolates with a confidence level of 0.9 (and above) could be considered as a positive identification. Even though some isolates appeared to be morphologically different, the system identified them as being the same organism.

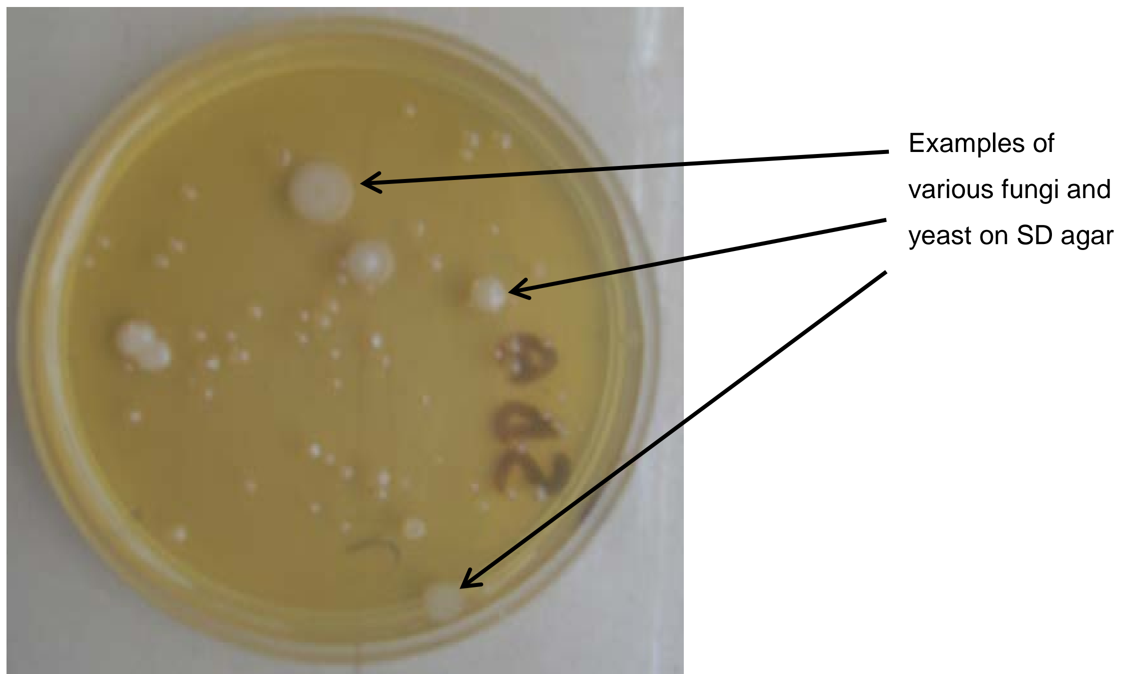


Figure 3.12 Different colonies cultured on SD agar from water samples collected from RR Section.

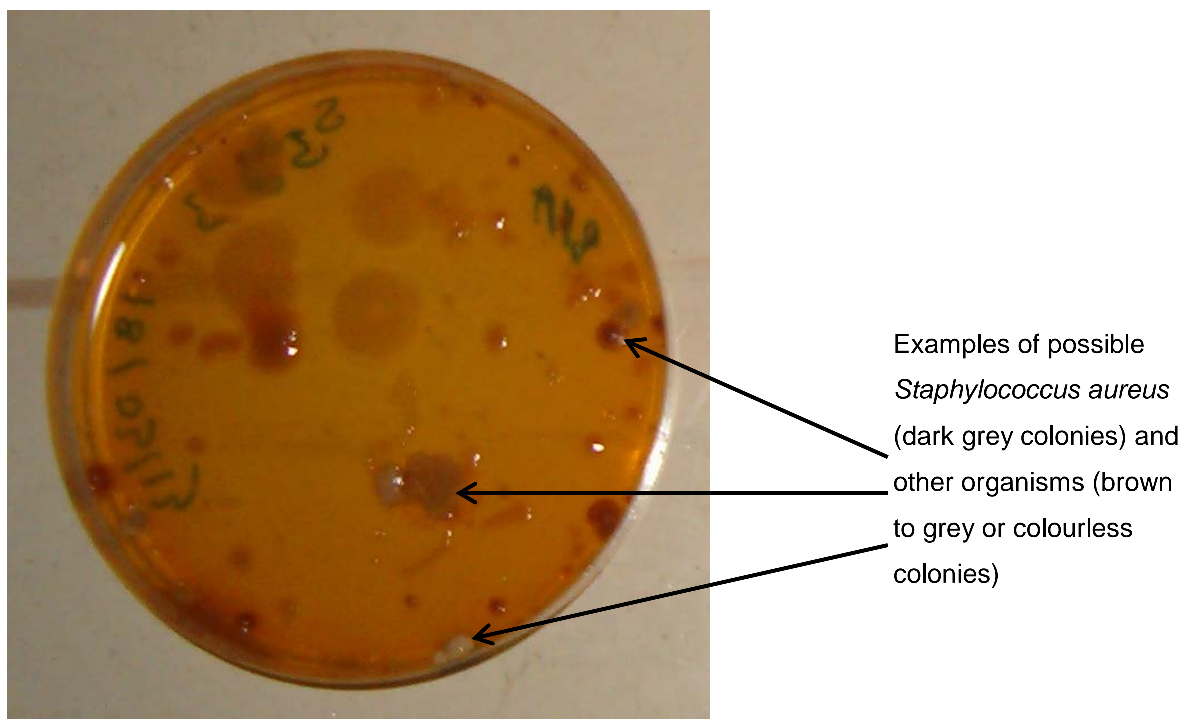


Figure 3.13 Different colonies cultured on BP agar from water samples collected from RR Section.

Although BP agar is usually used for the isolation of *S. aureus,* this organism was not identified during this study. Furthermore, organisms with typical colonial morphology of *S. aureus* were identified as *Bacillus* sp. and other species of the *Staphylococcus* genus. This is in agreement with a previous study where BP agar was used to isolate *S. aureus.*

Capita *et al.* (2001) found that some isolates with typical *Staphylococcus aureus* morphology on BP agar were identified as *Bacillus* spp. and other species of the *Staphylococcus* genus. **Table 3.8** tabulates organisms identified by the BBL Crystal™ GP ID system, while the detailed information on the identified organisms is presented in Appendix C.

Aerococcus, Bacillus, Brevibacillus, Corynebacterium, Gemella, Kocuria, Micrococcus, Staphylococcus and *Streptococcus* were the nine genera identified by this system. *Corynebacterium* species (16%), which included *C. bovis, C. diphtheria* and *C. pseudodiphtheriticum* were the most identified isolates.

Table 3.8 Gram-positive bacteria isolated form the six sampling sites.

Organism Identified by BBL Crystal™ GP ID
Aerococcus urinae
Bacillus cereus
Bacillus licheniformis
Brevibacillus brevis
Corynebacterium bovis
Corynebacterium diphtheria
Corynebacterium pseudodiphtheriticum
Corynebacterium species
Gemella morbillorum
Kocuria kristinae
Micrococcus luteus
Staphylococcus equorum
Staphylococcus sciuri
Streptococcus intermedius
Streptococcus porcinus
Streptococcus anginosus

Aerococcus urinae and *Bacillus cereus* accounted for 15%, respectively, of the organisms identified, while *Micrococcus luteus, Staphylococcus equorum* and *Streptococcus intermedius* accounted for 7% each of the identified species.

In a previous study conducted by Ashour *et al.* (2011), the BBL Crystal™ GD IP system was used to obtain genera such as *Staphylococcus, Micrococcus, Bacillus* and *Gemella morbillorum,* while Venter (2010) identified *Bacillus* spp. *and Kocuria rosea* using this identification system. Even though *Aerococcus* was one of the most isolated genera, only *Aerococcus urinae,* was identified. Site A recorded the highest genus diversity where *Aerococcus* sp., *Bacillus* sp., *Corynebacterium* sp., *Kocuria* sp., *Micrococcus* sp. and *Staphylococcus* sp. were identified. The BBL Crystal™ GP ID system could also identify various Gram-positive organism at Site B (*Brevibacillus* sp., *Corynebacterium* sp., *Gemella* sp. and *Streptococcus* sp.), Site C (*Aerococcus* sp., *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and *Streptococcus* sp.), Site D (*Bacillus* sp., *Corynebacterium* sp. and *Staphylococcus* sp.), Site E (*Bacillus* sp., *Corynebacterium* sp. and *Streptococcus* sp.) and Site F (*Aerococcus* sp., *Corynebacterium* sp. and *Streptococcus* sp.). Even though a wide variety of organisms were identified at the different sites throughout the sampling period, various strains (such as *Bacillus megaterium, Corynebacterium pseudogenitalium* and *Corynebacterium genitalium*) had a confidence level below 0.9, which according to the manufacturer's instructions could not be considered as a positive identification.

3.4.1 Health concerns linked to isolated microorganisms

Corynebacterium is an organism with Gram-positive bacilli (Bernard, 2012) commonly found in the environment as well as being part of the normal human skin microbiota and mucous membranes (Gomila *et al.*, 2012). Of all the *Corynebacterium* species, *C. diphtheria* is considered to be the most significant pathogen causing diphtheria and also regarded as one of the most feared childhood diseases (Wagner *et al.,* 2010). This organism was identified at Site D in week 6. Although diphtheria cases have significantly declined in South Africa (WHO & UNICEF 2013), Liebenberg *et al.* in 2009 reported a case of one death due to this disease in Cape Town. *Corynebacterium pseudodiphtheriticum* which was isolated at Site E in week 1, is increasingly associated with respiratory infections (Bittar *et al.*, 2010), despite it forming part of the microflora in the upper respiratory tract (Díez-Aguilar *et al.*, 2013). It was previously isolated from 13 children who had been diagnosed with cystic fibrosis (Bittar *et al.,* 2010). *Corynebacterium bovis,* on the other hand, was isolated at Site B in week 4. It is a Gram-positive bacillus (Meyer & Reboli, 2005), but is rarely labelled as a human pathogen (Dalal *et al.,* 2008). The organism has, however, been linked to rare cases of meningitis, leg

ulcer, chronic otitis media, endocarditis, an epidural abscess, ventriculojuglar shunt infection (Dalal *et al.,* 2008) as well as a prosthetic shoulder joint infection (Achermann *et al.,* 2009). Thus, it can be assumed that children playing with the greywater sampled, are at risk of contracting diseases particularly if they are inadequately vaccinated (Bernard, 2012).

Aerococci are Gram-positive cocci that mimic both staphylococcal and streptococcal characteristics (Shelton-Dodge *et al*., 2011; Rasmussen, 2013), thus making it difficult to accurately identify. *Aerococcus urinae* was one of the most isolated organisms during the course of this study. It was isolated from all sites except Sites D and E. Previously, this organism was thought to have low pathogenicity (Siera-Hoffman *et al*., 2005), but it has been reported to cause severe, sometimes fatal, infections of the urinary tract as well as endocarditis (De Jong *et al*., 2010; Ho *et al*., 2010). Various studies have isolated *Aerococcus urinae* from urine and blood samples from elderly patients (De Jong *et al*., 2010; Ho *et al*., 2010; Shelton-Dodge *et al*., 2011).

Bacillus cereus is a Gram-positive organism common in soil, dust and water (Saygin *et al*., 2013). Given its abundance in water and soil, *B. cereus* was only identified at Site C (week 4), Site D (week 1 and week 3) and Site E (week 4). *Bacillus cereus* is an opportunistic bacterium well known to cause foodborne outbreaks (Da Silva *et al.,* 2013). It has also been implicated in many opportunistic infections in immunocompromised as well as in healthy people (Bottone, 2010; Logan *et al*., 2011). In a study conducted by Venter (2010), where HPC bacteria in a drinking water distribution system was analysed, *Bacillus cereus* was the most common organism identified by the BBL Crystal™ GP ID system and confirmed by 16S rRNA gene sequencing. The presence of this organism in this study therefore, poses possible health risks to the RR Section community, especially where children play in close proximity to greywater or those that use the greywater for daily use.

