

ASSESSMENT OF METHODS FOR DETERMINING THE HEALTH-RELATED MICROBIOLOGICAL QUALITY OF UNTREATED SURFACE WATER IN THE MODDER RIVER CATCHMENT

LEATILE KOEKOE



Dissertation submitted by

in fulfilment of the requirements for the Degree:

in the

School of Agriculture and Environmental Sciences

within the

Faculty of Health and Environmental Sciences

of the

Central University of Technology, Free State

Supervisor: Prof. P. Jagals (D.Tech)

Co-Supervisor: Dr. E. Pretorius (Ph.D)

BLOEMFONTEIN

February 2004

DECLARATION OF INDEPENDENT WORK

I, **LEATILE KOEKOE**, Identity Number [REDACTED] and Student Number [REDACTED] do hereby declare that this research project, submitted to the Technikon Free State for the degree **MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH**, is my own independent work.

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


.....
SIGNATURE OF CANDIDATE

2004/03/31
.....
DATE

This study compared three *Escherichia coli* (*E. coli*) detection methods to determine an optimum method to use in assessing the health-related microbiological water quality in surface water of the Middle Modder River catchment. As an outcome, the study recommends an optimum method for two types (varying pollution status) of river water.

Faecal coliforms and *E. coli* were selected as microbiological indicator organisms. The three methods were based on colour changes to indicate the presence of the selected organism groups. Two of the methods were membrane-filtration based while the other was based on the Most Probable Number technique. The membrane-based methods used the reagent media M-FC (for faecal coliforms) and Chromocult Coliform[®] Agar for *E. coli* respectively. These were compared with the miniaturised liquid-based Most Probable Number (MPN) using the Quanti-Tray[®] / 2000 -Colilert 18 method for *E. coli*.

The Colilert method turned out to be the optimum method for the water selected for this study. Higher numbers of true indicator organisms were detected by this method than by the other two methods in all the selected waters.

Using the identified optimum method, the following was determined:

- ① The *E. coli* numbers in the water of the Modder River (at the 90th percentile compliance limit) exceeded recommended water quality guideline limits (South African Water Quality Guidelines, the Quality of Domestic Water Supplies: Assessment Guide as well as the National Microbial Monitoring Programme) for indicator organisms in water for domestic use (drinking,

food preparation, bath and recreation waters.

- ② The *E. coli* numbers did not exceed recommended water quality guideline limits for indicator organisms where the intended use was for crop irrigation.
- ③ Water in the Modder River was suitable for raw water extraction to treat for use as drinking water.

Met hierdie studie is drie *Escherichia coli* (*E. coli*) identifiseringsmetodes vergelyk om die mees aangewese metode te bepaal, wat gebruik kan word om die gesondheidsverwante mikrobiologiese gehalte van die water in die Middel Modderrivier opvangsgebied aan te dui. Die uitkoms van die studie dui op twee metodes wat gevolg kan word vir die twee tipes rivier water met wisselende besoedelings-status.

Fekale kolivorme en *E. coli* is gekies as mikrobiologiese indikator organismes. Die drie metodes is gebaseer op kleurveranderings om die aanwesigheid van die geselekteerde organisme groepe aan te toon. Die metodes is membraanfiltrasie en Mees Waarskynlike Getal metode. Die membraan-gebaseerde metodes gebruik onderskeidelik die reagent medium M-FC (vir fekale kolivorme) en Chromocult Coliform[®] Agar (vir *E. coli*). Hierdie metodes is vergelyk met die miniatuur vloeistof-gebaseerde Mees Waarskynlike Getal (MWG) met die gebruik van Quanti-Tray[®] / 2000 -Colilert 18 metode vir *E. coli*.

Die Colilertmetode blyk die beste metode te wees vir die water geselekteer vir die studie. Hoër tellings van ware indikator organismes is gevind deur die gebruik van hierdie metode, in vergelyking met die ander metodes.

Die geïdentifiseerde metode is gebruik om die volgende te bepaal:

- 1 Die *E. coli* tellings in die water van die Modderrivier (by die 90^{ste} voldoeninglimiet) die voorgestelde watergehalte riglyne (Suid Afrikaanse Watergehalteriglyne, die *Quality of Domestic Water Supplies: Assessment Guide* sowel as die Nasionale Mikrobiologiese Moniteringsprogram) vir

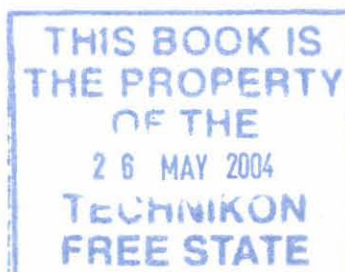
Uittreksel iii



indikator organisme vir huishoudelike gebruik (drink, voedselvoorbereiding, bad en wasgoed was) en ontspanningswater, oorskry het. Dit is vasgestel deur die gebruik van die optimale Colilertmetode.

- 2 Die *E. coli* tellings het egter nie die voorgestelde watergehalte riglyne vir indikator organismes vir gewas besproeiingswater oorskry nie.
- 3 Die water in die Modderivier is geskik vir rouwaterontrekking vir die suiwering van drinkwater.

- ◆ To the heavenly father, this was accomplished through your guidance.
- ◆ To my family Teboho, Maleloko, Leloko Koekoe and Mojalefa Matlole - thanks for not missing me too much during my study years, your encouragement and prayers kept me going strong.
- ◆ To my extended family Uncle Columbus Malebo and his family, thanks for your support and always having that care when it came to my studies.
- ◆ To my supervisor Professor Paul Jagals - this would not have happened without you. Thanks once again Prof.
- ◆ To the Central University of Technology, Free State for making this possible - especially the Faculty of Health and Environmental Sciences for providing the facilities.
- ◆ To the Institute of Water Quality Studies (DWAF) for allowing me to use their NMMP data.
- ◆ The National Research Foundation for the financial assistance - without you this would not have been possible.
- ◆ To all the "water babies", Mariette, Patience, Corinne, Maronel and Jemina, thanks ladies for your understanding, help and guidance especially during the long lab hours spent together.
- ◆ To all my friends Bontle, Deliwe, Linah, Lindi, Louisa, Neo and Pulane - thanks "bo-mati" for understanding the words "I cannot see you because I have to do my work".





LIST OF FIGURES	vi
LIST OF TABLES	vii
CHAPTER 1:INTRODUCTION	1-18
1.1 BACKGROUND	1
1.2 MICROBIOLOGICAL INDICATORS OF FAECAL WATER POLLUTION	5
1.2.1 Faecal coliforms	6
1.2.2 <i>Escherichia coli</i>	7
1.3 METHODS TO DETECT FAECAL COLIFORMS AND <i>E. coli</i>	8
1.3.1 Chromocult and M-FC methods - Membrane Filtration Technique	10
1.3.2 QuantiTray®/2000 Colilert 18 Method - Most Probable Number Technique	11
1.4 WATER USES AND QUALITY CRITERIA IN THE CATCHMENT	12
1.4.1 Domestic use	13
1.4.2 Abstraction for treatment	13
Rustfontein treatment facility	14
Mazelspoort treatment facility	14
1.4.3 Recreational users	15
1.4.1 Agricultural purposes	15
1.5 AIMS OF THE STUDY	16
1.5.1 Rationale	16
1.5.2 The research problem	17
1.5.3 The research aim	17
1.5.4 The scope of the study	17
1.5.5 Specific objectives	18



CHAPTER 2: METHODOLOGY

19-34

2.1	THE STUDY AREA	19
2.1.1	Point sources of surface water pollution	19
2.1.2	Non-point sources of surface water pollution	19
2.2	MONITORING SITES	20
2.2.1	Water categories	20
2.2.2	The Modder River	21
2.2.3	The Klein Modder River	23
2.2.4	The Sepane Spruit	25
2.3	WATER SAMPLING	25
2.4	WATER QUALITY ASSESSMENT	26
2.4.1	Faecal coliforms	26
2.4.2	<i>Escherichia coli</i>	26
2.4.3	Organism counting	27
2.4.4	Colony verification	27
2.4.5	Adapting data to false positives	28
2.4.6	Adapting faecal coliform data to <i>E. coli</i> data	29
2.5	STATISTICAL ANALYSIS OF WATER QUALITY DATA	29
2.5.1	Data analyses	29
2.5.2	Analyses of variance (ANOVA)	30
2.5.3	Statistical hypotheses	30
2 6	LAYOUT	30
2.6.1	Selection of optimal method to assess water quality of the Modder River	31
2.6.2	General microbiological water quality of the Modder River reflected by the optimal method	33

CHAPTER 3: RESULTS AND DISCUSSION

35-59

3.1	THE OPTIMUM <i>E. coli</i> DETECTION METHOD	35
3.1.1	<i>E. coli</i> and the M-FC method for faecal coliforms	36
3.1.2	The Chromocult method for <i>E. coli</i>	37
3.1.3	The Colilert method for <i>E. coli</i>	38
3.1.4	Comparing the three detection methods on water of the Modder River	38
3.1.4.1	Unpolluted water at MR1	38
3.1.4.2	Polluted water at MR2	39
3.1.4.3	Polluted water at MR3	40
3.1.4.4	Polluted waters at MR4 and MR5	41
3.1.5	The three methods and waters from the Klein Modder River	42
3.1.5.1	Unpolluted water of KM1	43
3.1.5.2	Water quality at KM2	44
3.1.5.3	Water quality at KM3	45
3.1.6	The three methods and water from the Sepane Spruit	46
3.1.7	The optimum method	47
3.2	ASSESSING THE HEALTH-RELATED MICROBIOLOGICAL WATER QUALITY OF THE MODDER RIVER USING OPTIMUM METHOD	48
3.2.1	Using Colilert to determine impact points on the Modder River	50
3.2.2	The Colilert and Membrane-based methods to assess Modder River water for various uses	52
3.2.2.1	Water use at MR1	52
3.2.2.2	Water use at MR2 and MR3	53
3.2.2.3	Water use at MR4 and MR5	55
3.2.3	The Colilert and Membrane-based methods to assess Klein Modder River water for various uses	56
3.3	GENERAL QUALITY OF MODDER RIVER	58



CHAPTER 4: SUMMARY, CONCLUSION AND RECOMMENDATIONS

60-62

4.1	SUMMARY	60
4.2	CONCLUSION	61
4.3	RECOMMENDATIONS FOR FUTURE WORK	62

REFERENCE LIST

63-72

APPENDICES:	Appendix A: Membrane Filtration Technique
	Appendix B: Most Probable Number: Quanti-Tray®-/2000 Colilert 18-method
	Appendix C: M-FC reagent medium for detecting faecal coliforms
	Appendix D: Chromocult® Coliformen Agar for detecting <i>Escherichia Coli</i>
	Appendix E: Confirming colonies
	Appendix F: Statistical analyses
	Appendix G: Comparison tables

LIST OF FIGURES

CHAPTER 1: INTRODUCTION	1-18
Figure 1.1 Middle Modder River Catchment	3
CHAPTER 2: METHODOLOGY	19-34
Figure 2.1 Map of study area	21
Figure 2.2 Framework of the result section	31
CHAPTER 3: RESULTS AND DISCUSSION	35-59
Figure 3.1 Faecal coliform detection on M-FC medium	36
Figure 3.2: <i>E. coli</i> detection on Chromocult Coliform medium	37
Figure 3.3a Method comparison for <i>E. coli</i> detection at MR1	39
Figure 3.3b Method comparison for <i>E. coli</i> detection at polluted MR2	40
Figure 3.3c Comparison of methods for <i>E. coli</i> detection at MR3	41
Figure 3.3d Method comparison for <i>E. coli</i> number detection at MR4	41
Figure 3.3e Method comparison for <i>E. coli</i> detection at MR5	42
Figure 3.3f Method comparison in unpolluted water at KM1	43
Figure 3.3g Method comparison in polluted water at KM2	45
Figure 3.3h Method comparison in polluted water at KM3	46
Figure 3.3i Method comparison for <i>E. coli</i> detection at TB9	46
Figure 3.4 <i>E. coli</i> numbers in the Modder River detected by the Colilert and the membrane based methods	49
Figure 3.5 <i>E. coli</i> numbers in the Klein Modder River detected by the Colilert and the membrane based methods	56

LIST OF TABLES

CHAPTER 1: INTRODUCTION 1-18

Table 1.1	Site-specific guidelines for health-related water quality	13
-----------	---	----

CHAPTER 3: RESULTS AND DISCUSSION 35-59

Table 3.1	Preferred method based on method comparison	47
Table 3.2:	Comparing Colilert-derived <i>E. coli</i> numbers detected at Modder River sample sites	50
Table 3.3	Determining overall the significantly different sites in the Modder River	51
Table 3.4	The significantly different sites of lesser polluted waters in the Modder River	51
Table 3.5	Water use in the Modder River	52
Table 3.6	General use of water in the Klein Modder River	57

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

South Africa's water resources have been under increasing threat of faecal pollution in recent years. This is due to an increase in human population density, which has led to the establishment of informal settlements lacking proper sanitation infrastructures (Fatoki, Gogwana and Ogunfowokan, 2003; Department of Water Affairs and Forestry [DWAF], 1996 [a]). Faecal contamination from human and animal wastes is the major cause of deteriorating water quality in many resource waters (Day, 1996; DWAF, 1996 [a]). Continual faecal pollution in resource water is a global problem that is particularly hazardous in rural communities that depend directly on untreated source water for all their domestic, recreational and food production water needs (Venter, 2001; Bokako, 2000 and Jagals, 2000). Communities dependent on such water resources for basic survival are more prone to water-borne diseases (Pegram, Rollins and Espey, 1998).