Bacillus licheniformis was isolated at Site A in week 9. Venter (2010), also found that all *B. licheniformis* isolates identified by the BBL Crystal™ GP ID system were identified as *B. thuringiensis* by 16S rRNA gene sequencing. It was therefore regarded as a misidentification by the BBL Crystal™ GP ID system. It could be due to the fact that the BBL Crystal™ GP ID system not recognising *B. thuringiensis* in its database. Thus, the system suggested the more closely related species as *B. licheniformis*. This suggests uncertainty when *B. licheniformis* is identified by BBL Crystal™ GR ID system, consequently a confirmation test needs to be done by a more sensitive method such as 16S rRNA gene sequencing.

Gemella spp. are Gram-positive cocci, which have not been allocated a family name (Euzéby, 2010). *Gemella* spp. are opportunists, especially to the immunocompromised individuals. *Gemella morbillorum* form part of the human flora in the upper respiratory tract, oral cavity as well as the intestinal tract (Collins, 2006). Not only is *G. morbillorum* primarily

hosted by humans, but domesticated animals such as goats, pigs, rabbits and horses are also primary hosts (Collins, 2006). In this study, this organism was isolated at Site B in week 3. This opportunistic organism has been detected from patients with measles, septic arthritis, meningitis and/or brain abscesses (Collins, 2006).

Streptococcus is a Gram-positive bacterium with cocci occurring in pairs or chains (Da Silva *et al.*, 2013). Three species of *Streptococcus, S. intermedius* (Site B, week 7 and Site F, week 8)*, S. porcinus* (Site E, week 6) and *S. anginosus* (Site C, week 5), were isolated during the sampling period. *Streptococcus intermedius* belongs to the *Streptococcus anginosus* group of bacteria (Petti *et al.,* 2008; Tran *et al.*, 2008). Although considered a commensal part of the microbial flora of the oral cavity (Neumayr *et al.*, 2010), this organism is considered to be the most pathogenic opportunistic member of the *Streptococcus anginosus* (Clarridge *et al.*, 2001; Rashid *et al.,* 2007). Even though *S. intermedius* causes deep-seated infections amongst middle-aged people and older individuals, Maliyil *et al.* (2011) reported a case of splenic abscesses and multiple brain abscesses caused by *Streptococcus intermedius* in a healthy young male with no previous history of illness.

Another organism of interest is *Staphylococcus sciuri,* which was isolated at Site C in week 1. It is common in nature and has been isolated from domesticated animals, wild animals as well as many food products of animal origin. According to Chen *et al.* (2007), *S. sciuri* is an opportunistic pathogen that causes a variety of diseases such as endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections. In a study conducted by Ahoyo *et al.* (2013), 45% of patients tested for *S. sciuri*-related bacteraemia, died from this disease.

Micrococcus spp. contains Gram-positive cocci and are commonly found in soil, water, dust and skin of humans and animals (Kocur *et al.*, 2006; Kao *et al.*, 2012). *Micrococcus luteus* are members of the *Microccoccus* family and is found in abundance on the human skin (Kocur *et al.*, 2006). This opportunistic organism (Kao *et al.*, 2012) was isolated at Sites A and C in weeks 2 and 7, respectively. Although rarely causing disease, it has been associated with various infections such as in immunocompromised individuals (Kao *et al.*, 2012).

Kocuria kristinae is a former member of the *Micrococcus* family and has been reclassified to the genus *Kocuria* (Bannerman & Peacock, 2007). *Kocuria kristinae* is a normal part of the human skin and oral cavity flora (Folic *et al.*, 2010), and was isolated at Site A in week 4. It has been reported as a frequently opportunistic organism to immunocompromised people where it causes infections such as peritonitis (Carlini *et al.*, 2011) and bacteraemia (La *et al.,* 2011). Even though the organism is usually affiliated with infections in immunocompromised people, a few cases have been reported where it has

been linked to infections in healthy people. Folic *et al.* (2010) reported on a case where a seven year old boy, without a history of illness, contracted synovitis and periariticular bursitis caused by *K. kristinae*, while Dunn *et al*. (2011) reported the first case of a healthy pregnant woman diagnosed with bacteraemia caused by *K. kristinae*.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

4.1 ENUMERATION OF BACTERIAL CONTAMINANTS IN STAGNANT WATER POOLS

South Africa is a water scarce country and its water sources have been under increasing threat of microbial contamination. Rapid urbanisation has resulted in increased establishment of informal settlements with inadequate sanitation and water quality. Microbial contamination from greywater in informal settlements has been recognised by the Department of Water Affairs as one of the major water pollution factors. In order to manage wastewater in informal settlements, the Department of Water Affairs has recognised the need for information on microbial quality of greywater in such settlements (DWAF, 2001a). Although communal taps and toilets have been provided in such areas, they are often broken or inadequately serviced. This renders the available amenities inadequate. Poor drainage around communal taps results in water pools forming at the base of the taps. This water then becomes microbially contaminated through faecal contamination, hand washing after toilet use, laundry and washing of vegetables and raw meat. Therefore, the main aim of this study was to determine the level of microbial contamination of stagnant water in RR Section, Khayelitsha, Western Cape. Enumeration techniques such as the Most Probable Number (MPN), Heterotrophic Plate Count (HPC) as well as Flow cytrometry (FCM) techniques were employed to determine the total microbial count in the stagnant water samples, while the API 20E, the RapID™ ONE and the BBL Gram Positive (GP) Identification (ID) systems were used to identify possible pathogenic organisms in the stagnant water samples.

Six sampling sites (**Figure 2.1**) were identified across RR Section and sampling was conducted for a period of five months. The Most Probable Number (MPN) technique was used to determine the level of all gas-producing microorganisms, which include the faecal coliforms as well as the *E. coli* within the stagnant water samples. The Heterotrophic Plate Count (HPC) technique was used to determine the number of all culturable microorganisms within the stagnant water samples, while the Flow cytrometry (FCM) analysis technique was used to assess the total bacterial counts within the stagnant water samples.

On average the total MPN counts (**Figure 3.1**) ranged from the lowest count of 4.5×10^4 microorganisms/100mℓ recorded at Site E (week 4), to the highest count of 1.6×10^8 microorganisms/100mℓ recorded at Site A (weeks 3 and 5) as well as at Site B (week 5). The corresponding faecal coliforms enumerated during the sampling period ranged from the lowest count of 1.3×10^3 microorganisms/100mℓ obtained at Site E in week 4, to the highest count of 4.7×10^6 microorganisms/100mℓ obtained at Site B (week 5). In comparison, the

E. coli counts ranged from a zero count observed at Site F (week 9), to the highest count of 1.8 x 10^6 microorganisms/100mℓ recorded at Site A (week 5) and Site F (week 5). These results are significantly ($p < 0.05$) higher than the DWAF (1996a; 1996b) and the SABS (2011) Water Quality Standards. Only the *E. coli* count of 70 microorganisms/100mℓ, obtained at Site F (week 9) met the DWAF (1996b) Water Quality Standard but not the SABS (2011) Water Quality Standards. No *E. coli* was detected at Site F, which met both the DWAF (1996b) and the SABS (2011) Water Quality Standards. *Escherichia coli* count of 70 microorganisms/100mℓ was detected at Site F week 9. This count met the allowable limit of DWAF (1996b) Water Quality Standard but not the SABS (2011) Drinking Water Standard. No *E. coli* was detected at Site F, which met both the DWAF (1996b) and the SABS (2011) Water Quality Standards.

The average HPC count results for the stagnant water samples are depicted in **Figures 3.2** and **3.3**. The HPC counts ranged from 1.1 x10^3 microorganisms/mℓ recorded at Sites D and E in weeks 8 and 7, respectively, to 2.9 x10^5 microorganisms/mℓ recorded at Sites C in week 4. When comparing the HPC counts to the highest MPN counts, the highest MPN counts yielded significantly ($p < 0.05$) higher results than the HPC counts, even though the MPN results represent only gas-producing organisms, which technically should only represent a fraction of the total microbial population as represented by the HPC results. Seventy percent of the MPN counts exceeded the HPC counts throughout the sampling period. When comparing percentage ratios of the HPC results to the MPN results, the HPC (**Table 3.2**) accounted for a small fraction of 0.25%, 0.12% and 0.32%, respectively, of the highest count of the MPN results recorded at Site A (weeks 3 and 5) and Site B (week 5). However, irrespective of which method yielded higher counts, both enumeration techniques displayed significantly ($p < 0.05$) higher counts than the SABS (2011) and DWAF (1996a; 1996b) guidelines for domestic and recreational water, which in itself may pose a major health risk to the inhabitants of RR Section.

The total cell counts recorded by the FCM technique for the analysed stagnant water samples are depicted in **Figure 3.6**. The highest total FCM count was 3.4 x 10^7 microorganisms/mℓ recorded at Site A in week 5. The FCM technique yielded significantly ($p < 0.05$) higher counts than both the MPN and the HPC techniques. The relative values in percentage ratio of the MPN to the total FCM are presented in **Table 3.3**. The MPN counts represented only 4.74% of the highest total FCM at Site A in week 5. In comparison, the highest MPN count was 1.6 x 10^8 microorganisms/100mℓ detected at Sites A (weeks 3 and 5) and B (week 5). These accounted for 72.33%, 4.74% and 17.99%, respectively, of the total FCM counts detected at the same sites during the sampling period.

In comparison, the HPC (**Table 3.4** - **3.5**) accounted for a fraction of 0.01% for both the total FCM and the viable FCM counts, as recorded at Site A in week 5. When comparing

the percentage ratios of the highest HPC count to the total and viable FCM counts, the highest count for HPC (2.9 x 10^5 microorganisms/mℓ), which was obtained at Site C in week 4 accounted for a fraction of 32.43% and 40.24% of both the total FCM and the viable FCM counts recorded at the same site during the sampling period. The results show that of the three techniques, the FCM is a more reliable technique as it is able to enumerate a total microbial count, which includes the dead microbial cells. Furthermore, the FCM technique is able to determine the total microbial count in aquatic environments as it is able to detect organisms that have entered into a viable-but-non-culturable state due to environmental stress, while the HPC can only enumerate organisms that are culturable.

Even though warmer temperatures usually result in higher microbial activity, high temperatures did not necessarily account for higher microbial counts during this study. Instead, higher microbial counts were recorded during the winter months compared to the summer months. The reason for this could not be established as nothing out of the ordinary could be observed during this time period. However, various assumptions can be drawn. In the Western Cape, the winter season is rainy, and in informal settlements rain water often mixes with excreta from broken toilets. The wastewater could thereby mix with stagnant water pools at the base of the communal toilet, thus increasing microbial activity. Poor drainage also creates an environment for microbial organisms to flourish. Food particles were often found in the stagnant water pools during the sampling period and this could have contributed to higher microbial counts. Due to a lack of proper sanitation facilities, the community members use night pots to defecate or urinate in at night and these pots are washed at the communal taps in the morning, thereby introducing microorganisms to the stagnant water at the base of the stand taps. Community members were observed on various occasions, washing intestines of slaughtered animals whose blood and faecal matter could have introduced microorganisms to the stagnant water.

4.2 IDENTIFICATION OF GRAM-NEGATIVE BACTERIA

The API 20E and RapID™ ONE systems were used to identify organisms cultured from the stagnant water samples. These organisms were initially isolated from selective media (MacConkey, Eosin Methylene Blue and MacConkey without salt agars). Based on their morphological differences, pure cultures were then re-streaked onto nutrient agar. Prior to inoculation into the respective biochemical wells of both the API 20E and the RapID™ ONE kits, an oxidase test and Gram stain were performed on each chosen colony. Only colonies that fit the manufacturers' instructions were then subjected to identification by the API 20E and the RapID™ ONE systems.

The identified organisms belonged to the *Enterobacteriaceae*, which includes the coliform groups (Table 3.7). The API 20E system identified 80% of the isolated colonies from the stagnant water samples in RR Section, while the RapID™ ONE system identified only 72%. The organisms frequently identified by API 20E system were *E. coli*, *K. pneumonia*, *K . oxytoca*, and *Acinetobacter baumannii/calcoaceticus.* The organisms identified most often by the RapID™ ONE system were *K. pneumonia*, *E. coli, Acinetobacter calcoaceticus* and *E. cloacae*. Compared to the RapID™ ONE system, the API 20E system proved to be more sensitive as it was able to identify greater genus diversity. The RapID™ ONE system proved to be more sensitive than the API 20E system in identifying *Salmonella* as it was able to identify more *Salmonella* species than the API 20E system. Although indicator organisms such as *E. coli* indicate faecal contamination, the presence of other *Enterobacteriaceae* groups such as *Acinetobacter, Enterobacter, Klebsiella, Salmonella* and *Serratia* species may pose a potential health risk to the community of RR Section, as most of these organisms may be opportunistic pathogens.

Although it took approximately 48 hours from initial cultivation to purification into single isolates before incubation into the respective biochemical wells, the RapID™ ONE system was much quicker in obtaining results than the API 20E system. It took approximately 5 hours of incubation in order to get results with the RapID™ ONE system, while it took approximately 18 – 24 hours of incubation in order to obtain results with the API 20E system. In addition, the RapID™ ONE system proved to be more user-friendly in results interpretation and required one reagent, compared to the API 20E system which was more challenging in results interpretation and required more reagents. Furthermore, not all isolates could be identified by both systems as they required further biochemical analysis.

4.3 IDENTIFICATION OF GRAM-POSITIVE BACTERIA

The BBL Crystal™ Gram-Positive (GP) Identification (ID) System was used for the identification of Gram-positive organisms cultured from the stagnant water samples. These organisms were initially isolated from selective media (Sabouraud Dextrose Agar and Baird Parker agars). Based on their morphological differences, pure cultures were then re-streaked onto nutrient agar. Prior to inoculation into the respective biochemical wells of the BBL Crystal™ GP ID kit, a Gram stain test was performed on each chosen colony. Only colonies that fit manufacturer's instructions were then subjected to identification by the BBL Crystal™ GP ID System. The BBL Crystal™ GP ID System could only identify 71 % of the isolates subjected for identification. The organisms identified most often by the BBL

Crystal™ GP ID System were *Corynebacterium* species, which included *C. bovis, C. diphtheria* and *C. pseudodiphtheriticum, Aerococcus urinae* and *Bacillus cereus*.

The organisms identified in this study by the three identification systems are mostly opportunistic organisms. Many of the Gram-negative microorganisms can be transmitted via water, while the majority of the Gram-positive bacteria have previously been isolated from aquatic environments. Therefore, the presence of these organisms in aquatic environments may pose a major health risk to the inhabitants of this informal settlement.

4.4 THE IMPLICATIONS OF POOR SANITATION IN RR SECTION

Many studies that assess greywater quality in informal settlements in South Africa concentrate on the chemical analysis. For microbial analysis the focus is usually on indicator organisms such as total coliforms, faecal coliforms and *E. coli*. This study, however, has given a much needed basis for understanding the pathogenic microbial organisms that could be present in greywater in informal settlements, and can therefore be utilised in informal settlement wastewater management.

Waterborne pathogens are of a great public concern, as they can cause diseases through drinking contaminated water, contact with contaminated water, or poor hygiene. The stagnant water pools are therefore a potential health risk to the children who use these areas as a playground and to the adults who were observed using this water to wash their hands instead of the water from the installed standpipes.

The inadequate drainage at the bottom of the standpipes creates a health hazard to the community of RR Section. As previously mentioned, the presence of faecal coliforms such as *E. coli* in the sampled water indicates faecal contamination (Payment & Locas, 2011). In addition, the presence of pathogenic microorganisms such as *Klebsiella pneumonia, Salmonella* spp., *Bacillus cereus* and *Micrococcus luteus* in the greywater at the base of the communal standpipes, is a possible health risk to the children playing in close proximity to the greywater at the base of the communal taps water as well as adults using this water for daily use such as hand washing.

These organisms could have been introduced via many routes. According to SJC (2011), raw sewage that leaks into the streets or walkways is a definite way in which faecal pollutants come into contact with the stagnant water pools at the base of communal taps in RR Section. In addition, the rinsing of night pots (which are used by the residents for sanitary purposed during the night) (Granfone *et al.,* 2008; SJC, 2011; Tokota, 2012) as well as washing and rinsing of intestines of slaughtered animals at the communal taps, may contribute to increased microbial numbers. Furthermore, soaking clothes and utensils for a

prolonged period of time could possibly increase the bacterial load in greywater (Nganga *et al.*, 2012). Nganga *et al.* (2012), as previously stated, reported that solid food particles and remnants washed from used food containers (which were observed on various occasions throughout the sampling period) provided an excellent substrate for microbial growth as it is rich sources of nutrients.

According to Carden *et al.* (2007) human behavioural patterns such as social dynamics, attitudes and behavioural patterns, are important factors in the management of wastewater. It was evident throughout the sampling period that a general understanding of behavioural patterns and its link to health issues was either lacking or very low when considering the general attitude and behavioural patterns among communities. The societal perception on sanitary wastes is an important aspect, which needs to be addressed in RR Section and similar communities in order to reduce waterborne infections or diseases. Apart from instilling the proper attitude and awareness amongst inhabitants, the problem may persist if the infrastructure in the area is not improved as well.

The City of Cape Town has developed Greywater Guidelines, which aim to provide guidance on how and where greywater from informal settlements within the City of Cape Town should be disposed of (City of Cape Town, 2005). These guidelines make a provision for soakaways to drain greywater from the communal standpipes. They also make provision for regular maintenance of these soakaways. It can therefore be assumed that these guidelines are not adhered to in RR Section as water still accumulates at the base of the standpipes as seen in Figure 2.1. The water accumulating at the base of the standpipes creates an ideal environment for microorganisms to flourish. According to Fry *et al.* (2008) the supply of piped, treated water to all households is equally important to public health as the provision of improved sanitation. Esrey (1996) suggested that the additional benefits of improved sanitation would be higher than only improving the water quality. In addition, the safe disposal of human excreta is far more effective than any amount of hand-washing to ensure protection against infectious disease transmission (Curtis *et al.,* 2000). Therefore, good sanitation, availability of good quality water, adequate disposal of human and animal excrement and public education in hygiene practices are major factors that reduce the impacts of diarrhoeal diseases (UNICEF & WHO, 2009). Furthermore, adequate sanitary facilities and drainage systems in RR Section and similar communities could to a significant extent alleviate the problem of microbially infested stagnant water pools at the base of communal standpipes.

4.5 RECOMMENDATIONS

Understanding microbial content in greywater in informal settlements is important when managing wastewater from these areas. There is a need for frequent detailed analysis of microbial content in such areas. Based on the results obtained in this study, it is recommended that:

- FCM technique is the most reliable technique in determining the accurate bacterial load in aquatic environments. It should therefore be used as one of the main techniques to determine if the water quality falls within the allowable limits.
- Biofilm material at the base of the communal taps should be analysed, as these materials are known to encourage the survival of organisms which might not be encountered in the water.
- The commercial identification methods used in this study should be used as preliminary identification, and confirmation tests should be done with more sensitive methods, like the Polymerase Chain Reaction technique and 16S rRNA gene sequencing.
- To ensure holistic sanitation management, it is important for a social study to be conducted whereby people's perception of greywater quality can be assessed. Furthermore, the way people interact with greywater also needs to be explored, as this is vital for securing comprehensive greywater management in informal settlements.

REFERENCES

Achermann, Y., Trampuz, A., Moro, F., Wüst, J. & Vogt, M. 2009. *Corynebacterium bovis* shoulder prosthetic joint infection: the first reported case. *Diagnostic Microbiology and Infectious Diseases*, 64(2): 213 – 215.

Ackermann, A. 2010. Assessment of microbial loads of the Plankenburg and Berg Rivers and the survival of *Escherichia coli* on raw vegetables under laboratory conditions. MSc Food Science thesis, University of Stellenbosch, South Africa.

Adams, B. L., Bates, T. C., & Oliver, J. D. 2003. Survival of *Helicobacter pylori* in a natural freshwater environment. *Applied and Environmental Microbiology*, 69(12): 7462 – 7466.

Ahmed, K.S., Khan, A.A., Ahmed, I., Tiwari, S.K., Habeeb, A., Ahi, J.D., Abid Z, Ahmed N, & Habibullah, C.M. 2007. Impact of household hygiene and water source on the prevalence and transmission of *Helicobacter pylori*: a South Indian perspective. *Singapore Medical Journal*, 48(6): 543 – 549.

Ahoyo, T.A., Yehouenou, P., Baba-Moussa, L., Tttolou, G.A., Boco, M. Dramane, K.L. & Aminou, T. 2013. *Staphylococcus sciuri* outbreak at tertiary hospital in Benin. *Journal of Medical Microbiology Diagnostics*, 2(3): 1 – 5.

Allen, M.J., Edberg, S.C. & Reasoner, D.J. 2004. Heterotrophic plate count bacteria – what is their significance in drinking water? *International Journal of Food Microbiology*, 92: 265 – 274.

Allen, M.E. 2013. MacConkey plates protocol. *American Society for Microbiology.* http://www.microbelibrary.org/component/resources/laboratory-test/2855-macconkey-agar-plates-protocols [15 September 2014].

Antonopoulos, P., Constantinidis, F., Charalampopoulos, G., Dalamarinis, K., Karanicas, I. & Kokkini, G. 2008. An emergency diagnostic dilemma: a case of *Yersinia enterocolitica* colitis mimicking acute appendicitis in a β-thalassemia major patient: the role of CT and literature review. *Emergency Radiology*, 15(2): 123 – 126.

Archer, B.N., Cengimbo, A., De Jong, G.M., Keddy, K.H., Smith, A.M., Sooka, A., Ntshoe, G. & Blumberg, L. 2009. Cholera outbreak in South Africa preliminary

descriptive epidemiology on laboratory-confirmed cases. 15 November 2008 to 30 April 2009. *Communicable Diseases Surveillance Bulletin*, 7(2): 3 – 8.

Arnone, R.D. & Walling, J.P. 2007. Waterbone pathogens in urban watersheds. *Journal of Water and Health* 5(1): 149 – 162.

Ashour, M.S.E.D., Mansy, M.S. & Eissa, M. E. 2011. Microbiological environmental monitoring in pharmaceutical facility. *Egyptian Academic Journal of Biological Sciences,* 3(1): 63 – 74.

Bannerman, T.L. & Peacock, S.J. 2007. *Staphylococcus, Micrococcus,* and other catalase-positive cocci. In Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. & Pfaller, M.A. (eds). *Manual of clinical microbiology,* 9th ed. Washington: ASM Press: 390 – 404.

Barcina, I. & Arana, I. 2009. The viable-but-non-culturable phenotype: a crossroads in the life-cycle of non-differentiating bacteria? *Reviews in Environmental Science and Bio/Technology*, 8(3): 245 – 255.

Barnes, J.M. 2010. Water Microbial Analysis, Khayelitsha. Unpublished Report. September, 2010.

Bartram, J., Cotruvo, J., Exner, M., Fricker, C. & Glasmacher, A. 2004. Heterotrophic plate count measurement in drinking water safety management. Report of an Expert Meeting Geneva, 24 – 25 April 2002. *International Journal of Food Microbiology*, 92: 241 – 247.

Becton Dickinson (BD). 2011. BD™ Baird-Parker agar. http://www.bd.com/resource.aspx?IDX=25497 [23 September 2014].