According to studies conducted by Fatoki, Muyima and Lujiza (2001) in the Umtata River catchment, Davie (2002) in the Jukskei River catchment, and Jagals (2000) in the Modder River catchment, residential areas with inadequate sanitation facilities often cause faecal pollution in receiving surface waters. This is due to land-deposited human faecal material that is flushed into these waters as urban run-off, especially after heavy rainstorms.

Discharges from point sources also contribute to faecal pollution. A study by Fatoki, Morrison, Persson and Ekberg (2001) also indicated that final effluent from a sewage works had an impact on the water quality of the Keiskammahoek River in the Eastern Cape.



Jagals (1997) reported faecal waste water treatment facilities in the Middle Modder River catchment.

Numerous studies, such as the one by Bezuidenhout, Mthembu, Puckree and Lin (2002), show that rural communities are directly dependent on the river water for all their basic needs which include drinking, washing, recreation and agriculture. Because of these demands, a constant need exists to detect faecal pollutants in these waters.

The Middle Modder River catchment, and in particular those sections draining along the Klein Modder River into the Modder River, is situated within the Mangaung region (South-Eastern Free State, South Africa). The catchment undergoes long periods of low rainfall, particularly during the winter months. The Modder River is the main watercourse draining the catchment, and mainly flows during summer months because of summer rainfall (Koning and Roos, 1999).

Faecal pollution levels tend to increase during rainy periods, when surface run-off from surrounding urban areas carries a variety of microbiological contaminants (Byamukama, Knasiime, Mach and Farnleitner, 2000; Solo-Gabriele, Wolfert, Desmarais and Palmer, 2000). This is also reported for the Modder River (Jagals, 2000; Pretorius, 1996). The Modder River is not a large river (Jagals, 2000) and its waters support several uses such as drinking water, recreation, livelihood fishing and agriculture (Steyn, 2003). The major cities in this catchment are Bloemfontein, Botshabelo and Thaba 'Nchu (Figure 1).

This study focused on the city of Botshabelo in particular and on run-off from the neighbouring Thaba 'Nchu to a lesser extent. Sources of faecal pollution from these cities include human faecal material from residential areas with inadequate

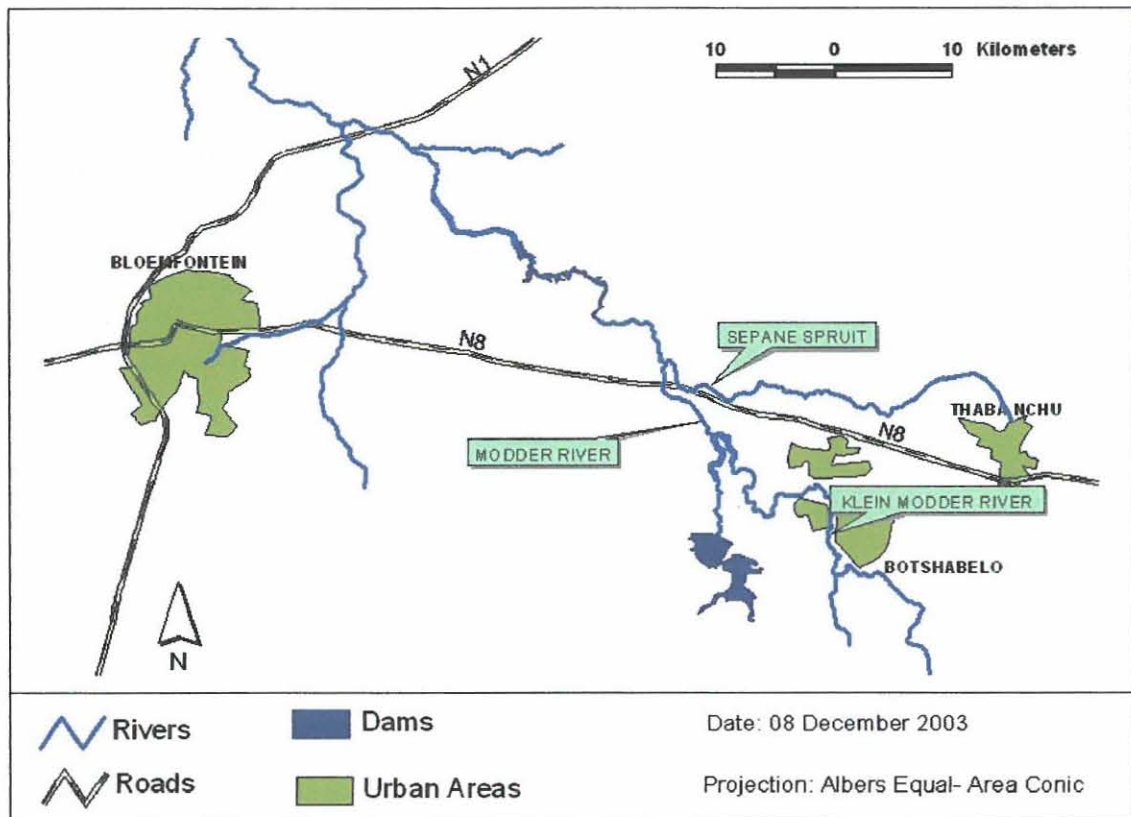


Figure 1.1 Middle Modder River catchment

Treated final effluent from two wastewater treatment facilities (Botshabelo and Thaba 'Nchu), as well as agricultural run-off from the surrounding areas also contributed to faecal water pollution. These sources of pollution are well described in studies conducted by Jagals (2000; 1997; 1994) and Ralikontsane (2000).

When water is faecally polluted, micro-organisms such as faecal coliforms and *Escherichia coli* are present (Standard Methods, 1998). These organisms are generally present with other pathogenic micro-organisms in faecally polluted waters and are generally used as indicator organisms for faecal pollution. They are easily detected by various methods if present in the water. When found in water



resources, it is accepted that coliforms indicate faecal pollution and are consequently a threat to human health (Grabow, 1996).

The most common reason for using indicator organisms instead of endeavouring to detect the actual pathogens in water is that the indicator organisms are a reliable indication of faecal pollution (Grant, 1997), and are more economical, safer and quicker to use (Steyn, 2003; Jagals, 2000). The methods used by Jagals (2000) for detecting the coliforms in different resource waters vary in approach, accuracy and reliability, which could potentially lead to problems as described below:

- ① Water quality assessment laboratories could be inconsistent in detecting and reporting microbiological indicator organism numbers from the same waters, and neither the public nor the service providers would know which results are accurate and reliable to use.
- ② Water laboratories would not know which detection method yields more reliable results when applied to detect these indicator organisms. This is because detection methods use different methodologies. This would create great uncertainty in reporting whether water posed a risk of infection to users, and to what extent such risk should be controlled.

In particular it would not be known to what extent the above mentioned two factors affected the quality of water assessment data collected from the Modder River catchment. The primary aim of this study was therefore to determine which of the traditional or more recently developed detection methods currently in use in this area could optimally assess the health-related microbiological quality of water in the catchment.



The term “health-related” refers to the polluted water could have on the health of individuals when used, without treatment, for domestic, recreational and agricultural purposes.

This study applied several methods to detect the presence of microbiological indicator organisms in the samples taken from selected waters, compared the results and finally described the health-related microbiological quality of water in the study catchment based on the most reliable method. For this study, the method considered the most reliable and therefore the optimum method, was the one that yielded the highest results with the least false positives.

1.2 MICROBIOLOGICAL INDICATORS OF FAECAL WATER POLLUTION

The use of microbiological indicators to measure the quality of water is widespread (Noble, Moore, Leecaster, McGee and Weisberg, 2002). Bacterial indicators are used instead to direct tests for pathogens because the methods used to detect bacterial indicator organisms are less expensive and the organisms are not dangerous to detect (Haile, Witte, Gold, Cressey, McGee, Millikan, Glasser, Harawa, Erwin, Harmon, Harper, Dermand, Alamillo, Barrett, Nides and Wing, 1999). Water quality assessment is practised to discover the microbiological quality of water in terms of faecal pollution, and to make available relevant information to various water users (Gregory and Frick, 2000).

Coliform bacteria belong to the family *Enterobacteriaceae*. Of these, the ones that best represent faecal pollution are faecal coliforms (FC) and *Escherichia coli* (*E. coli*) which are well-recognised bacterial organisms for assessing the health-related microbial water quality (Sartory and Watkins, 1999).



Faecal coliforms and *E. coli* analogously as indicators of faecal pollution (DWAF 2002; Jagals, Grabow, Griesel and Jagals, 2001).

This study therefore applied these microbiological indicators, faecal coliforms and *E. coli*, in assessing the level of faecal pollution in untreated surface water of the Modder River catchment.

1.2.1 Faecal coliforms

According to the South African Water Quality Guidelines (DWAF, 1996 [a]) as well as the Assessment Guide of the Water Research Commission (WRC, 1998), faecal coliforms indicate best the possible presence of microbiological pathogens responsible for transmission of infectious diseases such as gastroenteritis, dysentery, salmonellosis, typhoid fever and cholera (DWAF, 1996 [a]).

For studies done in the Modder River, faecal coliforms were used to determine trends of faecal pollution in the Modder River catchment (Griesel and Jagals, 2002; Jagals, 2000,1997; Jagals and Grabow, 1996; Pretorius 1996). These are also the indicators of choice currently used by the National Microbial Monitoring Programme (NMMP) (DWAF, 2002) in the same area.

In addition to *Enterobacter*, *Klebsiella*, and *Citrobacter*, the faecal coliform (FC) group also includes *Escherichia coli* (Alonso, Soriaono, Carbajo, Amoros and Garelick, 1999; DWAF, 1996 [b]). It is *E. coli* that gives the more reliable indication of faecal pollution (Jagals *et al.*, 2001; ISO, 1995 [a], South African Bureau of Standards [SABS], 1984; Grabow, 1990). However, the use of faecal coliforms instead of just *E. coli* has become commonplace because it traditionally needed only



a single detection step over traditional *E. coli* detection required several steps and took much longer to complete (Jagals, 2000).

For practitioners in the fields of environmental health and engineering, operating basic laboratory facilities, faecal coliforms (FC) were the most suitable indicator bacteria group for many years because of the single detection step. It is considered a simple and reliable (accurate and selective) test approach. The reason for using FC was that a small laboratory would not have required the more advanced technology and skills needed for the complex multi-step method then required for detecting *E. coli* (Yakub, Castric, Stadterman-Knauer, Tobin, Blazina, Heineman, Yee and Frazier, 2002; Jagals *et al.*, 2001; Jagals, 2000).

Non-expert analysts would tend to use only faecal coliform detection to assess faecal pollution of surface waters. This tendency, amongst others led formulators of the South African Water Quality Guidelines to use faecal coliforms instead of *E. coli* as indicators of water safety (WRC, 1998; DWAF, 1996 [a]). The presence of faecal coliforms in water is also used to indicate the risk of infection to consumers by the South African Water Quality Guidelines (DWAF, 1996 [a], [b] and [c]).

1.2.2 *Escherichia coli*

Although for some years not as popular as the faecal coliform group, *Escherichia coli* (*E. coli*) are still widely used in the evaluation of the quality of recreational waters, environmental fresh water sources, raw water for drinking water supply and wastewater effluents (Standard Methods, 1998).

The World Health Organisation Guidelines for Drinking Water (WHO, 1996) uses the presence of *E. coli* in water to indicate risk of infection to consumers, contrary to the South African guidelines, which uses only the faecal coliforms.



Efforts are constantly being made to develop methods for coliform analyses specifically to obtain more information in less time (Grant, 1997). Recent developments in chromogenic substrate testing for coliforms have made it possible to use *E. coli* in one single step by means of a highly selective media (IDEXX, 2000; Merck, 1996), used with the membrane filtration (MF) technique as well as the most probable number (MPN) technique for the detection of *E. coli* (ISO, 1994 [a]).

E. coli are more specific indicators of faecal pollution in water than faecal coliforms. Jagals *et al.* (2001) reported that some of the faecal coliform groups do not always meet the requirements of being true indicators of faecal pollution when found in water resources, whereas *E. coli* met this requirement.