Becton Dickinson (BD). 2012. BBL crystal™ identification systems gram-positive ID kit. http://www.bd.com/ds/technicalCenter/inserts/8809911JAA%20(01).pdf [04 February 2014].

Becton Dickinson (BD). 2013. BD ™ MacConkey agar without salt. http://www.bd.com/resource.aspx?idx=9012 [19 September 2014].

Bernard, K. 2012. The genus *Corynebacterium* and other medically relevant Coryneform-like bacteria. *Journal of Clinical Microbiology*, 50(10): 3152 – 3158.

Berney, M., Hammes, F., Bosshard, F., Welenmann, H.U. & Egli, T. 2007. Assessment and interpretation of bacterial viability by using the LIVE/DEAD *Bac*Light Kit in combination with flow cytometry. *Applied and Environmental Microbiology*, 73(10): 3283 – 3290.

Bien, J., Sokolova, O. & Bozko, P. 2011. Characterization of virulence factors of Staphylococcus aureus: Novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. *Journal of Pathogens*, 2011.http://www.hindawi.com/journals/jpath/2011/601905/ [14 August 2014].

Birks, R. Colbourne, J., Hills, S. & Hobson, R. 2004. Microbiological water quality in a large in-building, water recycling facility. *Water Science & Technology*, 50(2): 165 – 172.

Birks, R. & Hills, S. 2007. Characterisation of indicator organisms and pathogens in domestic grey water for recycling. *Environmental Monitoring Assessment,* 129: 61 – 69.

Bittar, F., Cassagne, C., Bosdure, E., Stremler, N., Dubus, J.C., Sarles, J., Reynaud-Gaubert, M., Raoult, D. & Rolain, J.M. 2010. Outbreak of *Corynebacterium pseudodiphtheriticum* infection in cystic fibrosis patients, France. *Emerging Infectious Diseases*, 16(8): 1231 – 1236.

Bitton, G. 2011. Wastewater microbiology. 4th ed. Hoboken, N.J.: Wiley-Blackwell.

Blossom, D.B., & Srinivasan, A. 2008. Drug-Resistant *Acinetobacter baumannii-calcoaceticus* complex: an emerging nosocomial pathogen with few treatment options. *Infectious Diseases in Clinical Practice*, 16(1): 1 – 3.

Bojer, M. S., Struve, C., Ingmer, H., Hansen, D. S., & Krogfelt, K. A. 2010. Heat resistance mediated by a new plasmid encoded Clp ATPase, ClpK, as a possible novel mechanism for nosocomial persistence of *Klebsiella pneumoniae*. *PloS One*, 5(11): e15467.
http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0015467&representation=PDF [16 August 2014].

Bottone, E.J. 2010. *Bacillus cereus,* a volatile human pathogen*. Clinical Microbiology Review, 23: 382 – 398.*

Bowie, V.L., Snella, K.A., Gopalachar, A.S. and Bharadwaj, P. 2004. *Listeria meningitis* associated with infliximab. *Annals of Pharmacotherapy*, 38: 58 – 61.

Braden, C. R. 2006. *Salmonella* enteric serotype enteritidis and eggs: A national epidemic in the United States. *Clinical Infectious Diseases*, 43(4): 512 – 517.

Bregman, J. 2011. Interview: discussing placement of standpipes located in RR Section in Khayelitsha. October, 2011.

Bullock, N.O. & Aslanzadeh, J. 2013. Biochemical profile-based microbial identification systems. In Tang, Y.W. & Stratton, C.W. (eds). *Advanced techniques in diagnostic microbiology*. New York: Springer: 87 – 121.

Burtscher, M.M., Zibuschka, F., Mach, R.L., Lindner, G. & Farnleitner, A.H. 2009. Heterotrophic plate count vs. in situ bacterial 16S rRNA gene amplicon profiles from drinking water reveal completely different communities with distinct spatial and temporal allocations in a distribution net. *Water SA*, 35(4): 495 – 504.

Cabral, J.P. 2010. Water microbiology. Bacterial pathogens and water*. International Journal of Environmental Research and Public Health*, 7(10): 3657 – 3703.

Callaway, T. R., Edrington, T. S., Anderson, R. C., Byrd, J. A., & Nisbet, D. J. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *Journal of Animal Science*, 86: E163 – E172.

Capita, R., Alonso-Calleja, C., Moreno, B. & del Camino García-Fernández, M. 2001. Assessment of Baird-Parker agar as screening test for determination of *Staphylococcus aureus* in poultry meat. The *Journal of Microbiology-Seoul, Korea*, 39(4): 321 – 325.

Carden, K., Armitage, N., Sichone, O., Winter, K. & Rivett, U. 2006. Management options for the safe use and disposal of greywater in the non-sewered areas of South Africa. Paper presented at the third international conference on ecological sanitation, 23 - 26 May 2005, Durban, South Africa.

http://www.ewisa.co.za/literature/files/243%20Carden.pdf [06 June 2014].

Carden, K., Armitage, N., Winter, K., Sichone, O. & Rivett, U. 2007. *Understanding the use and disposal of greywater in the non-sewered areas of South Africa*. Pretoria,, South Africa: Water Research Commission.

Carden, K., Armitage, N., Winter, K., Sichone, O. & Rivett, U. 2008. The management of greywater in the non-sewered areas of South Africa. *Urban Water Journal*, 5(4): 329 – 343.

Carlini, A., Mattei, R., Lucarotti, I., Bartelloni, A & Rosati, A. 2011. *Kocuria kristinae*: an unusual cause of acute peritoneal dialysis related infection. *Peritoneal Dialysis International*, 31(1): 105 – 107.

Castaño-Boldú, E. & Comas-Riu, J. F. 2012. Flow cytometry in the analysis of cellular populations: Handbook of instrumental techniques for materials, chemical and biosciences research. *Biosciences Technologies (BT)*, 14. http://diposit.ub.edu/dspace/bitstream/2445/32082/1/BT09%20-%20Flow%20cytometry_ed2.pdf [30 May 2014].

Center for Disease Control and Prevention. 2013. *Legionella* (Legionnaires' Disease and Pontiac Fever). http://www.cdc.gov/legionella/about/signs-symptoms.html [11 June 2014].

Chen, S., Wang, Y., Chen, F., Yang, H., Gan, M. & Zheng, S.J. 2007. A highly pathogenic strain of *Staphylococcus sciu*ri caused fatal exudative epidermitis in piglets. *PloS One*, 1: e147. http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0000147&representation=PDF [12 August 2014].

Choi, S.H., Lee, J.E., Park, S. J., Kim, M.N., Choo, E.J., Kwak, Y.G., Jeong, J.Y., Woo, J.H., Kim, N.J & Kim, Y. S. 2007. Prevalence, microbiology, and clinical characteristics of extended-spectrum β-lactamase-producing *Enterobacter* spp., *Serratia marcescens*, *Citrobacter freundii*, and *Morganella morganii* in Korea. *European Journal of Clinical Microbiology & Infectious Diseases*, 26(8): 557 – 561.

City of Cape Town. 2005. City of Cape Town Grey Water Guidelines Version - 20 May 2005

http://webcache.googleusercontent.com/search?q=cache:UH8Z-IGYIE4J:www.capetown.gov.za/en/Water/WaterservicesDevPlan/Documents/4.3_GOTO%25209.2_Grey%2520Water%2520Guidelines%2520-%252020%2520May%252005.doc+&cd=1&hl=en&ct=clnk&gl=za [07 June 2005].

City of Cape Town. 2009. Memorandum: Abahlali Basemjondolo of the Western Cape – 29 July 2009. www.capetown.gov.za [10 November 2011].

City of Cape Town. 2011. Water services development plan (WSDP) for City of Cape Town 2011/12 – 2015/16 – Executive summary. Draft 2.4. http://www.capetown.gov.za/en/Water/WaterservicesDevPlan/Documents/WSDP_2011_2012/WSDP_Exec_Sum_Sector_plan_18Feb11_printable.pdf [11 September 2012].

Claridge, J.E., Attorri, S., Musher, D.M., Hebert, J. & Dunbar, S. 2001. *Streptococcus intermedius, Streptococcus constellatus*, and *Streptococcus anginosus* (*Streptococcus milleri* group) are of different clinical importance and are not equally associated with abscess. *Clinical Infectious Diseases*, 32(10): 1511 – 1515.

Cleveland, K.O., Mazumder, S.A., & Gelfand, M.S. 2014. Association of *Raoultella* bacteremia with diseases of the biliary tract. *Scandinavian Journal of Infectious Diseases*, 46(7): 541 – 542.

Collins, M.D. 2006. The genus *Gemella*. In Dworkin, M., Falkow, S., Rosenber, E., Schleifer, K.H. & Stackebrandt, E (eds). *The prokaryotes, 3rd* ed. New York: Springer: 511 – 518.

Community Organisation Resource Centre [CORC]. 2013. RR Section. water and sanitation distribution.

Correa, A.L., Mazo, L.M., Valderrama, M.P., Restrepo, A. & Jaimes, F. 2012. An outbreak of *Leclercia adecarboxylata* bacteremia likely associated to contaminated heparin vials. Abstract. *Infection*, 16(2): 117 – 121. http://www.scielosp.org/scielo.php?pid=S0021-25712012000400013&script=sci_arttext&tlng=pt [25 September 2013].

Cossart, P. & Toledo-Arana, A. 2008. *Listeria monocytogenes*, a unique model in infection biology: an overview. *Microbes and Infection*, 10: 1041 – 1050.

Cowan, M. K. & Tolaro, K. P. 2009. Microbiology: a systems approach. 2nd ed. New York: McGraw-Hill Companies, Inc.

Crump, J.A. & Mintz, E.D. 2010. Global trends in typhoid and paratyphoid fever. *Clinical Infectious Diseases*, 50(2): 241 – 246.

Curtis, V., Cairncross, S. & Yonli, R. 2000. Review: Domestic hygiene and diarrhoea – pinpointing the problem. *Tropical Medicine & International Health*, 5(1): 22 – 32.

Da Silva, N. Taniwaki, M.H., Junqueira, V.C.A., Silveira, N.F.A., do Nascimento, M.S. & Gomes, R.A.R. 2013. *Microbiological examination methods of food and water. A laboratory manual*. London: Taylor & Fransis Group.

Dalal, A., Urban, C., Ahluwalia, M. & Rubin, D. 2008. Corynebacterium bovis line related septicemia: A case report and review of the literature. *Scandinavian Journal of infectious Diseases*, 40(6-7): 575 – 577.

Davenport, P. & Land, K. J. 2007. Isolation of Leclercia adecarboxylata from the blood culture of an asymptomatic platelet donor. *Transfusion*, 47(10): 1816 – 1819.

De Jong, M.F., Soetekouw, R., Reinier, W. & Veenendaal, D. 2010. *Aerococcus urinae*: severe and fatal bloodstream infections and endocarditis. *Journal of Clinical Microbiology*, 48(9): 3445 – 3447.

De Roy, K., Clement L., Thas, O., Wang, Y & Boon, N. 2012. Flow cytometry for fast microbial community fingerprinting. *Water Research*, 4: 907 – 919.

Department of Monitoring & Evaluation (DPME) & Department of Human Settlement (DHS). 2012. Sanitation service – quality of sanitation in South Africa. Report on the status of sanitation services in South Africa. http://www.sahrc.org.za/home/21/files/Quality%20of%20sanitation%20-%20Exec%20Summary%20(Fin).pdf%20III.pdf [17 October 2014].

Department of Water Affairs and Forestry (DWAF). 1996a. South African Water Quality Guidelines. Volume 1. Domestic Water Use. 2nd ed. Pretoria: DWAF. http://www.dwaf.gov.za/IWQS/wq_guide/domestic.pdf [15 November 2011].

Department of Water Affairs and Forestry (DWAF). 1996b. South African Water Quality Guidelines. Volume 2. Recreational Water. 2nd ed. Pretoria: DWAF.

Department of Water Affairs and Forestry (DWAF). 2001a. Managing the Water Quality Effects of Settlements – National Strategy. Pretoria: DWAF.

Department of Water Affairs and Forestry (DWAF). 2001b. Managing the Water Quality Effects of Settlements – Technical Supporting Document. Pretoria: DWAF.

Department of Water Affairs and Forestry (DWAF). 2013. Media statement on the signing of a bilateral agreement on water resources management and water supply between the minister of Water Affairs and her Cuban counterpart. http://www.dwaf.gov.za/Communications/PressReleases/2013/MEDIA%20STATEMENT%20-%20CUBA.pdf [16 August 2014].

Diab, A.M., Al-Turk, I.M., Ibrahim, M.K. & Al-Zhrany, K.D. 2008. Tracing of Gram-negative antibiotic-resistant bacteria in hospitals final effluent at Al-Madinah Al-Mounwwarah. *Journal of Taibah University for Science*, 1, 24-33.

Díaz, M., Herrero, M., García, L. A. & Quirós, C. 2010. Application of flow cytometry to industrial microbial bioprocesses. *Biochemical Engineering Journal*, 48(3): 385 – 407.

Díez-Aguilar, M., Ruiz-Garbajosa, P., Fernández-Olmos, A., Guisado, P., Del Campo, R., Quereda, C., Cantón, R. & Meseguer, M. A. 2013. Non-diphtheriae Corynebacterium species: an emerging respiratory pathogen. *European Journal of Clinical Microbiology & Infectious Diseases*, 32(6): 769 – 772.

Dorrington, R.E. 2005. Projection of the population of the city of Cape Town. http://www.capetown.gov.za/en/stats/CityReports/Documents/Population%20Projection/Population_Projection_for_Cape_Town_2001-2021_Full_Report_2192006105338_359.pdf [11 May 2012].

Dunn, O.J & Clark, V.A. 1987. *Applied statistics: analysis of variance and regression* (2nd ed). New York: Wiley.

Dunn, R., Bares, S., & David, M. Z. 2011. Central venous catheter-related bacteremia caused by *Kocuria kristinae*: case report and review of the literature. *Annals of Clinical Microbiology and Antimicrobials, 10*(31).

http://www.biomedcentral.com/content/pdf/1476-0711-10-31.pdf [16 August 2014].

Dusserre, E., Ginevra, C., Hallier-Soulier, S., Vandenesch, F., Festoc, G., Etienne, J., Jarraud, S. & Molmeret, M. 2008. A PCR-based method for monitoring Legionella pneumophila in water samples detects VBNC that can recover their cultivability. *Applied and Environmental Microbiology*, 74: 4817 – 4824.

Engelkirk, P.G. & Duben-Engelkirk, J. 2008. *Laboratory diagnosis of infectious disease: essentials of diagnostic microbiology*. Baltimore: Lippincott Williams & Wilkins, a Wolters Kluwer business.

Environmental Protection Agency (EPA). 2013. Health risks from microbial growth and biofilms in drinking water distribution systems. http://www.epa.gov/ogwdw/disinfection/tcr/pdfs/whitepaper_tcr_biofilms.pdf [10 September 2013].

Epstein, S. S. 2009. Microbial awakenings. *Nature*, 457(7233): 1083 – 1083.

Eriksson, E., Auffarth, K., Henze, M. & Ledin, A. 2002. Characteristics of grey wastewater. *Urban Water,* 4: 85 -104.

Escalante, G., Campos, L., Valenzuela, C., Yanẽz, J., Zaror, C. & Mondaca, A. 2009. Arsenic Resistant Bacteria Isolated from arsenic contaminated river in the Atacama Desert (Chile). *Bulletin of Environmental Contamination and Toxicology*, 83: 657 – 661.

Esrey, S.A. 1996. Water, waste, and well-being: a multicountry study. *American Journal of Epidemiology*, 143(6): 608 – 623.

Euzéby, J. P. 2010. List of bacterial names with standing in nomenclature. *International Journal of Systematic Bacteriology*, 47, 13. http://www.bacterio.cict.fr/m/micrococcus.html [11 August 2014].

Fakruddin, M., Mannan, K.S.B. & Andrews, S. 2013. Viable-but-non-culturable Bacteria: food safety and public health perspective. *International Scholarly Research Notices Microbiology*: 1 – 6.

Fangtham, M. & Wilde, H. 2008. Emergence of *Salmonella paratyphi A* as a major

cause of enteric fever: need for early detection, preventive measures, and effective vaccines. *Journal of Travel Medicine*, 15(50): 344 – 350.

Fatoki, O.S., Muyima, N.Y.O. & Lujiza, N. 2001. Situational analysis of water quality in the Umtata River catchment. *Water SA*, 27(4): 467 – 474.

Feasey, N. A., Archer, B. N., Heyderman, R. S., Sooka, A., Dennis, B., Gordon, M. A., & Keddy, K. H. 2010. Typhoid fever and invasive nontyphoid salmonellosis, Malawi and South Africa. *Emerging Infectious Diseases*, 16(9): 1448 – 1451.

Folic, M., Janković, S., Ružić-Zečevič, D., Pajeveć, V., Rosić, N & Nikolić, P. 2010. Synovitis and periarticular bursitis of the coxofemoral joint caused by *Kocuria kristinae*: a case report. *Scientific Journal of the Faculty of Medicine in Niś*, 27(1): 51 – 54.

Food and Drug Administration. 2012. Bad Bug Book: handbook of foodborne pathogenic microorganisms and natural toxins. http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf [12 June 201].

Friedrich, E., Pillay, S., & Buckley, C. A. 2009. Carbon footprint analysis for increasing water supply and sanitation in South Africa: a case study. *Journal of Cleaner Production*, 17(1): 1 – 12.

Fry, L.M., Mihelcic, J.R. & Watkins, D.W. 2008. Water and nonwater-related challenges of achieving global sanitation coverage. *Environmental, Science & Technology*, 42(12): 4298 – 4304.

Ganesan, B., Stuart, M.R. & Weimer, B.C. 2007. Carbohydrate starvation causes a metabolically active-but-non-culturable state in *Lactococcus lactis*. *Applied and Environmental Microbiology,* 73: 2498 – 2512.

Gemmell, M.E. & Schmidt, S. 2010. Potential links between irrigation water quality and microbiological quality of food in subsistence farming in Kwazulu-Natal, South Africa. *Current research, technology and education topics in applied microbiology and microbial biotechnology,* 2: 1190 – 1195.

Gião, M.S., Azevedo, N F., Wilks, S.A., Vieira, M.J. & Keevil, C.W. 2008. Persistence

of *Helicobacter pylori* in heterotrophic drinking-water biofilms. *Applied and Environmental Microbiology*, 74(19): 5898 – 5904.

Global Water, Sanitation and Hygiene (WASH) Cluster. 2009. The human right to water and sanitation in emergency situations: the legal framework and guide to advocacy. http://www.worldwatercouncil.org/fileadmin/wwc/Right_to_Water/Project_8_HRTWS_in_Emergencies_Handbook.pdf [03 October 2014].

Goldberg, K. 2009. The Water Dialogues: Cape Town case Study. http://ww.waterdialogues.org/south-africa/documents/p.46CapeTownCaseStudySummary.pdf [16 October 2011].

Gomila, M., Renom, G., Del Carmen, M., Garau, M., Guerrero, D., Soriano, J.B. & Lalucat, J. 2012. Identification and diversity of multiresistant *Corynebacterium striatum* and clinical isolates by MALDI-TOF mass spectrometry and by a multigene sequencing approach. *BioMed Central Microbiology*, 12:1 – 8.

Gordon, M. A. 2008. *Salmonella* infections in immunocompromised adults. *The Journal of Infection*, 56, 413 – 422.

Govender, T., Barnes, J.M. & Pieper, C.H. 2011. Contribution of water pollution from inadequate sanitation and housing quality to diarrheal disease in low-cost housing settlements of Cape Town, South Africa. *American Journal of Public Health,* 101(7). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3110231/pdf/e4.pdf [21 September 201].

Granfone, M., Lizewski, C. & Olecki, D. 2008. Water and Sanitation. http://wpi-capetown.org/wp-content/uploads/atlas/08Water.pdf [10 December 2011].

Griesel, M. & Jagals, P. 2002. Faecal indicator organisms in the Renoster Spruit system of the Modder-Riet River catchment and implications for human users of the water. *Water SA,* 28(2): 227 – 234.

Gross, A., Kaplan, D. & Baker, K. 2007. Removal of microorganisms from domestic greywater using a recycling vertical flow constructed wetland (RVFCW). *Ecological Engineering*, 32, 107 – 114.

Gutierrez, K. 2005. Bone and joint infections in children. *Pediatric clinics of North America*, 52(3): 779 – 794.

Haller, L., Poté, J., Loizeau, J. L., & Wildi, W. 2009. Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Ecological Indicators*, 9(3): 540 – 547.

Hammes, F.A. & Egli, T., 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. *Environmental Science and Technology*, 39: 3289 – 3294.

Hammes, F., Berney, M., Wang, Y., Vital, M., Köster, O. & Egli, T. 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Research*, 42(1), 269 – 277.

Hammes, F., Berger, C., Köster, O. & Egli, T. 2010. Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system. *Research and Technology – AQUA*, 59(1): 31 – 40.

Hare, J. 2008. Sabouraud Agar for fungal growth protocols. *American Society for Microbiology*. http://www.microbelibrary.org/library/laboratory-test/3156-sabouraud-agar-for-fungal-growth-protocols [11 September 2014].

Hart, C. A. 2006. *Klebsiella, Citrobacter, Enterobacter* and *Serratia* spp. In Gillespie, S.H. & Hawkey, P.M. (eds). *Principles and practice of clinical bacteriology*. West Sussex: John Wiley & Sons, Ltd: 377 – 386.

Hess, B., Burchett, A., & Huntington, M. K. 2008. *Leclercia adecarboxylata* in an immunocompetent patient. *Journal of Medical Microbiology,* 57(7): 896 – 898.

Hill, D. D., Owens, W. E., & Tchounwou, P. B. 2005. Comparative assessment of the physico-chemical and bacteriological qualities of selected streams in Louisiana. *International Journal of Environmental Research and Public Health*, 2(1): 94 – 100.

Ho, E., Coveliers, J., Amsel, B.L., Stockman, B., Walopt, J., Ieven, M. & Rodrigus, I. 2010. A case of endocarditis due to *Aerococcus urinae. Journal of Heart Valve Disease*, 19: 264 – 266.

Hoffman, L.C., Britz, T.J. & Schnetler, D.C. 2010. Prevalent organisms on ostrich carcasses found in a commercial abattoir. *Journal of the South African Veterinary Association*, 81(3): 151 – 155.

Högenauer, C., Langner, C., Beubler, E., Lippe, I. T., Schicho, R., Gorkiewicz, G., Krause,R., Gerstgrasser, N., Krejs, G.J. & Hinterleitner, T. A. 2006. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *New England Journal of Medicine*, 355(23): 2418 – 2426.

Housing Development Agency (HDA). 2013a. South Africa: Informal Settlements Status.
http://www.thehda.co.za/uploads/images/HDA_South_Africa_Report_lr.pdf [12 June 2014].

Housing Development Agency (HDA). 2013b. Western Cape: Informal Settlements Status.
http://www.thehda.co.za/uploads/images/HDA_Western_Cape_Report.pdf [12 June 2014].

Jabeen, S., Mahmood, Q., Tariq, S., Nawab, B. & Elahi, N. 2011. Health impact caused by poor water and sanitation in district Abbottabad. *Journal of Ayub Medical College Abbottabad,* 23(1): 47 – 50.

Jamshidi, A., Bassami, M.R. & Afshari-Nic, S. 2009. Identification of *Salmonella spp.* and *Salmonella typhimurium* by a multiplex PCR-based assay from poultry carcasses in Mashhad- Iran. *International Journal of Veterinary Research*, 3(1): 43 – 48.