Simultaneous detection of faecal coliforms and *E. coli* would, therefore, have practical benefits such as confirming whether faeces from warm-blooded animals had caused the faecal pollution detected in water resources (Jagals *et al.*, 2001; Grabow, 1996).

1.3 METHODS TO DETECT FAECAL COLIFORMS AND *E. coli*

If there is a potential for any body of water being used for recreation or for public water supply to contain a massive variety of micro-organisms, then there will be a need to develop improved detection methods to indicate whether certain microbiological indicator organisms are present in these water resources or not (Hanko, 2000).

In South Africa, water quality assessment laboratories generally use a variety of methods and techniques for faecal coliforms and *E. coli* detection and enumeration.



Such methods have also been used in a study by Brenner, Rankin, Roybal, Stelma, Scarpino and Dufour (1993).

Jagals *et al.* (2001) did a study in the same study region and the most popular methods used to detect indicator organisms were:

- ① faecal coliform detection with M-FC medium and membrane filtration technique;
- ② *E. coli* detection with a chromogenic substrate medium agar-based Chromocult® Coliform and membrane filtration; and
- ③ *E. coli* detection with a Most-Probable-Number technique (MPN) using the liquid chromogenic substrate chromogenic enzyme substrate 4-methyl-umbelliferyl- β -D-glucuronide (MUG) (ISO, 1994 [a]) in 96-well micro-titre plates.

This study continued by further comparing the M-FC (faecal coliforms) and Chromocult® coliform method (*E. coli*) using the membrane filtration technique, although this time not using the 96-well micro-titre plate technique but the recently-developed QuantiTray / 2000 Colilert® MPN method for *E. coli* detection.

Although Eckner (1998) compared the Colilert method to the membrane filtration method for the detection of *Escherichia coli* and Enterococci used in drinking and bathing water quality monitoring, in Sweden this method has not yet been tested on its own or in combination with the M-FC and Chromocult® coliform methods.

The three methods used by Jagals *et al.* (2001) and described in Standard Methods, (1998) are quantitative methods, representing high levels of sensitivity and can be used to evaluate various waters including treated/untreated drinking waters,



r. The methods are relatively easy to use, even by non-expert water laboratory analysts (Yakub *et al.*, 2002).

For this study, the membrane filtration technique was used to detect *Escherichia coli* and faecal coliforms. The Most Probable Number technique, which uses the QuantiTray®/ 2000 Colilert 18 method for *E. coli* detection, was also used in this study. Both methods are described below.

1.3.1 Chromocult and M-FC methods using the membrane filtration technique

- ① The Chromocult® Coliform Agar is a one-step selective medium used for the detection of *Escherichia coli* (*E. coli*). This medium enables one-step identification of this indicator organism by means of a chromogenic response (Merck, 1996). The enzyme β -D-glucuronidase is characteristic for *E. coli* (Standard Methods, 1998). The substrate X-glucuronide (Merck, 1996) is used for the identification of β -D-glucuronidase in *E. coli* which cleaves the substrate X-glucuronide, causing positive colonies to turn dark-blue to violet in colour, thus providing (according to Merck (1996) unequivocal) one-step identification for *E. coli*.
- ② The M-FC Agar method also uses a selective medium for the detection of faecal coliforms in various waters (Hanko, 2000; Biolab, 1997). The medium contains aniline blue dye, which causes positive colonies to turn dark-blue in colour. The M-FC method is not designed to detect only *E. coli*. The percentage of the true positive *E. coli* was derived from the results of the M-FC method. The *E. coli* of the counted faecal coliforms will be used in this study. These are indicated in Appendix G in Table 1.



Both of these selective methods in combination with the membrane filtration technique. This technique, which has been approved by the Environmental Protection Agency (EPA), makes it possible for large volumes of water to be examined. By filtering the water sample through a sterile membrane filter, bacteria in the water is retained. The filter is then placed on the appropriate selective nutrient media and incubated at an appropriate optimum temperature for growth of indicator organisms (EPA, 1999).

The advantages of using the membrane filtration technique is the increases in sensitivity, accuracy and reliability (Tamagnini and Gonzalez, 2001) while allowing reduction of time, labour, equipment, space and material (Hanko, 2000). The technique is considered highly reproducible, yields rapid quantitative results and is not costly (Lifshitz and Joshi, 1997). Unfortunately, there are several drawbacks associated with this technique, in particular the two to five days it takes to confirm positive results (Hanko, 2000).

1.3.2 QuantiTray®/ 2000 Colilert 18 method - Most Probable Number technique

The use of the QuantiTray®/2000 Colilert 18 has quite recently become widespread in use in many countries around the world (Landre, Gavriel and Lamb, 1998). The method uses a commercial, defined-substrate medium (Palintest, 2001), which is a pre-dispensed dry-blended reagent formulation containing the enzyme 4-methylumbelliferyl- β -D-glucuronide (MUG) and which specifically detects the presence of *Escherichia coli*, (EPA, 2001; Van Pouke and Nelis, 2000).



The Colilert method also use of multiple wells to estimate the most probable number (MPN). Although it is not the actual count of the bacteria present, the MPN is a statistical estimate of the number of bacteria that, more probable than any other, would give the observed result (Hanko, 2000).

This method was developed for monitoring the quality of drinking water and recreational waters (Elmund, Allen and Rice, 1999), and is much simpler to use than the membrane filtration technique. This allows a laboratory to analyse more water samples at a lower cost and with less chance of human error when recording results (Budnick, Howard and Mayo, 1996). This method has also recently been gaining popularity amongst water laboratory analysts, over the classic analytical methods for indicator organism number detection in various water resources (Elmund *et al.*, 1999).

For the purposes of this study, only the *Escherichia coli* results yielded with this method were considered (not the total coliform results that the method can also yield), as the main focus of this study was aimed at detecting faecal pollution of water. The Colilert method does not produce any false negative results.

1.4 WATER USES AND QUALITY CRITERIA IN THE SELECTED CATCHMENT

Table 1.1 represents the specific water quality guideline limits to be used in this study to determine potential health effects that could be expected as a result of various water uses.

**Table 1.1** Site-specific guideline related water quality for this study

Water use category	Faecal coliforms / <i>E. coli</i> counts / 100 ml	
Domestic	Drinking	0 = Not detectable ^(a)
	Food preparation	≤1 = Insignificant chance of infection ^(b)
	Bathing (contact risk)	≤10 = Insignificant chance of infection ^(b)
	Laundry (contact risk)	≤10 = Insignificant chance of infection ^(b)
Abstraction	For drinking water after limited treatment	≤2,000 = Potential health risk ^(c)
Agriculture (consumption risk)	Vegetables and salad eaten uncooked, livelihood fishing and livestock watering	≤1,000 = Insignificant chance of infection ^(d)
Recreation (consumption risk)	Full contact recreation	≤130 = Risk of gastrointestinal effects excepted ^(e)

a) World Health Organisation (1996). Guidelines for drinking water quality.

b) Water Research Commission (1998). Quality of Domestic Water Supplies Vol 1: Assessment Guide.

c) Department of Water Affairs and Forestry (2002). National Microbial Monitoring Programme for Surface Waters.

d) South African Water Quality Guidelines – Vol 4: Agricultural Use (1996); Jagals and Steyn (2002)

e) South African Water Quality Guidelines – Vol 2: Recreational Use (1996).

1.4.1 Domestic use

Due to a rapid increase in human population density, people are often forced to reside in overcrowded and informal settlements situated along river banks - often with inadequate water and sanitation infrastructures (Bezuidenhout *et al.*, 2002). People living under these circumstances often have no alternative but to use untreated surface water as domestic water for drinking, bathing and washing clothes, especially when they experience municipal water cuts in their residential areas (Fatoki, *et al.*, 2003; Pretorius, 1996). The Modder River catchment reflects this condition, especially in areas located along the Klein Modder River, which flows through Botshabelo and into the Modder River (Figure 1.1).

1.4.2 Abstraction for treatment of drinking water

The National Microbiological Monitoring Programme for Surface Waters in South Africa recommends that raw water extracted to be fully treated for drinking water

contain $\leq 2,000$ faecal coliform (IAF, 2002). Water is abstracted from the Modder River (at sites **MR1** and **MR5**; Chapter 2; Section 2.2.1) to be treated for drinking water at two drinking water treatment facilities, namely Rustfontein and Mazelspoort.

Rustfontein treatment facility

This facility is located in the upper reaches of the Modder River. The water to be treated for drinking water is withdrawn from the Rustfontein impoundment in the Modder River. Jagals (2000) described the water quality at the impoundment as unpolluted water, meaning that the water is not impacted by any anthropogenic sources of pollution, since there are no human activities upstream that can affect the water quality.

Water extracted from the impoundment was treated by going through the process of sedimentation, filtration and disinfection, in order to reduce the risk of infection and to make the water suitable for drinking. Jagals (2004) found water at the impoundment suitable to use for such purposes. The facility supplies water to Botshabelo Thaba 'Nchu and Bloemfontein (Figure 1.1).

Mazelspoort treatment facility

This drinking water treatment facility is located downstream from the Rustfontein drinking water treatment facility. The river water extracted from the Modder River to the treatment facility was described as polluted river water because it was polluted by surface discharges from upstream urban areas and poorly managed wastewater treatment facilities from Botshabelo and Thaba 'Nchu (Jagals, 2000).

Jagals (2004) reported that, although polluted, the *E. coli* numbers in the river water were not as high as may have been expected from the upstream pollution impacts.



After the water has been fully treated, it provides treated water to people in the city of Bloemfontein.

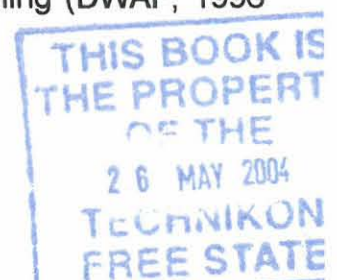
1.4.3 Recreational users

In the catchment, people practise water-related activities such as full (swimming), intermediate (canoeing) and non-contact recreations such as boating and relaxing near water when picnicking, for instance. People using the water for intermediate and non-contact recreation in this area are generally from areas with proper water and sanitation infrastructure. Jagals (2000) reported that such persons could tend to underestimate the risk of infections associated with their recreational activities due to absence of water-borne diseases in their domestic environment.

Children, living within the particularly poor parts of the catchment where there are little or no recreational facilities, often use the water for recreation, especially during hot summer days. Steyn (2003) found that children are exposed to a risk of infection whilst swimming or playing in the water as they may ingest the polluted water. The South African Water Quality Guidelines recommend that the acceptable guideline limit for recreational purposes of surface water be set at ≤ 130 *E. coli* or faecal coliform / 100 ml for full body immersion such as swimming (DWAF, 1996 [b]).

1.4.4 Agricultural purposes

The agricultural sectors increasingly use polluted surface water for producing food (Jagals and Steyn, 2002). Many residents in the surrounding residential areas engage in small farming activities for sustenance and survival (Nel, 2002). These activities can be grouped in two categories.





- ① Firstly, poor people grow vegetables that they use for supplying themselves with food. They often use polluted untreated surface water to irrigate these crops (Jagals and Steyn, 2002; Jagals, 1997). These crops can pose a threat to human health especially if eaten raw or undercooked (Blumenthal, Peasey, Ruiz-Palacois and Mara (1999). The South African Water Quality Guidelines (1996) as well as Jagals and Steyn (2002), recommend the limit of $\leq 1,000$ faecal coliforms / 100ml for the purposes described above.
- ② Secondly, people fish in the waters. Fish harvested from polluted waters may pose a threat to human health (Jagals and Steyn, 2002; Blumenthal *et al.*, 1999). The livestock of the people close to the river banks drink the water. According to the South African Water Quality Guidelines for water used in agriculture (DWAF, 1996 [c]), ingesting water of an unacceptable microbiological quality may adversely affect the animal.

1.5 AIMS OF THE STUDY

1.5.1 Rationale

In the Modder River catchment microbiological indicator organism numbers were mostly detected and enumerated using the M-FC and Chromocult and other most probable number methods (Jagals, 2000). However, none of these studies compared the results from these methods in terms of optimally indicating the potential risk of infection to users of the various waters. The Colilert method has not been tested previously in the waters of the study area. It was considered necessary to establish the optimum method in order to measure the results it yielded against health-related water quality guidelines. This was to predict, as optimally as the

selected method would allow health effects that such water could induce.

This study was a first attempt to compare the new QuantiTray® / 2000-Colilert 18 to the M-FC and Chromocult methods for the detection of microbiological indicator organism numbers within the Modder River catchment.

1.5.2 The research problem

It was uncertain which of the three detection methods would yield the most reliable health-related microbiological water quality results when used to assess the occurrences of microbiological indicator organisms in the various waters used by people in the catchment.