Ji, Z.G. 2008. Hydrodynamics and water quality: modeling rivers, lakes and Estuaries. New Jersey (NJ): John Wiley & Sons Inc.

Johnson, E.A. 2009. *Clostridia*. In Schaechter, M (ed). *Encyclopedia of Microbiology*. 3rd Ed. Oxford, Elsevier: 87 – 93.

Kao, C.C, Chiang, C.K. & Huang J.W. 2012. *Micrococcus* species-related peritonitis in patients receiving peritoneal dialysis. *International Urology and Nephrology,* 46(1): 261 – 264.

Kaper, J.B., Nataro, J.P., & Mobley, H.L. 2004. Pathogenic *Escherichia coli*. *Nature*

Reviews Microbiology, 2(2): 123 – 140.

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., Muyodi, J., Githinji, J.W., Kagendo, D., Munyalo, A. & Hart, A.C. 2006. Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: Zoonotic or anthroponotic transmission? *Journal of Medical Microbiology*, 55: 585 – 591.

Keddy, K. 2013. Diarrhoeagenic *Esherichia coli* (DEC*). Communicable Diseases Surveillance Bulletin*, 11(3): 74 – 76.

Khalaphallah, R. & Andres, Y. 2012. The effect of various abiotic factors on the survival growth of *Escherichia coli* and *Pseudomonas aeruginosa* in bathroom greywater. *Journal of Water, Reuse and Desalination*, 2(2): 92 – 101.

Khalil, M.T., Skakir, S.H., Saad, A.A., El-Shabrawy, G.M. & Hassan, M.M. 2008. Physico-chemical environment of Lake Edku, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, 12(2): 119-132.

Kitch, T.T. Jocobs, M.R. & Appelbaum, P.C. 1994. Evaluation of RapID™ onE system for identification of 379 strains in the family *Enterobacteriaceae* and oxidase-negative, gram-negative nonfermenters. *Journal of Clinical Microbiological*, 32(4): 931 – 4.

Kocur, M., Kloos, W.E. & Schleifer, K.H. 2006. The genus *Micrococcus*. *Prokaryotes*, (3): 961 – 971.

Koola, J. & Zwane, A.P. 2014. Water supply and sanitation. In A. J. Culyer (ed.) *Encyclopedia of Health Economic.* San Diego: Elsevier: 477 – 482.

Koola, J. and Zwane, A. P. (2014). Water Supply and Sanitation. In A. J. Culyer (Ed.), Encyclopedia of Health Economics (pp. 477-482).

La, C.C., Wang, J.Y., Lin, S.H., Tan, C.D., Wang, C.Y., Liao, C.H., Chou, C.H., Huang, Y.T., Lin, H.I & Hsueh, P.R. 2011. Catheter-related bacteraemia and infective endocarditis caused by *Kocuria* species. *Clinical Microbiology and Infection*, 17(2): 190 – 192.

Lal, A. & Cheeptham, N. 2007. Eosin-methylene blue agar plates protocol. *American Society for Microbiology*.

http://www.microbelibrary.org/component/resource/laboratory-test/2869-eosin-methylene-blue-agar-plates-protocol [15 September 2014].

Lambertz, S. T., Nilsson, C., Hallanvuo, S., & Lindblad, M. 2008. Real-time PCR method for detection of pathogenic Yersinia enterocolitica in food. *Applied and environmental microbiology*, 74(19): 6060 – 6067.

Lemarchand, K., Masson, L. & Brousseau, R. 2004. Molecular biology and DNA microarray technology for microbial quality monitoring of water. *Critical Reviews in Microbiology*, 30: 145 – 172.

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S. & Tandoi, V. 2012. *Salmonella* in surface and drinking water: occurrence and water-mediated transmission. *Food Research International*, 45(2): 587 – 602.

Liebenberg, S.J.R., White, W.A. & Fagan, J.J. 2009. A case report of an 11 year old boy with a fatal sore throat: *Corynebacterium diphtheriae*-does it still occur? *Southern African Journal of Epidemiology and Infection*, 24(4): 40 – 42.

Lister, P.D., Wolter, D.J. & Hanson, N.J. 2009. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews*: 582 – 610.

Liu, G., Van der Mark, J.Q.J.C. & Van Dijk, J.C. 2013. Flow cytometry total cell counts: a field study assessing microbiological water quality and growth in unchlorinated drinking water distribution systems. *BioMed Research International*. http://www.hindawi.com/journals/bmri/2013/595872/ [10 August 2014].

Logan, N.A., Hoffmanster, A., Shadomy, S.V & Stauffer, K. 2011. *Bacillus* and related genera. In Versalovic, J., Carroll, K.C., Funke, G., Jorgensen, J.H., Landry, M.L. & Warnock, D.W. (eds). *Manual of clinical microbiology*. 3rd ed. Washington D.C: American Society for Microbiology: 381 – 402.

Luyt, C.D., Tandlich, R., Muller, W.J. & Wilhelmi, B.S. 2012. Microbial monitoring of surface water in South Africa: an overview. *International Journal of Environmental Research and Public Health*, 9: 2669 – 2693.

Macey, M.G. 2007. Principles of flow cytometry. In Macey (ed). *Flow cytometry:*

principles and applications. Totowa, NJ: Humana Press Inc.: 1 – 15.

Madigan, M.T. & Martinko, J.M. 2006. Brock biology of microorganisms. 11th ed. New Jersey (NJ): Prentice Hall.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Fazil, A. & Hoekstra, R.M. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*, 50(6): 882 – 889.

Malaty, H.M. 2006. Epidemiology of *Helicobacter pylori* infection*. Best Practice & Research Clinical Gastroenterology*, 21(2): 205 – 214.

Maliyil, J., Caire, W., Nair, R., & Bridges, D. 2011. Splenic abscess and multiple brain abscesses caused by *Streptococcus intermedius* in a young healthy man. *Proceedings (Baylor University. Medical Center)*, 24(3): 195 – 199.

McKenzie, R., Bourgeois, A.L., Frech, S.A., Flyer, D.C., Bloom, A., Kazempour, K. & Glenn, G. M. 2007. Transcutaneous immunization with the heat-labile toxin (LT) of enterotoxigenic *Escherichia coli* (ETEC): Protective efficacy in a double-blind, placebo-controlled challenge study. *Vaccine*, 25: 3684 – 3691.

Mels, A., Castellano, E., Braadbaart, O., Veenstra, S., Dijkstra, I., Meulaman, B., Singels, A. & Welsenach, J.A. 2010. Sanitation services for the informal settlements of Cape Town, South Africa. *Desalination*, 250: 330 – 337.

Meyer D.K. & Reboli A.C. 2005. Other coryneform bacteria and *Rhodococcus*, In Mandell GL, Bennett JE, Dolin R, editors. (eds). *Principles and practice of infectious disease.* 6th ed. Philadelphia: 2465 – 2478.

Mihelcic, J. R., Lauren, M.F., Myre, E.A., Philips, L.D. & Barkdoll, B.D. 2009. Field guide to environmental engineering for development workers: water, sanitation, and indoor air. Virginia: ASCE Publications.

Moe, C.L. & Rheingans, R.D. 2006. Global challenges in water, sanitation and health. *Journal of water and Health.* 04: 4 – 57.

Mofokeng, N.M. 2008. The greywater situation in informal settlements of the eKurhuleni Metropolitan Municipality – eastern region (Gauteng, South Africa).

Masters in Environmental Management thesis, North-West University, Potchefstroom. http://dspace.nwu.ac.za/bitstream/handle/10394/4140/mofokeng_nm.pdf?sequence=3 [11 October 2012].

Monnet, D., Fremey, J, Brun, Y., Boeufgras, J. & Fleurette, J. 1991. Dificulties in identifying *Klebsiella* strains of clinical origin. *Zentrlbl Bakteriol*, 274: 465 – 464.

Morakinyo, K.O., Ogunrayewa, M.O., Olalekan, K.B. & Adenubi, O.O. 2012. Urban slums as spatial manifestations of urbanization in sub-Saharan Africa: a case study of Ajegunle slum settlement, Lagos, Nigeria. *Developing Country Studies*, 2(11): 1 – 10.

Morpeth, S. C., Ramadhani, H. O., & Crump, J. A. 2009. Invasive non-typhi *Salmonella* disease in Africa. *Clinical Infectious Diseases*, 49(4): 606 – 611.

Muanda, C. 2009. Investigation of anaerobic up-flow batch reactor for treatment of greywater in un-sewered settlements. MTech thesis, Cape Peninsula University of Technology. http://digitalknowledge.cput.ac.za/xmlui/bitstream/handle/11189/1436/C%20Muanda%20MTech%20thesis%20final%20dated%2012012010%20Library%20copy.pdf?sequence=1 [13 October, 2014].

Murata, T., Iida, T., Shiomi, Y., Tagomori, K., Akeda, Y., Yanagihara, I., Mushiake, S., Ishiguro, F. & Honda, T. 2001. A large outbreak of foodborne infection attributed to *Providencia alcalifaciens*. *Journal of Infectious Diseases*, 184(8): 1050-1055.

Murray, P., Holmes, B. & Auken H. 2005. *Citrobacter, Klebsiella, Enterobacter, Serratia*, and other *Enterobacteriaceae*. In: Borriello P, Patrick R, Funke G, (eds). *Tropley and Wilson's Microbiology and microbial Infections.* London: Hodder Arnold:1474 –1507.

Myers, D.N., Stoeckel D.M., Bushon R.N., Francy D.S. & Brady A.M.G. 2014. Fecal indicator bacteria: US geological Survery Techniques of water resources investigation, book 9, chapter A7 Section 7.1 (version 2.1). https://water.usgs.gov/owq/FieldManual/Chapter7-Archive/Chapter7.1/NFMChap7_3_FIB.pdf [10 February 2015].

Mzini, L.L. 2013. The effects of greywater irrigation on vegetable crops and soil. MSc

thesis. University of Cape Town: Cape Town. https://open.uct.ac.za/bitstream/handle/11427/6609/thesis_sci_2013_mzini_loyiso.pdf?sequence=1 [16 October 2014].

Ndlovu, T. 2013. Comparison of diagnostic tools and molecular based techniques for the RapID™ identification of *Escherichia coli* and coliforms in contaminated river water. MTech thesis, Cape Peninsula University of Technology, Cape Town.

Neumayr, A., Kubitz, R., Bode. J.G., Bilk, P. & Häussinger, D. 2010. Multiple liver abscesses with isolation of *Streptococcus intermedius* related to a pyogenic dental infection in an immunocompetent patient. *European Journal of Medical Research*, 15: 319 – 322.

.

Nganga, V.G., Kariuki, F.W. & Kotut, K. 2012. A comparison of the physico-chemical and bacteriological quality of greywater from water deficient households in Homabay Town and Githurai Estates in Kenya. *The Open Environmental Engineering Journal,* 5: 110 – 118.

Nucera, D.M., Maddox, C.W., Hoien-Dalen, P. & Weigel, R.M. 2006. Comparison of API 20E and invA PCR for identification of *Salmonella enterica* isolates from swine production units. *Journal of Clinical Microbiology*, 44(9): 3388 – 3390.

O'Hara, C.M. 2005. Manual and automated instrumentation for identification of *Enterobacteriaceae* and other aerobic gram-negative bacilli. *Clinical Microbiology Reviews*, 18(1):147–162.

O'Sullivan, J, Bolton, D.J., Duffy, G., Baylis, C., Tozzoli, R., Wasteson, Y. & Lofdahl, S. 2007. Pathogenic *Escherichia coli* network: methods for detection and molecular characterisation of pathogenic *Eschericia coli*. Dublin: Ashtown Food Research Centre.