1.5.3 The research aim

The primary aim of this study was to determine the optimal method by comparing the performance of the three methods of detecting the number of indicator organisms. The secondary aim of the study was to use the optimum method, once determined, to describe the risk related to the occurrence of indicator organisms in untreated surface waters used for various purposes in the catchment.

1.5.4 The scope of the study

- ① The basis of comparing the three methods was the percentage of false positives yielded by each method.
- ② The enumeration data values were to be adapted downwards (where applicable) with the percentage of false positives, and the method that then



gave the highest count as the optimal method for each water (use) category as described in Section 1.4 of this chapter in Table 1.1.

1.5.5 Specific Objectives

To achieve the aim of the study, the following objectives had to be attained:

- ① assess the true positive occurrence of the selected microbiological indicator organisms at various monitoring sites within the catchment using the three methods;
- ② adapt the original data values downwards to assume the true values;
- ③ by comparing the results obtained by the three methods, determine the optimal method to assess the quality of the particular water;
- ④ describe the fitness for use of the water in the surface resources based on the results obtained from the optimum method; and
- ⑤ use the results from the optimum method to describe the risk of infection to exposed water users in the catchment based on water quality guideline values as stated.

2.1 THE STUDY AREA

The study was conducted in the Modder River catchment (Figure 2.1), in particular in the areas where the Klein Modder River drains into the Modder River and further downstream in the Modder River. The cities (Botshabelo and Thaba 'Nchu) are situated within this catchment. These are low socio-economic urban development areas with a population density of 201,400 inhabitants (World Gazette, 2003).

2.1.1 Point sources of surface water pollution

The cities have some water-borne sanitation (although these are very limited) and a wastewater treatment facilities (WWTF). The WWTF discharge final effluent into the Klein Modder River (Botshabelo) and Sepane Spruit (Thaba 'Nchu) just downstream from the urban areas.

2.1.2 Non-point sources of surface water pollution

Non-point sources of pollution to the surface water in the study area include informal settlements with pit latrine sanitation in and around the two cities and also faecal wastes from farming livestock kept within the residential area. Pit latrines also mostly service the formal residential areas. There were also areas that did not have adequate pit latrines at the time of this study. This often led to polluted run-off to the rivers, especially during heavy rainy periods (Jagals, 2000; Ralikontsane, 2000 and Pretorius, 1996).

The monitoring sites for this study are shown in Figure 2.1. These were selected by Jagals and Koekoe (2002) to reflect different activities upstream, midstream and downstream of the city. The sites have also been reported by Jagals (2000; 1997) as sites where the activities adversely affect the health-related microbiological quality of water in the two rivers.

2.2.1 Water categories

For this study, waters were divided into two categories:

- ① **Unpolluted water** was used as a category for the sites MR1 in the Modder River and KM1 in the Klein Modder River. These sites are not subject to pollution from urban developments, but will carry faecal indicator organisms from natural diffuse sources such as birds and wildlife in the vicinities of the sites.
- ② Sites MR2, MR3, MR4, and MR5 (Modder River); KM2 and KM3 (Klein Modder River) and TB9 from the (Sepane Spruit) represent **polluted urban surface run-off water**. Waters at these sites receive various sources of pollution from wastewater treatment discharges and surface urban discharges. Sites such as MR4 and MR5 are assumed to be less polluted as the pollution levels decrease downstream. For this study however, these sites were categorised as polluted.

2.2.2 The Modder River (Fi

Monitoring sites **MR1, MR2, MR3, MR4, and MR5** were selected along the Modder River to reflect the health-related microbiological quality of the water, varying from its upstream unpolluted state, to its polluted state after receiving various surface water discharges from the Klein Modder River which flows through Botshabelo.

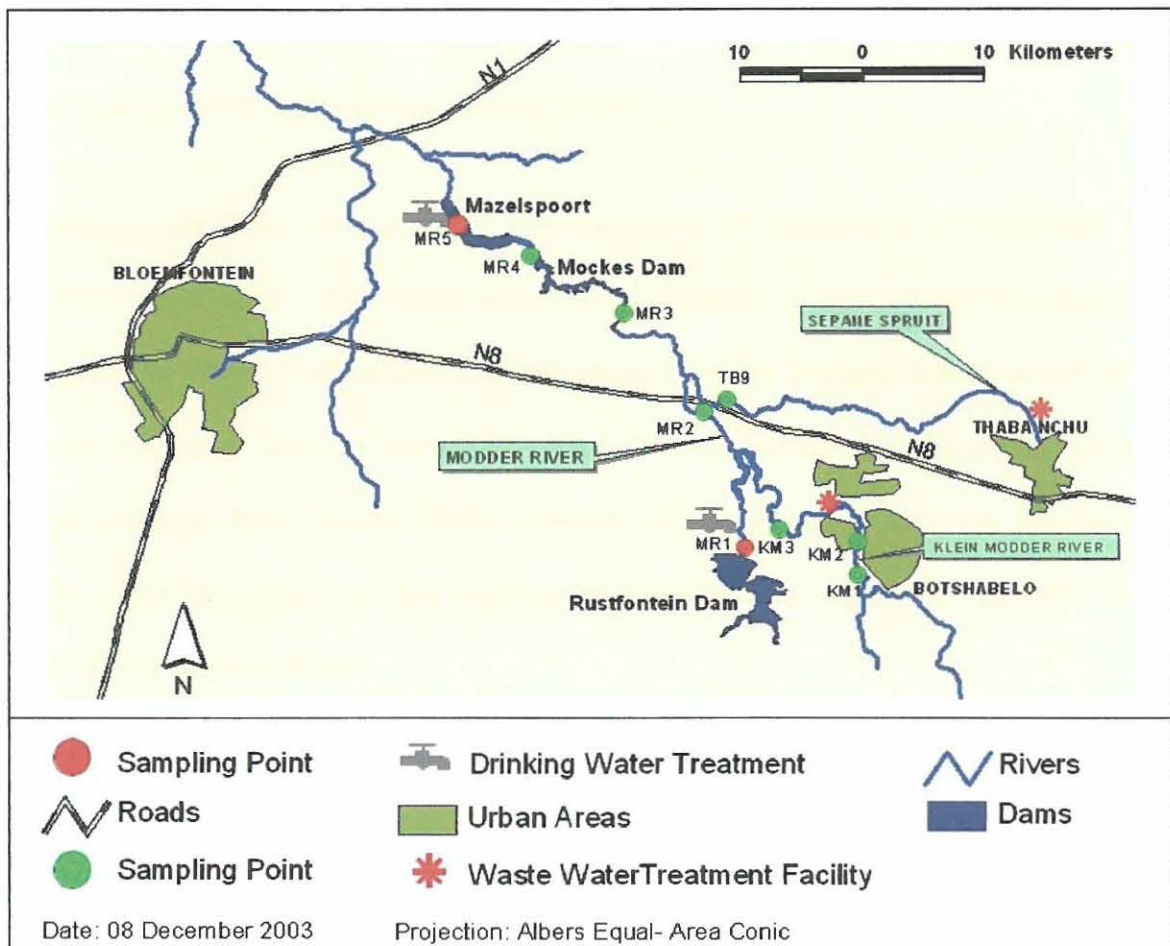


Figure 2.1 Map of study area

MR1 is the unpolluted water reference point at the overflow from the Rustfontein Dam in the Modder River. Rustfontein Dam stores resource water to be treated for drinking water at the Rustfontein drinking water treatment facility, situated adjacent to the dam wall. The facility supplies treated water on demand to the major cities in the local municipal area of Mangaung, i.e. Botshabelo, Thaba 'Nchu and



Bloemfontein (Figure 2.1).

at this site by Jagals (2000; 1994) reflected a natural microbiological quality of water normally not influenced by any surface water discharges from urban developments.

MR2 was downstream from the confluence of the Modder and the Klein Modder Rivers, about 16 kilometres downstream from **MR1**. This site was selected to determine the impact of the Klein Modder River on the health-related microbiological water quality of the water of the Modder River.

Monitoring site **MR3** was situated approximately 18 kilometres downstream from monitoring site **MR2**. Site **MR3** represented impacts of agricultural developments and other natural run-off as well as discharges from the Sepane Spruit, which drains the city of Thaba 'Nchu (Figure 2.1). The site was selected to determine whether faecal pollution from Thaba 'Nchu (Section 2.2.3 below) would, via the Sepane Spruit, have an impact on the microbiological indicator organism numbers in the water of the Modder River.

Site **MR4** is approximately 20 kilometres downstream from **MR3** at the sluice outlet from the Mocke's Dam. Mocke's Dam is the holding dam for treatment water abstracted at the Mazelspoort barrage. It is also a recreational area mainly hosting recreational activities such as intermediate contact (canoeing and fishing) and non-contact (picnicking).

The site was selected to determine whether water from **MR3** had an impact on the microbiological water quality at **MR4** from where the water flows to **MR5** to be extracted for drinking water treatment.



MR5 is further downstream a dam where the Modder River is impounded to form a bulkhead for raw water intake for treatment water to supply residential areas in Bloemfontein. The resource water extracted from the Modder River to the Mazelspoort drinking water treatment facility was described by Jagals (2000) as polluted river water because the water was often severely polluted by discharges from upstream urban areas and also by inadequately managed wastewater treatment facilities from Botshabelo and Thaba 'Nchu.

The dam behind the Mazelspoort weir is a popular recreational area hosting activities that include full-contact (full-body immersion with a strong possibility of water ingestion) as well as intermediate contact with water (fishing, international barefoot water skiing and canoeing) and also non-contact recreation such as picnicking.

2.2.3 Klein Modder River (Figure 2.1)

The Klein Modder River (meaning Small Mud River) drains a variety of surface run-offs from Botshabelo. At the time of the study, approximately five megalitres (Mℓ) of treated final effluent from the city's wastewater treatment facility was released into the Klein Modder River daily from a facility designed to treat twenty megalitres (Mℓ) per day. Due to an increase in human population density it is expected that the effluent volume will increase. At times when treatment operations were not at a proper level, this facility discharged increased numbers of microbiological indicator organisms to the Modder River (Jagals, 2000; 1997; Koning and Roos, 1999).

Jagals (2000; 1997) conducted studies in the Klein Modder River to determine the health-related microbiological quality of its water. Results obtained indicated that

wastes of human and animal origin polluted the water to such an extent that the water became unsuitable for domestic use or even for non-potable purposes such as recreation, agriculture and livelihood fishing.

For this study, monitoring sites **KM1**, **KM2** and **KM3** were selected along the Klein Modder River (KMR) in Botshabelo. These three sites are also the sites selected for the National Microbial Monitoring Programme (NMMP) (DWAF, 2002) in the region. The programme was basically developed to monitor the status and trends of faecal pollution in various regions. The waters at the KMR sampling points were monitored to determine the numbers of faecal coliforms in the KMR before it flows into the Modder River. This was done to determine whether water coming from the KMR had an impact on the health-related microbiological water quality of the Modder River.

Monitoring site **KM1** was situated upstream from where the Klein Modder River enters Botshabelo. The site was selected to determine the microbiological quality of the water before receiving any surface water discharges from urban developments.

KM2 was situated downstream from the city, immediately upstream from where the river receives the discharge from the wastewater treatment facility (WWTF). This site was selected to represent the health-related microbiological water quality in surface water run-off from the largest part of the city before receiving treated final effluent discharges from the WWTF.

Monitoring site **KM3** was approximately 600 metres downstream from site **KM2**. The site was selected to determine the microbiological quality of water after receiving the various surface water discharges from the city and its WWTF.

2.2.4 The Sepane Spruit (I

Monitoring site TB9 is in the low flowing Sepane Spruit which flows from Thaba 'Nchu. Thaba 'Nchu is located about 15 km east of Botshabelo. The rural area around Thaba 'Nchu is currently used for small rural settlements as well as for cattle farming. The Sepane Spruit is mainly polluted by waste from cattle (Ralikontsane, 2000) and also by final treated effluent discharged from a wastewater treatment facility.

2.3 WATER SAMPLING

Twenty-three (23) sampling sessions were conducted over a period of a year from March until November 2002, covering all seasons at the nine sampling points. Five samples were collected from the Modder River, three in the Klein Modder River and one in the Sepane Spruit as discussed in Section 2.2 above. Samples were collected at least every second week. The number of samples collected was 233.

Additional water samples were taken following rainstorms. Griesel and Jagals (2002) found in the Renoster Spruit and Bezuidenhout *et al.* (2002) in the Kwazulu Natal area, that there was an increase in indicator organism numbers due to rainfall. For this study, samples taken during these weather conditions were also used, although the rainfall periods that occurred were not compared to one another in this study. Samples were taken to determine whether surface water run-off from rainfall could cause a significant change in the health-related microbiological water quality and also to determine which of the detection methods yielded the more reliable indicator organism results.



Water samples were collected in airlocks[®], placed in cooler bags at temperatures less than 10°C, and transported immediately to and analysed at the Water Quality Laboratory of the Water and Health Research Group of the Central University of Technology, Free State.

2.4 WATER QUALITY ASSESSMENT

Faecal coliforms (FC) and *Escherichia coli* (*E. coli*) were detected and enumerated using the membrane filtration technique (Appendix A) (Standard Methods, 1998) as well as the most probable number (MPN) technique (Appendix B).

2.4.1 Faecal coliforms (FC)

Faecal coliforms (FC) are microbial bacterial indicators of faecal pollution that can contribute to transmission of infectious diseases such as cholera and typhoid fever (Department of Water Affairs and Forestry (DWAF), 1996 [b]). These organisms were enumerated on membrane Faecal coliform Agar (M-FC) (Appendix C) using the membrane filtration technique.

2.4.2 *Escherichia coli*

Escherichia coli (*E. coli*) are bacterial indicators that give a more specific indication of human and warm-blooded animal faecal pollution in water than do faecal coliforms (DWAF, 1996 [b]). These indicator organisms were detected by two methods in this study.

- ① The membrane filtration technique (Appendix A) using the Chromocult[®] Coliform Agar (CCA) (Merck Corporation, 1996) (Appendix D).
- ② The QuantiTray[®] / 2000 Colilert 18 (IDEXX, 2000) using the Most Probable Number (MPN) technique (Appendix B).

2.4.3 Organism counting

After the prescribed incubation period for organism growth, colonies on the membranes were counted according to the colour generally specified by the commercial supplier of the media (Merck Corporation, 1996)

For the QuantiTray® / 2000 Colilert 18 the number of positive (fluorescing) wells were counted (IDEXX, 2000) (Appendix B).

The maximum range of detection for the Colilert system is ≤ 2420 organisms per 100 ml. It was expected that some of the polluted waters might have higher *E. coli* numbers and the water samples used in the systems were diluted accordingly. An explanation of the dilution approach is provided in Appendix B.

2.4.4 Colony verification (Appendix E)

Non-indicator organisms often find the selectivity of a specific growth medium accommodating, and then grow in the colours prescribed to the analyst for colony verification. These organism colonies are referred to as false positives (Standard Methods, 1998).

False positives are a problem for this type of assessment routine (Jagals, 2000). According to Standard Methods (1998), false positives are non-indicator organisms that are capable of growing on the selective growth medium within the same colour range normally used to identify the true indicator organisms. This often leads to substantial misinterpretation of the actual indicator organism numbers, especially when using the membrane filtration technique.



For this study, estimating the faecal coliform and *Escherichia coli* colonies grown on the filter membranes was done by using an analytical profile index procedure and API® 20E identification kits by bioMerieux® (Appendix E). Between 12% and 40% of the entire target colonies cultured on various selective growth media were randomly selected for verification (Standard Methods, 1998). This was done by streaking out and growing single colonies from the selective media on plate count agar (Standard Methods, 1998; Appendix E).

Verification on the selected colonies was not done because there was doubt about the manufacturer's interpretation of single step identification, but because there was doubt about the colour interpretation amongst the analysts.

2.4.5 Adapting data to false positives

From the confirmation step, the percentage of false positives yielded by each method for each water (use) category was determined. The enumeration data were then adapted downwards (where applicable) with the percentages of false positives and the method that then gave the highest counts was accepted as the optimal method to utilise in the selected waters of the study area.

Verifications were not done on the positive wells produced by the QuantiTray® Colilert 18 method. Studies done by Poucher, Devriese, Hernandes, and Delattre (1991), as cited in Jagals *et al.* (2001) who also used other miniaturised liquid MPN techniques based on chromogenic substrates, have shown that the fluorescence, produced by the enzyme reaction in the selective medium used inside the wells, is highly specific for *E. coli*, therefore minimising misinterpretation. This was also assumed valid for the Colilert method.

2.4.6 Adapting faecal coli *coli* data

The faecal coliform data were adapted downward as suggested by Jagals *et al.* (2001), firstly to the lower true positive faecal coliforms so confirmed, and then lower still to *E. coli* based on the percentage of actual *E. coli* identified amongst the true faecal coliforms during the confirmation process of the faecal coliforms.

2.5 STATISTICAL ANALYSIS OF WATER QUALITY DATA (APPENDIX F)

2.5.1 Data analyses

Data were entered into Microsoft Excel (XP 2000) spread sheets. The data were transformed to $\log_{10}$ values to remove excessive variance and to get the data more symmetrical (Helsel and Hirsch, 1995). Data were then described statistically for the sample size, mean of the logs (geometric mean), and compliance at the 90th percentile.

This 90th percentile compliance level was selected to indicate the levels of risk of infection to people who are exposed (by ingestion) to the test waters. This was done by comparing the results obtained to water quality guidelines.

The statistical programme SigmaStat[®] v2.04 (1997) was used to calculate and test for sample size, normality and analysis of variance (ANOVA) between data groups. SigmaPlot[®] v8 (2001) was used to plot the data into line and boxplots. Line graphs illustrate the differences in the health-related microbiological quality of water of the Klein Modder and Modder Rivers. Boxplots (illustrated in Appendix F) provide visual summaries of all the data obtained as well as showing the mean and the 90th percentile.

2.5.2 Analyses of variance (Appendix F)

For this study, all data were assumed non-parametrical (Appendix F). Analyses of variance are parametric and non-parametric tests that determine whether groups of data differ significantly or not. The data were subjected to non-parametric ANOVA since differences between data groups formed the statistical basis of this study.

The non-parametric Kruskal-Wallis ANOVA on Ranks was selected throughout this study, as three or more groups were continually compared (Helsel and Hirsch, 1995; Sigma Stat®, 1997).

2.5.3 Statistical hypotheses

These hypotheses are the statistical scrutiny that researchers decide upon up-front for their research sub-problem statements (Helsel and Hirsch, 1995). The objective of analysing the data statistically is to test whether these problem statements (hypothetically based) are of true value, based on the evidence provided by the data. The result of this is generally a statement on whether a statistical hypothesis should be substantiated, modified or rejected (Helsel and Hirsch, 1995).

For the purpose of this study, various problem statements and their related statistical hypothesis were formulated for each section in Chapter 3: Results and Discussion and illustrated in Figure 2.2.

2.6 LAYOUT

Figure 2.2 illustrates the layout used in Chapter 3: Results and Discussion. The results are discussed in two major sections in Chapter 3.

The first section deals with the optimal method and the second section with the health-related microbiological quality of the surface using the optimal method.

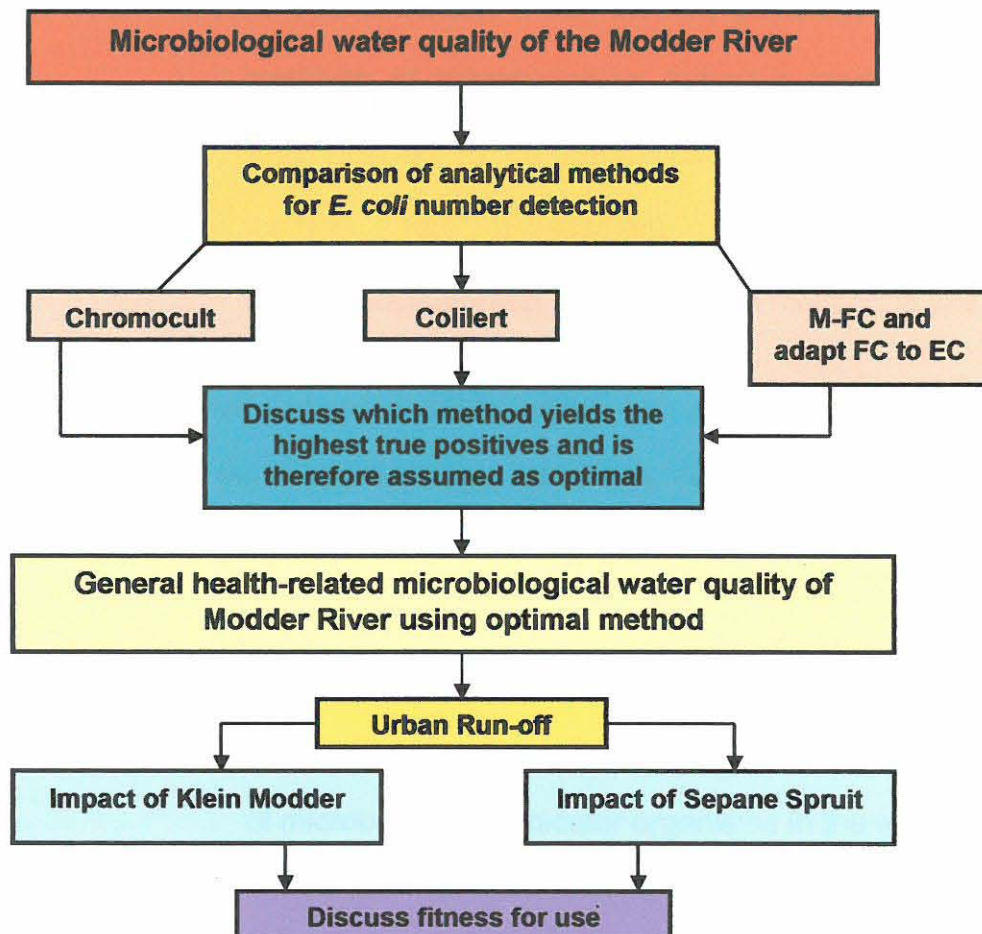


Figure 2.2 Framework of the result section

2.6.1 Selecting the optimal method to assess the microbiological water quality of the Modder River (Section 3.1, Chapter 3)

The objectives of this section were:

- ① to assess the occurrence of the selected microbiological indicator organisms at various selected monitoring sites within the catchment using the three assessment methods;



- ② to adapt the original data; and
- ③ to compare the results obtained by the three methods, and determine the optimal method for the particular water use.

Problem statement 1: It was uncertain which method yielded the highest true positives of microbiological indicator organism numbers. This needed to be determined to select the optimum method for assessment of the health-related microbiological water quality at each monitoring site.

The Kruskal-Wallis ANOVA of Ranks tested for any statistically significant differences between the three methods after the data were adapted (where required) according to the percentages of false positives.

Null hypothesis (H_0): There will be no statistically significant difference in the results from the three methods used to detect the number of microbiological indicator organisms in the waters.

Interpreting the findings

- ① Acceptance of the H_0 would indicate that any of the three methods could be used to detect microbiological indicator organism numbers at each monitoring site.
- ② Rejection of the H_0 would mean that statistically significant differences had occurred in the three methods. This would imply that methods detected microbiological indicator organisms differently at each monitoring site depending on the source of pollution. The method that gave the highest true positive organism numbers is the optimal method.



2.6.2 General microbiological quality of the Modder River using the optimal method (Section 3.2, Chapter 3)

In this section, the method determined (as in 2.6.1) as the optimal method would be used to give a general overview of the health-related microbiological water quality in the Modder River catchment obtained during the study period. The section comprises of two subsections.

The *first subsection* was to determine whether surface run-off from the Klein Modder River consisting of diffuse discharges from Botshabelo (including treated final effluent from Botshabelo's WWTF), had any impact on the health-related microbiological water quality of the Modder River.

The *second subsection* was to determine whether surface run-off from the Sepane Spruit coming from Thaba 'Nchu, had any impact on the health-related microbiological water quality of the Modder River.

Problem statement 1: It was uncertain to what extent waters from the Klein Modder River and Sepane Spruit impacted on the health-related microbiological water quality in the Modder River catchment.

The Kruskal-Wallis ANOVA on Ranks was applied to test for the statistically significant differences between the data of all the monitoring sites.

Null hypothesis (H_0): There will be no statistically significant differences in the numbers of microbiological indicator organisms detected at the various monitoring sites.

Interpreting the findings

- ① Acceptance of the H_0 would indicate that there was no statistically significant difference in the numbers of microbiological indicator organisms detected in the water of the Modder River. This would imply no impact from the two respective urban drains (Klein Modder and Sepane).
- ② Rejection of the H_0 would indicate that statistically significant differences in the numbers of microbiological indicator organisms occurred as a result of surface run-off from the Klein Modder River from Botshabelo and the Sepane Spruit from Thaba 'Nchu. If the numbers are significantly higher (as expected) it would imply that the diffuse charges from Botshabelo as well as Thaba 'Nchu, had a negative impact on the health-related microbiological quality of the Modder River.



CHAPTER 3: RESULTS AND DISCUSSION

This chapter is divided into two sections:

- 3.1 The selection of the optimum method, by using the results obtained for each monitoring site to determining which method yields the highest true positives, and is therefore optimal to use in the selected waters of the study area. The data representing the comparison of these three methods (Chromocult, Colilert and M-FC) are shown in boxplots throughout this chapter. The tables summarising the data are presented in Appendix G.
- 3.2 A discussion of the health-related microbiological quality of water in the Modder River catchment using the results obtained by the optimum method, in order to describe the fitness for use to water users for various purposes.