Obasohan, E.E., Agbonlahor, E.E. & Obano, E.E. 2010. Water pollution: a review of microbial quality and health concerns of water, sediment and fish in the aquatic ecosystem. *African Journal of Biotechnology*, 9(4): 423 – 427.

Odjadjare, E.E., Obi, L.C. & Okoh, A.I. 2010. Municipal wastewater effluents as a source of listerial pathogens in the aquatic milieu of the Eastern Cape province of South Africa: a concern of public health importance. *International Journal of*

Environmental Research and Public Health, 7(5): 2376 – 2394.

Okwori, A.E., Martínez, P.O., Fredriksson-Ahomaa, M., Agina, S.E., & Korkeala, H. 2009. Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. *Food Microbiology*, 26(8): 872 – 875.

Oliver, J.D. 2005. The viable-but-non-culturable state in bacteria. *Journal of Microbiology*, 43(1): 93 – 100.

Osamwonyi, O. U., Obayagbona, O. N., Aborishade, W., Olisaka, F., Uwadiae, E., & Igiehon, O. N. 2013. Bacteriological quality of vegetable salads sold at restaurants within Okada Town, Edo State, Nigeria. *African Journal of Basic & Applied Sciences*, 5(1): 37 – 41.

Ovchinnikova, O.G., Kocharova, N.A., Bialczak-Kokot, M., Shashkov, A.S., Rozalski, A. & Knirel, Y.A. 2012. Structure of the O-polysaccharide of *Providencia alcalifaciens* O22 containing D-glyceramide 2-phosphate. *European Journal of Organic Chemistry*, 18: 3500 – 3506.

Paillard, D., Dubois, V., Thiebaut, R., Nathier, F., Hoogland, E., Caumette, P. & Quentin, C. 2005. Occurrence of Listeria spp. in effluents of French urban wastewater treatment plants. *Applied and Environmental Microbiology*, 71(11): 7562 – 7566.

Parkinson, J. 2003. Drainage and stormwater management strategies for low-income urban communities. *Environment & Urbanization*, 15(2): 115 – 126.

Paterson, D. L. 2006. Resistance in gram-negative bacteria: *Enterobacteriaceae*. *The American Journal of Medicine*, 119(6): S20 – S28.

Paulse, A.N., Jackson, V.A. & Khan, W. 2007. Comparison of enumeration techniques for the investigation of bacterial pollution in the Berg River, Western Cape, South Africa. *Water SA*, 33(2): 165 – 173.

Paulse, A. N., Jackson, V. A. & Khan, W. 2009. Comparison of microbial contamination at various sites along the Plankenburg: and Diep Rivers, Western Cape, South Africa. *Water SA*, 35(4): 469 – 478.

Payment, P. & Locas, A. 2011. Pathogens in water: value limits of correlation with microbial indicators. *Ground Water*, 49(1): 4 -11.

Percival, S.L., Walker, J.T. & Hunter, P.R. 2000. *Microbiological aspects of biofilms and drinking water*. Florida: CRC Press.

Percival, S.L. & Williams, D.W. 2014. *Acinetobacter*. In Percival, S.L., Yates, M.V., Williams, D.D, Chalmers, R., & Gray, N. (eds). *Microbiology of waterborne diseases: microbiological aspects and risks.* 2nd ed. London: Academic Press: 35 – 48.

Petti, C.A., Simmon, K.E., Bender, J., Blaschke, A., Webster, KA, Conneely, M.F., Origitano, T.C. & Challapalli, M. 2008. Culture-negative intracerebral abscesses in children and adoloscents from *Streptococcus anginosus* group infection: a case series. *Clinical Infectious Diseases*, 46(10): 1578 – 1580.

Pillai, S.D. & Rambo, C.H. 2014. Routine techniques for monitoring and viral contaminants. In Bart, CA & Tortorello, M.L (eds). *Encyclopaedia of food microbiology.* 2nd ed. London: Elsevier Ltd: 766 – 772.

Pindi, P.K., Raghuveer Yadav, P. & Shiva Shanker, A. 2013. Identification of opportunistic pathogenic bacteria in drinking water samples of different rural health centers and their clinical impacts on humans. *BioMed Research International*, 2013. http://www.hindawi.com/journals/bmri/2013/348250/ [07 July 2014].

Popovic, N.T., Coz-Rakovac, R. & Strunjak-Perovic, I. 2007. Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria: a review. *Veterinaria Medicina*, 52(2): 49 – 53.

Prescott, M.L., Harley, J.P. & Klein, D.A. 2005. Microbiology. 6th ed. New York: McGraw Hill Companies.

Prüss-Üstün, A., Bos, R., Gore, F. & Bartma, J. 2008. *Safer water, better health: costs, benefits and sustainability of interventions to protect and promote health*. Geneva: WHO Press.

Raffatellu, M., Wilson, R.P., Winter, S.E. & Baumler, A.J. 2008. Clinical pathogenesis of typhoid fever. *Journal of Infection in Developing Countries*, 2(4): 260 – 266.

Rahn, K., DeGrandis, S., Clarke, R. & Mcewen, S. 1992. Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella. Molecular and Cellular Probes*, 6: 271 – 279.

Ramamurthy, T. & Albert, M.J. 2012. Epidemiology, pathogenesis and genetics of diarrheagenic *Escherichia coli* infections. In Faruque, S.M (ed*). Foodborne and waterborne bacterial pathogens. Epidemiology, evolution and molecular biology.* Norfolk: Caister Academic Press: 11 – 62.

Rashid, R.M., Salah, W. & Parads, J.P. 2007. '*Streptococus milleri*' aortic valve endocarditis and hepatic abscess. *Journal of Medical Microbiology*, 56(2): 280 – 282.

Rasmussen, M. 2013. *Aerococci* and aerococcal infections. *Journal of Infection,* 66(6), 467 – 474.

.

Rocha-e-Silva, R.C., Cardoso, W.M., Teixeira, R.S.C., Albuquerque, Á.H., Horn, R.V., Cavalcanti, C.M., LopesI, E.S. & Gomes Filho, V. J.R. 2013. *Salmonella Gallinarum* virulence in experimentally-infected Japanese quails (*Coturnix japonica*). *Revista Brasileira de Ciência Avícola*, 15(1): 39 – 45.

Rodda, N., Carden, K. & Armitage, N. 2010. Sustainable use of greywater in small-scale agriculture and gardens in South Africa (No. 1639/1). http://www.wrc.org.za/Knowledge%20Hub%20Documents/Research%20Reports/1639-1-11.pdf [06 July 2014].

Rompre, A., Servais, P., Baudart, J., De-Roubin, M.R. & Laurent, P. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of Microbiological Methods*, 49: 31 – 54.

Rusin, R., Enriquez, C.E., Johnson, D. & Gerba, C. P. 2000. Environmentally transmitted pathogens. In Maier, R.M., Pepper, I. L. & Gerba, C.P (eds). *Environmental microbiology.* California, USA: Academic Press: 447 – 489.

Sabae, S.Z. & Rabeh, S.A. 2007. Evaluation of the microbial quality of the River Nile waters at Damietta Branch, Egypt. *Egyptian Journal of Aquatic Research*, 33(1): 301–311.

Sadowsky, M. J. & Whitman, R. L. 2011. *The faecal bacteria*. Washington DC: ASM

Press.

Salukazana, L., Jackson, S., Rodda, N., Smith, M., Gounden, T., McLeod, N. & Buckley, C. 2006. Re-use of greywater for agricultural irrigation. In *Third international conference on ecological sanitation. 23 – 26 May 2005*, Durban, South Africa. http://www.waterinformation.co.za/literature/files/242%20Salukazana.pdf [22 June 2014].

Saygin, P. Yakartas, B. & Tuter, M. 2013. The Effects of UV Treatment on Microbiological disinfection of household greywater. *Journal of Selcuk University Natural and Applied Science*, 190 – 198.

Scheutz, F. & Strockbine, N.A. 2005. Genus *Escherichia*. In Brenner D.J., Krieg N.R. & Staley J.T., (eds). *Bergey's manual of systematic bacteriology.* 2nd ed. New York (NY): 607 – 623.

Senesi, S. & Ghelardi, E. 2010. Production, secretion and biological activity of *Bacillus cereus* enterotoxins. *Toxins*, 2(7): 1690 – 1703.

Shaikh, M. M. & Morgan, M. 2011. Sepsis caused by *Raoultella terrigena*. *Journal of the Royal Society of Medicine Short Reports*, 2(6): 49 – 51.

Shelton-Dodge, K., Vetter, E.A., Kohner, P.C., Nyre, L.M. & Patel, R. 2011. Clinical significance and antimicrobial susceptibilities of *Aerococcus sanguinicola* and *Aerococcus urinae*. *Diagnostic Microbiology and Infectious Disease*, 70(4): 448 – 451.

Siebel, E., Wang, Y., Egli, T., & Hammes, F. 2008. Correlations between total cell concentration, total adenosine tri-phosphate concentration and heterotrophic plate counts during microbial monitoring of drinking water. *Drinking Water Engineering and Science,* 1(1): 1 – 6.

Siera-Hoffman, M., Watkins, K., Jinadatha, C., Fader, R. & Carpenter, J.L. 2005. Clinical significance of *Aerococcus urinae*: a retrospective review. *Diagnostic Microbiology and Infectious Disease*, 53: 289 – 292.

Silber, G. 2011. Water and Sanitation Provision in RR Section. [Personal interview, 12 January]. Cape Town. (Unpublished).

Social Justice Coalition (SJC). 2011. Standing for safety & security for all – clean and safe sanitation campaign. Cape Town. (Unpublished).

South African Bureau of Standards. 2008. South African national standard: water quality – sampling. Part 1: guidance on the design of sampling programmes and sampling techniques. 2nd ed. Pretoria: SABS Standards Division.

South African Bureau of Standards. 2011. South African national standard. Drinking water. Part1: microbiological, physical, aesthetic and chemical determinants. Pretoria: SABS.

South African Institute of Race Relations. 2013. South Africa goes with the urbanisation flow. file:///C:/Users/leutaq/Downloads/Urbanisation%20-%2022_Jan_2013.pdf [10 June 2014].

Stern, P., Mokgatle, M. & Mayosi, B. 2004. The acceptability of dry sanitation: A preliminary study in two informal settlements in Khayelitsa, Cape Town. *Cape Town:* Equity Gauge.

Stovcik, V., Javorsky, P. & Pristas, P. 2008. Antibiotic resistance patterns and resistance genes in *Enterococci* isolated from sheep gastrointestinal tract in Slovakia. *Bulletin of the Veterinary Institute in Pulawy*, 52: 53 – 57.

Talbot, G.H., Bradley, J., Edwards, J.E., Gilbert, D., Scheld, M. & Bartlett, J.G. 2006. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clinical Infectious Diseases*, 42(5): 657 – 668.

Teplitski, M., & Butler, J. D. 2008. Indicator organisms: what every Floridian may want to know about microbiological water quality. http://edis.ifasufl.edu/ss482 [11 June 2014].

Tokota, L. 2012. Water and sanitation in RR Section. [Personal interview, 07 February]. Cape Town. (Unpublished).

Toranzos, A.A., McFeters, G.A. & Borrego. 2002. Detection of microorganisms in environmental freshwaters and drinking waters. In Hurst, C. J., Crawford, R. L.,

Knudsen, G.R., mcInerney, M.J & Stetzenbach, LD. (eds). *Manual of environmental Microbiology*. 2nd ed. Washington, D.C.: ASM Press: 205 – 219.

Tortorello, M.L. 2003. Indicator organisms for safety and quality – uses and methods for detection: minireview. *Association of Official Analytical Chemists International*, 86(6): 1208 – 1217.

Tran, M.P., Caldwell-McMillan, M., Khalife, W. & Young, V.B. 2008. *Streptococcus intermedius* causing infective endocarditis and abscesses: a report of three cases and review of the literature. *BMC Infectious Diseases*, 8(1). http://www.biomedcentral.com/content/pdf/1471-2334-8-154.pdf [10 August 2014].