3.1 THE OPTIMUM *E. coli* DETECTION METHOD

This section compares the three methods used for *E. coli* detection in two categories of water (unpolluted and polluted) as discussed in Chapter 2, Section 2.2.4. Although the M-FC method was not designed to detect only *E. coli*, the percentages of true positive *E. coli* were determined from results of the M-FC method. The *E. coli* of the counted faecal coliforms will be used in this study. These are indicated in Appendix G in Table 1.

For the results and discussions in Sections 3.1.1 and 3.1.2, the data were adapted downwards with the percentages of false positives to reflect, as closely as possible, the true values that the various methods are capable of yielding. The resulting data were then transformed into logarithmic numbers (Chapter 2, Section 2.5.1).

Figures 3.1 and 3.2 illustrate expressed per 100% recovery for the various water categories.

The results obtained from the Colilert method were not categorised. The positive wells produced by this method have shown that the fluorescence produced by the enzyme reaction in the selective medium used inside the wells, is highly specific for *E. coli*, therefore minimising the probability of misinterpretation (IDEXX, 2000).

The method that gave the highest counts was assumed to be the optimal method to use.

3.1.1. *E. coli* and the M-FC method for faecal coliforms

Table 1 (Appendix G) shows the verified results of the true *E. coli* amongst the counted faecal coliform (FC) colonies grown on membranes using the M-FC Agar. The details of the M-FC detection method are described in Appendix C. Figure 3.1 shows the true *E. coli* colonies after the data were adapted as described in Chapter 2, Section 2.4.6.

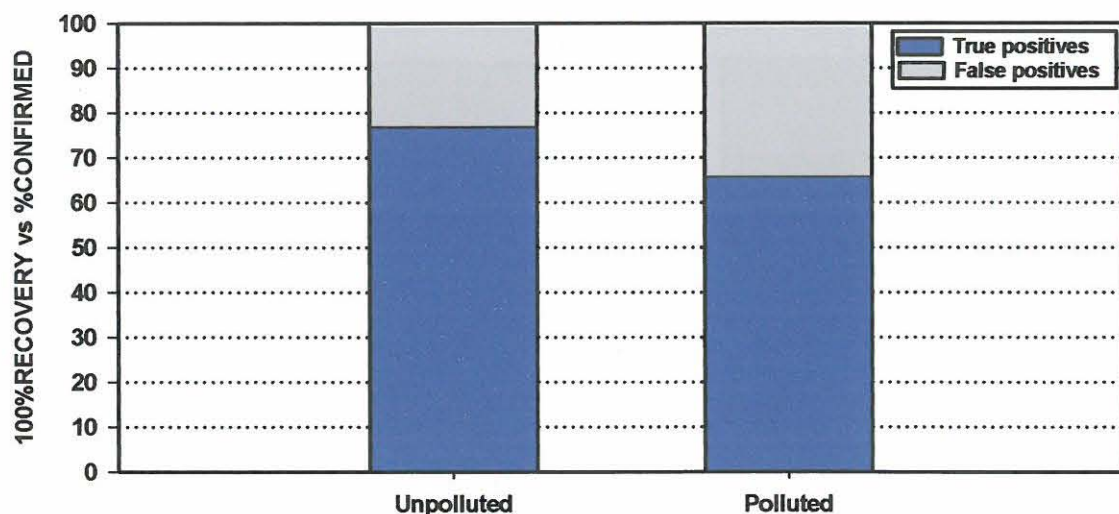


Figure 3.1 Faecal coliform detection on M-FC medium

The percentages of confirmed *E. coli* within the faecal coliform group remained around the 77% mark in unpolluted water and 66% for polluted water. It seemed that the method was more effective in detecting true *E. coli* in unpolluted water than when used for water with high levels of pollution. In a similar study conducted by Jagals (2000) in the same waters, the opposite was reported with the percentage of true positive *E. coli* within the faecal coliform group varying around 90% for polluted water, and lower in the case of unpolluted water. This discrepancy could be due to the fact that in this study more colonies of presumptive *E. coli* cultured from samples of polluted urban run-off were confirmed than those of unpolluted water. This is indicated in Table 1 (Appendix G).

3.1.2 The Chromocult method for *E. coli*

Table 2 (Appendix G) shows the results of *E. coli* verification of colonies grown on membranes using the Chromocult Coliform[®] Agar. The details of the Chromocult detection methodology used are described in Appendix D. Figure 3.2 shows the *E. coli* colonies after the data were adjusted downwards with the percentages of false positives in the selected waters.

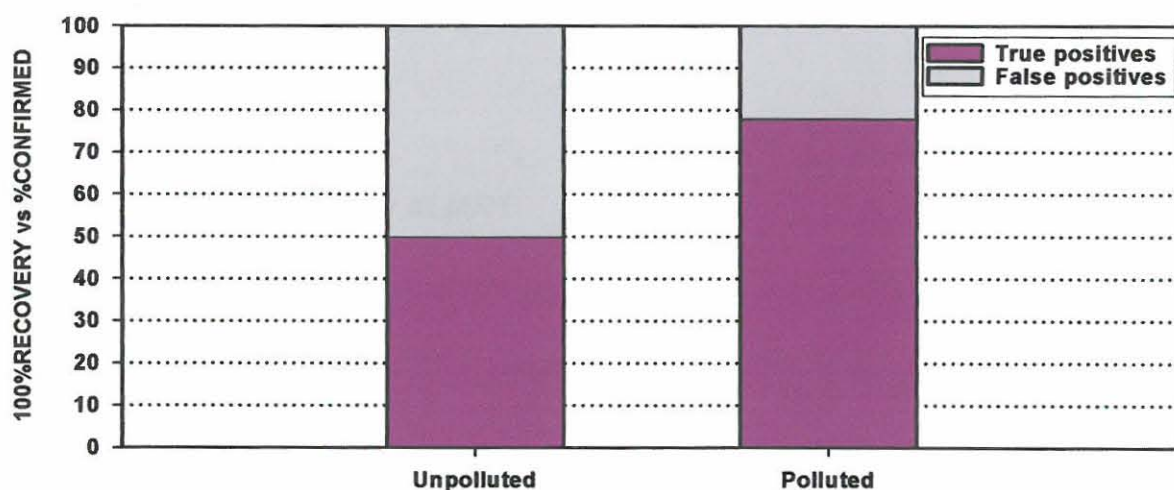


Figure 3.2 *E. coli* on Chromocult Coliform[®] medium



The purple colour shows true positive *E. coli* colonies on the Chromocult Coliform[®] Agar. The grey colour represents the false positive *E. coli* colonies after colony verification.

The percentages of confirmed *E. coli* varied around 80% for polluted water but decreased, as the water became less polluted until in water in an unpolluted state, only 50% of the colonies assumed to be *E. coli* on Chromocult Coliform[®] medium actually turned out to be *E. coli*. The method appeared more effective in polluted water as 78% of the colonies turned out to be *E. coli* than in water with low levels of pollution. Similar findings were reported by Jagals *et al.* (2001) in the same catchment.

3.1.3 The Colilert method for *E. coli*.

The *E. coli* numbers obtained using the Colilert method were not confirmed for the reasons explained in Chapter 2, Section 2.4.4.

3.1.4 Comparing detection method efficiency with Modder River water

The boxplots in Figures 3.3 (a, b, c, d., and e) compare the three methods (Table 3, Appendix G). "ND" on the Y-axes denotes the level where the organisms were "not detected".

3.1.4.1 Unpolluted water at MR1

The Colilert method produced significantly higher mean (yellow line in the boxes) results for *E. coli* than the Chromocult[®] Coliform Agar and the *E. coli* detected by the M-FC media. These differences were also significant at the 90th percentile ($P=0.002$) (note that the 90th percentile on the boxplots is the upper whisker). The

Figure 3.3 (a) illustrates the log-transformed results of the three methods.

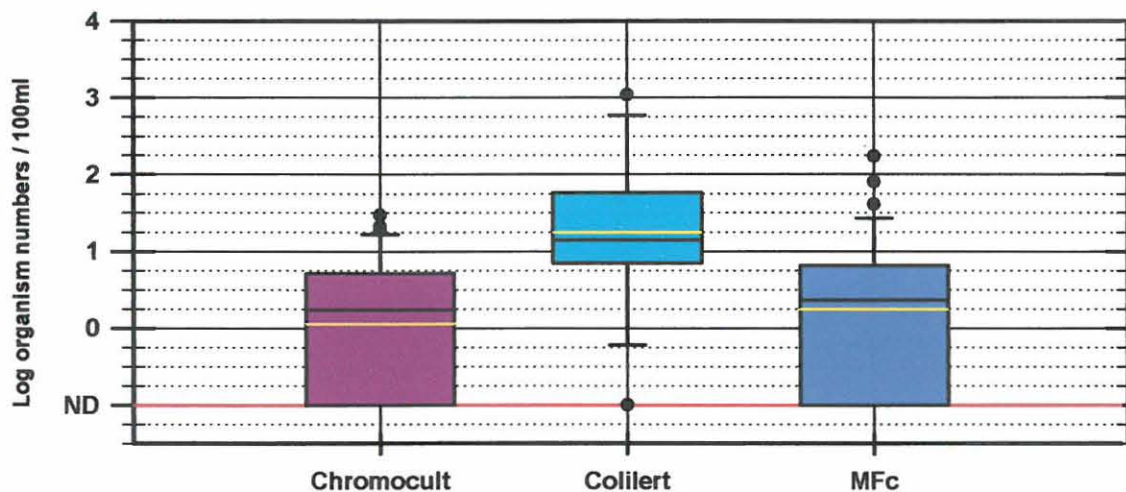


Figure 3.3(a) Method comparison for *E. coli* detection in unpolluted water at MR1

Jagals *et al.* (2001) conducted a similar study using the same water category, but the Sanofi[®] micro-titre plate method instead of Colilert (which also uses an MPN technique as well as the same chromogenic substrate as the Sanofi[®] method). They reported that the Sanofi[®] method yielded lower *E. coli* numbers than the Chromocult method.

Because of the higher yield, analysts should opt to use the Colilert method rather than the Chromocult and M-Fc methods to detect indicator organisms in unpolluted surface water.

3.1.4.2 Polluted water at MR2

Water sampled at MR2 represented polluted urban run-off from Botshabelo. Figure 3.3(b) shows the numbers of *E. coli* detected by the three methods. From the figure, it is clear that the Colilert results were higher than those obtained by the

The results of the Colilert method were significantly higher than the results of the *E. coli* detected by the M-Fc method. Results from the Chromocult method were higher than the M-Fc, even though there was no significant difference.

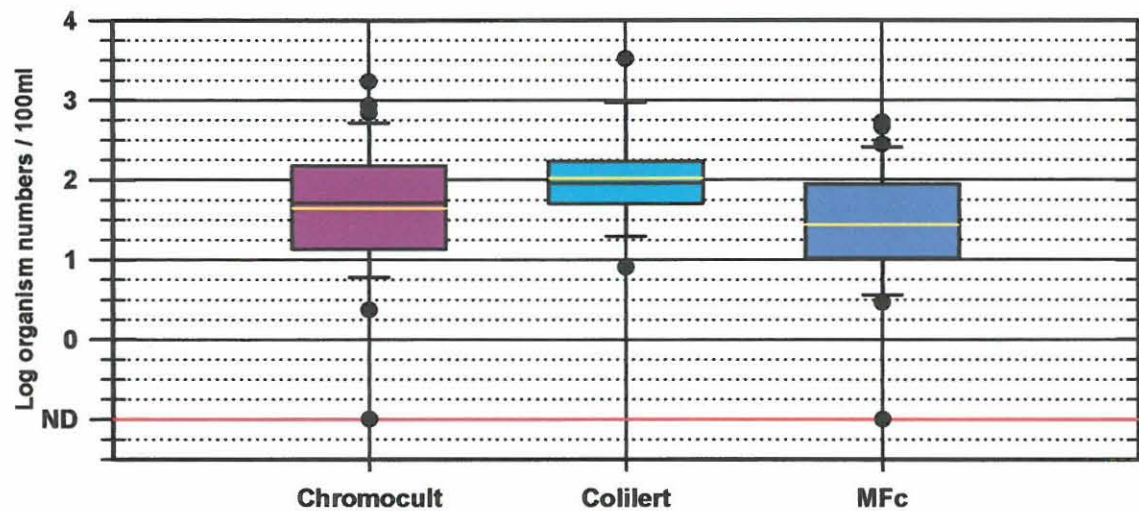


Figure 3.3(b) Method comparison for *E. coli* detection in polluted water at MR2

This means that both the Colilert and the Chromocult methods could be used reliably to detect *E. coli* in polluted water since they do not differ significantly. Even though there were no significant differences between the Colilert and Chromocult methods, the Colilert method detected more *E. coli* in polluted waters than did the other two assessment methods. According to a study by Jagals *et al.* (2001), the Chromocult method yielded higher *E. coli* numbers than the M-Fc and the Sanofi® methods in polluted water.

3.1.4.3 Polluted water at MR3

Figure 3.3(c) shows *E. coli* numbers below the confluence of the Sepane Spruit from Thaba 'Nchu. These waters were even more polluted than the water at MR2 as shown in Table 3 Appendix G.

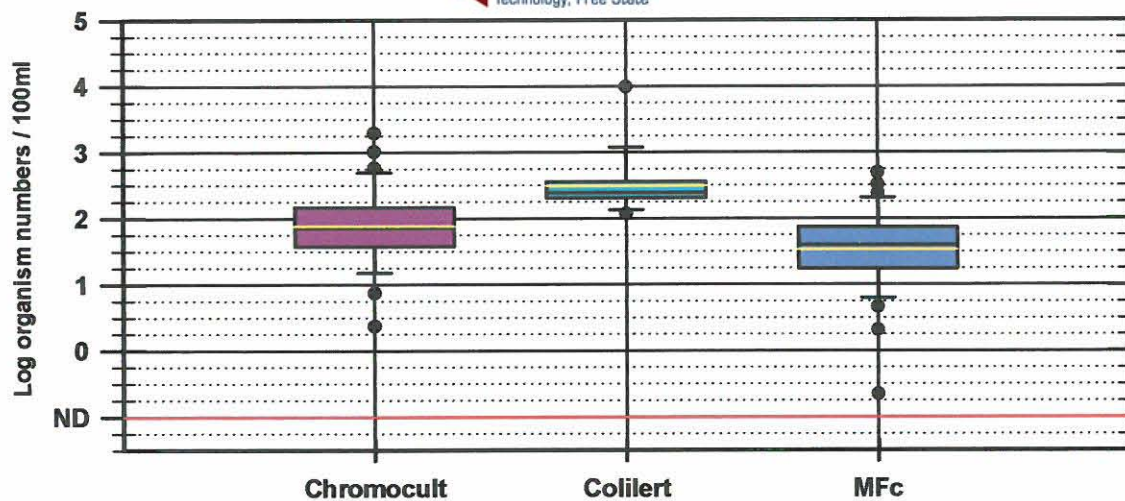


Figure 3.3(c) Method comparison for *E. coli* detection in polluted water at MR3

As with MR2, the Colilert results were significantly higher than the Chromocult and M-FC methods. The latter two methods did not differ significantly. Based on this, the Colilert method would be the method of choice to detect *E. coli* numbers.

3.1.4.4 Polluted waters at MR4 and MR5

Results in Table 3 (Appendix G) showed that pollution level measured at these sites decreased as the downstream part of the river assimilated the pollution. Results are illustrated separately for MR4 (Figure 3.3(d)) and MR5 (Figure 3.3 (e)).

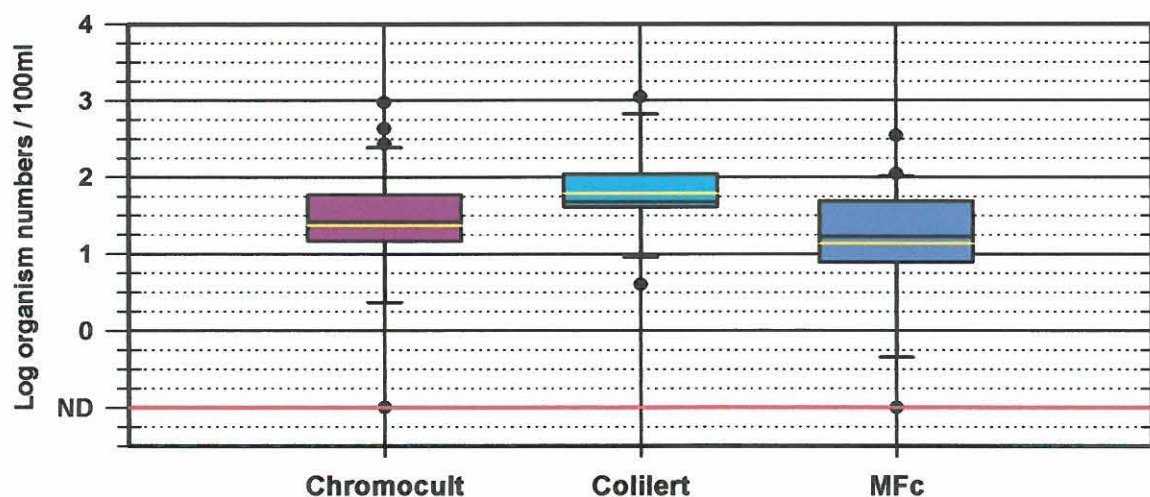


Figure 3.3(d) Method comparison for *E. coli* detection in polluted water at MR4

The results at MR4 differed significantly at the 90th percentile ($P = 0.011$). The Colilert results were significantly higher than those of the M-FC but did not differ from the Chromocult, which in turn, did not differ significantly from the M-FC. However, it was the Colilert method that still produced the highest numbers of *E. coli* at this site even as the water became less polluted.

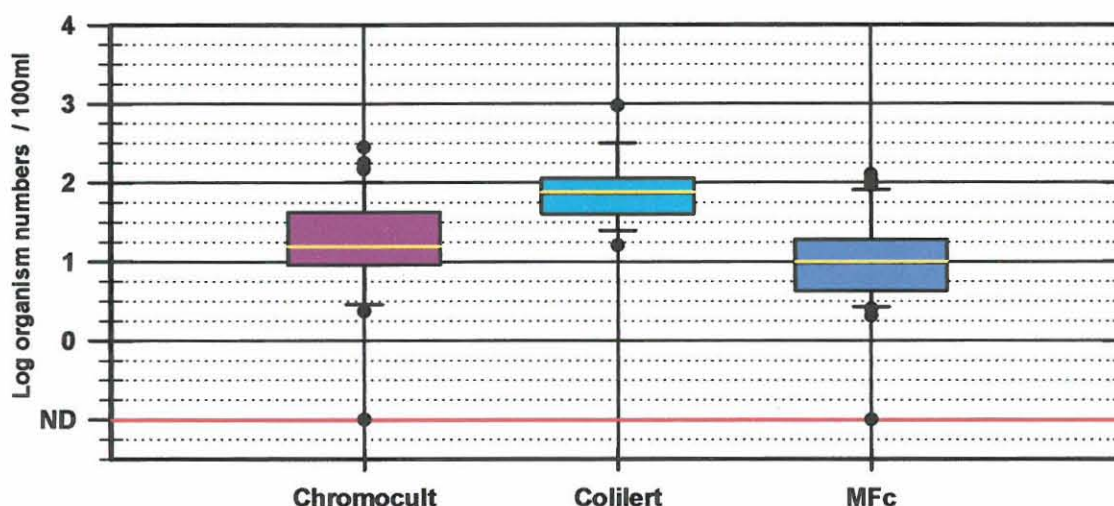


Figure 3.3(e) Method comparison for *E. coli* detection in polluted water at MR5

At MR5 (Figure 3.3(e)), there was a significant difference between the Chromocult, Colilert and M-Fc methods ($P \leq 0.001$). No difference was found between the Chromocult and M-Fc method even though the Chromocult yielded higher results. Nevertheless a difference was noted between the three methods with the Colilert method the optimum one to use. In both instances, preference would therefore ultimately be given to the Colilert method to detect *E. coli* numbers at both sites.

3.1.5 The three methods and waters from the Klein Modder River

The three sites selected in this river are also being used to collect health-related microbiological water quality data for the National Microbial Monitoring Programme (NMMP) (DWAF, 2002), as discussed in Chapter 2, Section 2.2.2. For NMMP data

The faecal coliform data used in this study for these three sites were generated by the candidate and submitted unadjusted (as requested), to the central data office of the NMMP. As can be seen from earlier discussions, unadjusted FC numbers were overestimated and therefore did not reflect a realistic situation for the occurrence of specifically *E. coli*. To see the effect of this, a separate discussion now follows on the efficiency of the three methods in the waters of the Klein Modder River. The results are in Table 4 (Appendix G) with Figures 3.3 (f, g, and h) as illustrations.

3.1.5.1 “Unpolluted” KM1

KM1 is situated where the upstream Klein Modder River enters Botshabelo. This site was assumed to be unpolluted in the initial stages of this study as there were no urban developments surrounding the site. The site was also selected as the official “unpolluted” monitoring point for NMMP data (Van Niekerk, Jagals, Molapo and Koekoe, 2001) as discussed in Chapter 2, Section 2.2.2. Figure 3.3 (f) shows the log-transformed results of true positive *E. coli* numbers detected by the three methods.

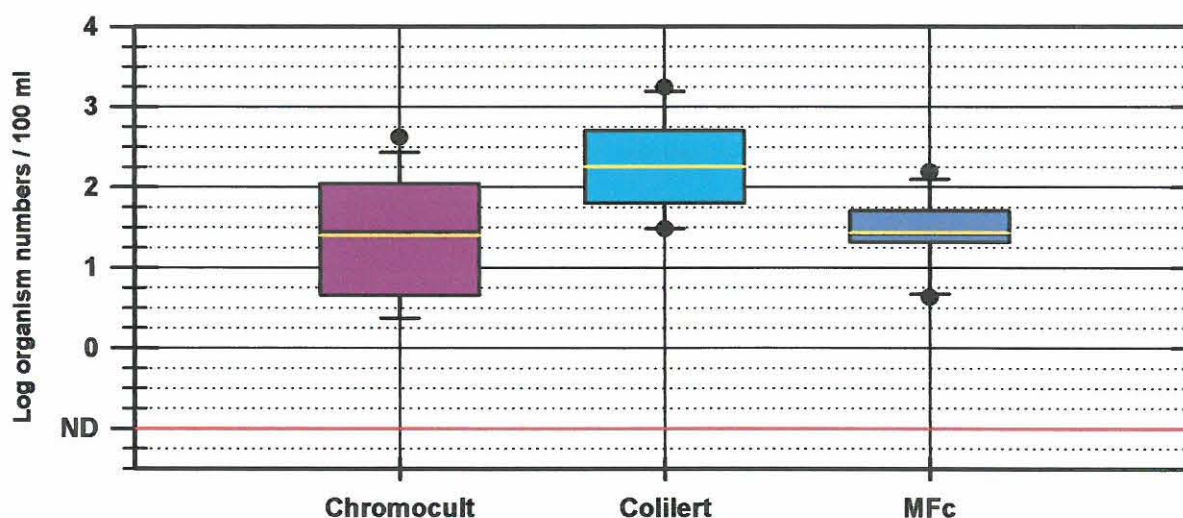


Figure 3.3(f) Method comparison for *E. coli* detection in unpolluted water at KM1



However, the results show that the water was polluted as indicated by the relatively high numbers of *E. coli* detected by all three methods. Jagals (1994) found higher (but unadjusted) M-Fc counts, which if adjusted according to the equation used in this study, would be quite similar to what has been found for this study. In his findings, Jagals described the water at this point as polluted despite it being upstream from the city. Pollution at this site was possible even though upstream, as the site was close to the city. Children and animals are mostly seen around the site.

No significant difference can be seen between the Chromocult and M-Fc methods for detecting *E. coli* numbers at this site but there were significant differences between the Chromocult/Colilert and also for the Colilert/M-Fc methods. However, the Colilert produced significantly higher *E. coli* numbers than the other two methods, judged by the yellow mean line in the boxplot as well as at the 90th percentile. The Colilert was the optimum method for detecting *E. coli*.

3.1.5.2 Water quality of KM2

Water at KM2 represents the Klein Modder River before receiving final treated effluent from an adjacent wastewater treatment facility (Figure 2.1, Chapter 2). Table 4 (Appendix G) shows that there was a significant difference ($P=0.025$) between the Colilert, Chromocult and M-Fc methods. There was a significant difference between the Colilert and M-Fc methods. No significant differences were observed between the Colilert and Chromocult and the Chromocult and M-Fc. Figure 3.3 (g) illustrates the results.

The Colilert produced significantly higher *E. coli* numbers than the other two methods (again judged by the yellow mean line in the boxplot and at the 90th

percentile). This indicated that the Colilert method was the optimum method for detecting *E. coli* numbers in the polluted water selected for this study.