Tsinda, A., Abbott, P., Pedley, S., Charles, K., Adogo, J., Okurut, K., & Chenoweth, J. 2013. Challenges to achieving sustainable sanitation in informal settlements of Kigali, Rwanda. *International Journal of Environmental Research and Public Health*, 10(12): 6939 – 6954.

UN-Habitat. 2003a. Guide to Monitoring Target 11: Improving the lives of 100 million slum dwellers. Progress towards the Millennium Development Goals. http://ww2.unhabitat.org/programmes/guo/documents/mdgtarget11.pdf [11 September 2012].

UN-Habitat. 2003b. The challenge of the slums: Global report on human settlements. Nairobi: United Nations.

UN-Habitat. 2003c. Water and Sanitation in the world's cities – local action for global goal*s*. London: United Nations Human settlement Programme.

UN-Habitat. 2013. State of the World's Cities 2012/2013. Prosperity of Cities. mirror.unhabitat.org/pmss/getElectronicVersion.aspx?nr=3387&alt=1 [11 October, 2014].

United Nations [UN] Special Rapporteur. 2014. UN Special Rapporteur on the Human Right to Safe Drinking Water and Sanitation Catarina de Albuquerque. http://reliefweb.int/sites/reliefweb.int/files/resources/BOOK-1-INTRO-WEB-LR.pdf [05 October 2015].

United Nations Children's Fund (UNICEF). 2008. *UNICEF Handbook on Water*

Quality. New York: UNICEF.

United Nations Children's Fund (UNICEF) & World Health Organization (WHO). 2009 Diarrhoea: why children are still dying and what can be done. Geneva: WHO Press.

United Nations Children's Fund (UNICEF) 2012. Cities and Children: The Challenge of Urbanisation in Tanzania. http://www.unicef.org/infobycountry/files/Cities_and_Children_-_FINAL.pdf [11 October 2014].

United Nations Development Programme (UNDP). 2006. Human development report 2006 – beyond scarcity: power, global poverty and the global water crisis. New York (NY): Macmillan.

United Nations Population Division (UNPD). 2002. World urbanization prospects: the 2001 revision. http://www.un.org/esa/population/publications/wup2001/wup2001dh.pdf [March 2012].

Van der Wienlen, P.W.J. & Kooij. 2010. Effects of water composition, distance and season on the adenosine triphosphate concentration in unchlorinated drinking water in the Netherlands. *Water Research*, 44: 4860 – 4867.

Venter, L. 2010. Presence of potentially pathogenic heterotrophic plate count (HPC) bacteria occurring in a drinking water distribution system in the North-West Province, South-Africa. MSc dissertation, North-West University: Potchefstroom. http://dspace.nwu.ac.za/bitstream/handle/10394/4380/Venter_L.pdf;jsessionid=14F9F926A9E52A317A7635DFDB390135?sequence=2 [16 January 2014].

Wagner, K.S., White, J. M., Crowcroft, N.S., De Martin, S., Mann, G. & Efstratiou, A. 2010. *Diphtheria* in the United Kingdom, 1986–2008: the increasing role of *Corynebacterium ulcerans*. *Epidemiology and Infection*, 138(11), 1519 – 1530.

Wang, Y., Claeys, L., van der Ha, D., Verstraete, W. & Boon, N. 2010. Effects of chemically and electrochemically dosed chlorine on *Escherichia coli* and *Legionella beliardensis* assessed by flow cytometry. *Applied Microbiology and Biotechnology*, 87:331 – 341.

Whalen, J.G., Mully, T.W., & English, J.C. 2007. Spontaneous *Citrobacter freundii*

infection in an immunocompetent patient. *Archives of Dermatology*, 143(1): 115 – 126.

Whitaker, J.A., Franco-Paredes, C., Del Rio, C., & Edupuganti, S. 2009. Rethinking typhoid fever vaccines: implications for travellers and people living in highly endemic areas. *Journal of Travel Medicine*, 16(1): 46 – 52.

Winward, G. P., Avery, L. M., Frazer-Williams, R., Pidou, M., Jeffrey, P., Stephenson, T., & Jefferson, B. 2008. A study of the microbial quality of grey water and an evaluation of treatment technologies for reuse. *Ecological Engineering*, 32(2): 187 – 197.

Wood, A., Uchronska, W. & Valashiya, G. 2001. Greywater Management in Dense, Informal Settlements in South Africa. WRC Report No. 767/1/01.

World Health Organisation (WHO) & United Nations Children's Fund (UNICEF). 2013. Progress toward global immunization goals. Summary representation of key indicators. Updated July 2013. http://www.who.int/immunization/monitoring_surveillance/SlidesGlobalImmunization.pdf [15 August 2014].

World Health Organisation (WHO) & United Nations Children's Fund (UNICEF). 2014. Progress on Sanitation and Drinking – water. 2013 update. Geneva: WHO Press.

World Health Organisation (WHO). 2006. WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater, Volume IV, Excreta and Greywater Use in Agriculture. Geneva: WHO Press.

World Health Organisation (WHO). 2008. Guidelines for drinking- water quality: incorporating 1st and 2nd addenda, vol.1, recommendations. 3rd ed. Geneva: WHO Press.

World Health Organisation (WHO). 2011a. Enterohaemorrhagic *Escherichia coli* (EHEC). Fact sheet No 125. http://www.who.int/mediacentre/factsheets/fs125/en/ [11 June 2014].

World Health Organisation (WHO). 2011b. Guidelines for drinking water. 4th ed. Geneva: WHO Press.

World Health Organisation (WHO). 2013. Diarrhoeal diseases. Factsheet No. 330. http://www.who.int/mediacentre/factsheets/fs330/en/ [10 June 2014].

Yoh, M., Matsuyama, J., Ohnishi, M., Takagi, K., Miyagi, H., Mori, K., Park, K.S., Ono, T. & Honda, T. 2005. Importance of *Providencia* species as a major cause of travellers' diarrhoea. *Journal of Medical Microbiology*, 54(11): 1077 – 1082.

Zamxaka, M, Pironcheva, G & Muyima, N.Y.O. 2004. Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. *Water SA,* 30(3): 333 – 340.

APPENDIX A

Appendix A: Organisms identified from RR Section greywater samples using the API 20E System

Week	Isolate No	API 20E
Site A		
1	1	*Enterobacter asburiae* - low discrimination – thus not taken as positive identification
	2	could not identify
	3	*Escherichia coli 1*
2	4	*Enterobacter sakazakii*
	5	*Pantoea spp 2*
3	6	*Klebsiella pneumoniae ssp pneumoniae*
	7	*Acinetobacter baumannii/calcoaceticus*
5	8	*Salmonella spp*
	9	*Serratia marcescens*
Site B		
1	10	Could not identify
	11	*Enterobacter asburiae*
2	12	*Enterobacter cloacae*
	13	*Escherichia coli 1*
3	14	*Escherichia coli 1*
	15	*Klebsiella oxytoca*
	16	*Pseudomonas oryzihabitans* - low discrimination – thus not taken as positive identification
5	17	*Klebsiella pneumoniae ssp pneumoniae*
	18	*Salmonella ser. paratyphi A*
Site C		
2	19	Could not identify
	20	*Klebsiella oxytoca*
	21	*Escherichia coli 1*
4	22	*Klebsiella oxytoca*
	23	*Klebsiella pneumoniae ssp pneumoniae*
5	24	*Pantoea spp 2*
	25	*Serratia marcescens*
Site D		
1	26	Could not identify
	27	Could not identify
	28	*Klebsiella pneumoniae ssp pneumoniae*
4	29	*Enterobacter aerogenes*

	30	*Escherichia coli 1*
Site E		
1	31	*Acinetobacter baumannii/calcoaceticus*
2	32	Could not identify
	33	Could not identify
	34	Enterobacter cloacae
	35	*Enterobacter aerogenes*
	36	*Enterobacter aerogenes*
3	37	Enterobacter cloacae
	38	*Escherichia coli 1*
5	39	*Klebsiella oxytoca*
	40	*Raoultella terrrigena*
	41	*Leclercia adecarboxylata*
Site F		
1	42	*Pantoea spp 2*
	43	*Klebsiella pneumoniae ssp pneumoniae*
3	44	*Escherichia coli 1*
4	45	*Escherichia coli 1*
	46	*Acinetobacter baumannii/calcoaceticus*
	47	*Klebsiella oxytoca*
5	48	could not identify
	49	*Acinetobacter baumannii/calcoaceticus*

APPENDIX B

Appendix B: Organisms identified from RR Section greywater samples using the RapID ONE System

Week	Isolate No	RapID ONE
		Site A
1	1	Could not identify
	2	*Salmonella gallinarum*
	3	*Escherichia coli 1*
2	4	*Enterobacter sakazakii*
	5	Could not identify
3	6	*Klebsiella pneumoniae*
5	7	*Salmonella 1 (Most)*
	8	*Serratia marcescens*
		Site B
1	9	*Acinetobacter calcoaceticus*
	10	*Enterobacter asburiae (EG 17)*
2	11	*Enterobacter cloacae*
	12	*Escherichia Coli*
3	13	Could not identify
5	14	*Klebsiella pneumoniae*
	15	*Salmonella paratyphi A*
		Site C
2	16	*Acinetobacter calcoaceticus*
	17	Could not identify
	18	*Escherichia coli 1*
4	19	Could not identify
	20	*Klebsiella pneumoniae*
5	21	Could not identify
	22	*Serratia marcescens*
		Site D
1	23	*Citrobacter freundii*
	24	*Providencia alcalifaciens*
	25	*Klebsiella pneumoniae*
		Site E
2	26	*Citrobacter freundii*
	27	Could not identify
	28	*Enterobacter cloacae*

	29	*Enterobacter aerogenes*
	30	*Enterobacter aerogenes*
3	31	*Enterobacter. cloacae*
5	32	Could not identify
	33	Could not identify
Site F		
1	34	Could not identify
	35	*Klebsiella pneumoniae*
5	36	*Acinetobacter calcoaceticus*

APPENDIX C

Appendix C: Organisms identified from RR Section greywater samples using the BBL Crystal™ GP ID System

Week	Isolate No	Organism
Site A		
1	1	*Staphylococcus equorum*
2	2	*Micrococcus luteus*
3	1	could not identify
4	2	*Kocuria kristinae*
5	3	*Corynebacterium bovis*
7	4	*Aerococcus urinae*
9	5	*Bacillus licheniformis*
Site B		
1	1	*Corynebacterium species*
3	2	*Gemella morbillorum*
4	3	*Brevibacillus brevis*
6	4	*Corynebacterium species*
7	5	*Steptococcus intermedius*
Site C		
1	1	*Staphylococcus sciuri*
2	2	*Bacillus megaterium* – confidence level below 0.9 – thus not taken as positive identification
3	3	could not identify
4	4	*Bacillus cereus*
5	5	*Streptococcus anginosus*
7	6	*Micrococcus luteus*
8	7	*Aerococcus urinae*
Site D		
1	1	*Bacillus cereus*
2	2	could not identify
	3	could not identify
3	4	*Bacillus cereus*
6	5	*Corynebacterium diphtheria*
9	6	*Corynebacterium pseudogenitalium* – confidence level below 0.9 – thus not taken as positive identification
	7	*Staphylococcus equorum*
Site E		
1	1	*Corynebacterium pseudodiphtheriticum*
2	2	could not identify
5	3	could not identify

4	4	*Bacillus cereus*
6	5	*Streptococcus porcinus*
8	6	could not identify
Site F		
1	1	*Aerococcus urinae*
2	2	could not identify
4	3	*Aerococcus urinae*
5	4	*Corynebacterium genitalium* – confidence level below 0.9 – thus not taken as positive identification
7	5	*Corynebacterium* species
8	6	*Streptococcus intermeddius*