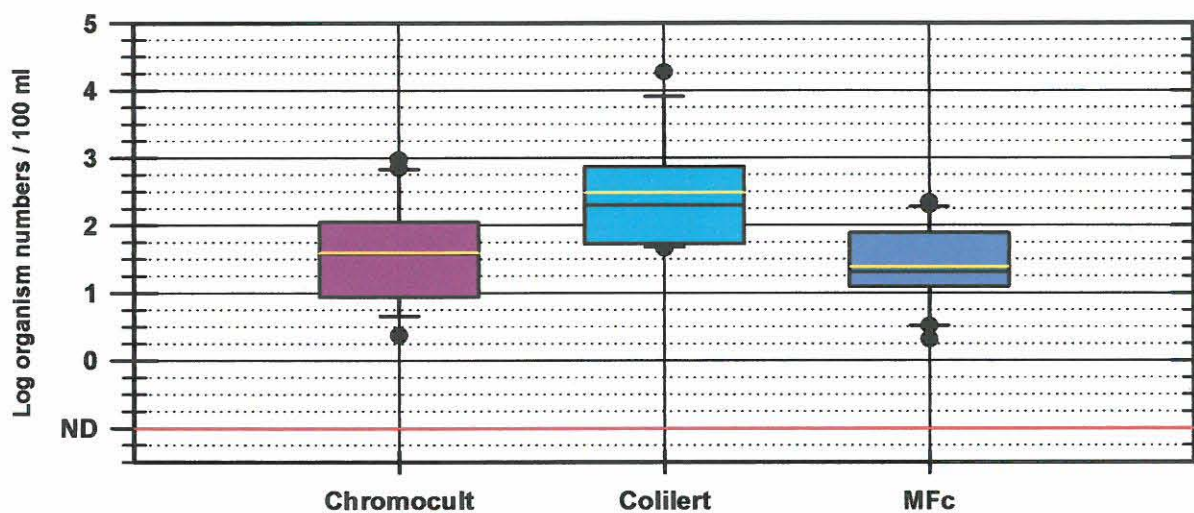


Figure 3.3(g) Method comparison for *E. coli* detection in polluted water at KM2

3.1.5.3. Water quality at KM3

E. coli numbers detected at this site were intended to determine whether the numbers detected by the optimum method would have an impact on the health-related microbiological quality of water in the Modder River. Results are illustrated in Figure 3.3 (h).

Table 4 (Appendix G) shows a significant difference ($P=0.002$) between the three methods. There was a significant difference between the Colilert and M-Fc methods and also for the Colilert and Chromocult methods. No significant differences could be seen between the Chromocult and M-Fc methods.

High *E. coli* numbers were produced by the Colilert method, indicating that this method was the optimum one for detecting *E. coli* numbers as the pollution levels in the river were assimilated and reduced.

percentile). This indicated that the Colilert method was the optimum method for detecting *E. coli* numbers in the polluted water selected for this study.

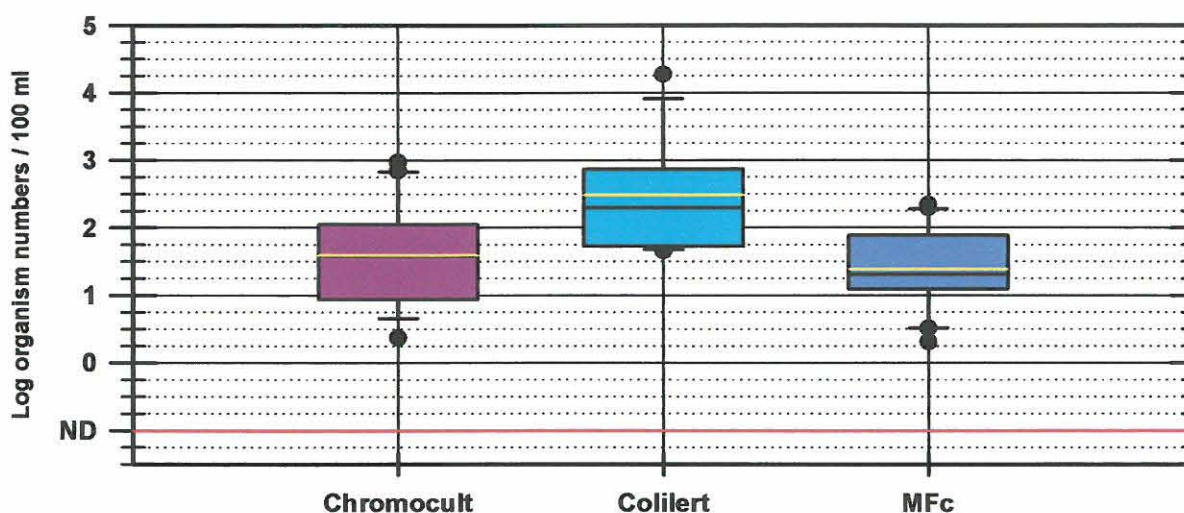


Figure 3.3(g) Method comparison for *E. coli* detection in polluted water at KM2

3.1.5.3. Water quality at KM3

E. coli numbers detected at this site were intended to determine whether the numbers detected by the optimum method would have an impact on the health-related microbiological quality of water in the Modder River. Results are illustrated in Figure 3.3 (h).

Table 4 (Appendix G) shows a significant difference ($P=0.002$) between the three methods. There was a significant difference between the Colilert and M-Fc methods and also for the Colilert and Chromocult methods. No significant differences could be seen between the Chromocult and M-Fc methods.

High *E. coli* numbers were produced by the Colilert method, indicating that this method was the optimum one for detecting *E. coli* numbers as the pollution levels in the river were assimilated and reduced.

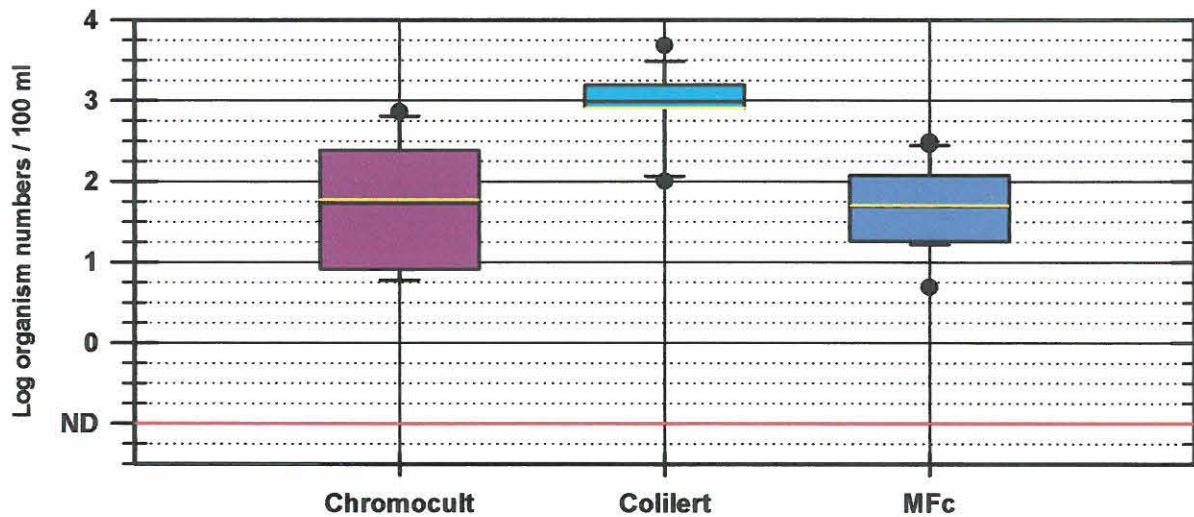


Figure 3.3(h) Method comparison for *E. coli* detection in polluted water at KM3

3.1.6. The three methods and water from the Sepane Spruit

Figure 3.3 (i) (Table 5, Appendix G) illustrates the results obtained from the Sepane Spruit. The Spruit, flowing from Thaba 'Nchu, receives final treated effluent discharged from a waste water treatment facility and is also polluted by cattle waste. This was discussed in Chapter 2, Section 2.2.3. There was not a significant difference between the Chromocult and Colilert methods although there was a significant difference between Colilert and M-FC methods.

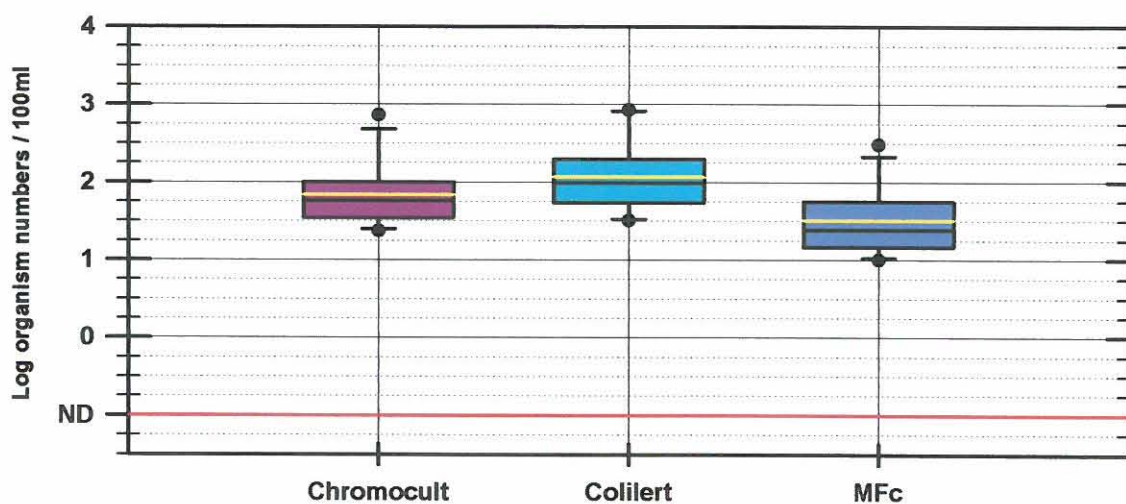


Figure 3.3(I) Method comparison for *E. coli* detection in polluted water at TB9

Clearly, the Colilert produced higher numbers than the other two methods. This was also the trend on the Modder River as illustrated by Figure 3.4 in Section 3.2 to follow. This indicated that in this instance the Colilert method was also the optimum method for detecting *E. coli*.

3.1.7. The optimum method

Table 3.1 summarises the findings for detection of method comparisons according to the results discussed above. It may be concluded that, in the waters of the Modder River catchment, the Colilert method, which uses the most probable number (MPN) technique, in general yielded the highest *E. coli* numbers at all sites and was therefore assumed to be the optimum method.

Table 3.1 Preferred method based on method comparison

Water type	Colilert MPN	Chromocult Coliform [®]	M-FC
Unpolluted river water	Optimum	No	No
Polluted urban surface run-off	Optimum	Alternative	No

The Colilert method provides an attractive replacement for the Chromocult and M-Fc methods due to its ability to detect *E. coli* numbers in just one easy step, with no confirmation steps required. Heavily polluted water does not affect the Colilert method as it does to the Chromocult and M-Fc methods. According to a study by Eckner (1998), the Colilert method provides more rapid, easier and more accurate counts than other detection methods as it is quicker to use, requires shorter incubation periods and is not as affected by turbidity in test waters as are the methods using membrane filtration.

In the 2001 study conducted by Jagals *et al.*, in the same catchment, the opposite was found for both unpolluted and polluted waters. The Sanofi[®] micro-titre plate



was then not as efficient to use the Chromocult method types, with the Chromocult method generally yielding the higher results. The reason why the Sanofi® (now BioRad®) and Colilert methods differed was not clear. Although the studies were conducted at very different times, the same sites were used. There could have been a change in the inherent characteristics of the pollution in the waters over the years. Nevertheless, a future study to compare the BioRad® and Colilert methods is recommended.

The Colilert method was used to assess the suitability for use of the various waters tested during this study. The data generated by the Colilert method were used in the discussions of next section of this chapter. Data from the membrane-based methods were also used in the following section to illustrate the level of potential underestimation when these methods are used or, conversely, when the Colilert method is not used.

3.2 ASSESSING THE HEALTH-RELATED MICROBIOLOGICAL WATER QUALITY OF THE MODDER RIVER USING THE OPTIMUM METHOD

This section gives a general overview of the health-related microbiological quality of water in the Modder River catchment using data from the optimum Colilert (C-method). For improved perspective, the other membrane-based methods (M-methods), and the *E. coli* data sets were combined (they were nearly always not statistically different) and included to illustrate to what extent the other two methods could potentially underestimate the true positive *E. coli* in the waters.

The discussions are twofold: 1) a brief discussion of the impact sites and 2) the ability of the two method sets (C and M) to estimate suitability of use.

Figure 3.4 illustrates the log-*E. coli* numbers in the Modder River water. The green symbols and line represent *E. coli* numbers detected using the C-method. The blue symbols and line show results from the M-method combination, which tended to underestimate *E. coli* numbers in the Modder River. For the sake of illustration, data from only the C-method are used to show the impact points where the Klein Modder River from the city of Botshabelo (indicated by red triangle) and Sepane Spruit from the city of Thaba 'Nchu (indicated by pink circle) join the Modder River.

The upper whiskers show the 90th percentile. The coloured lines represent the various health-related microbiological water quality guidelines proposed in Table 1.1, Chapter 1.

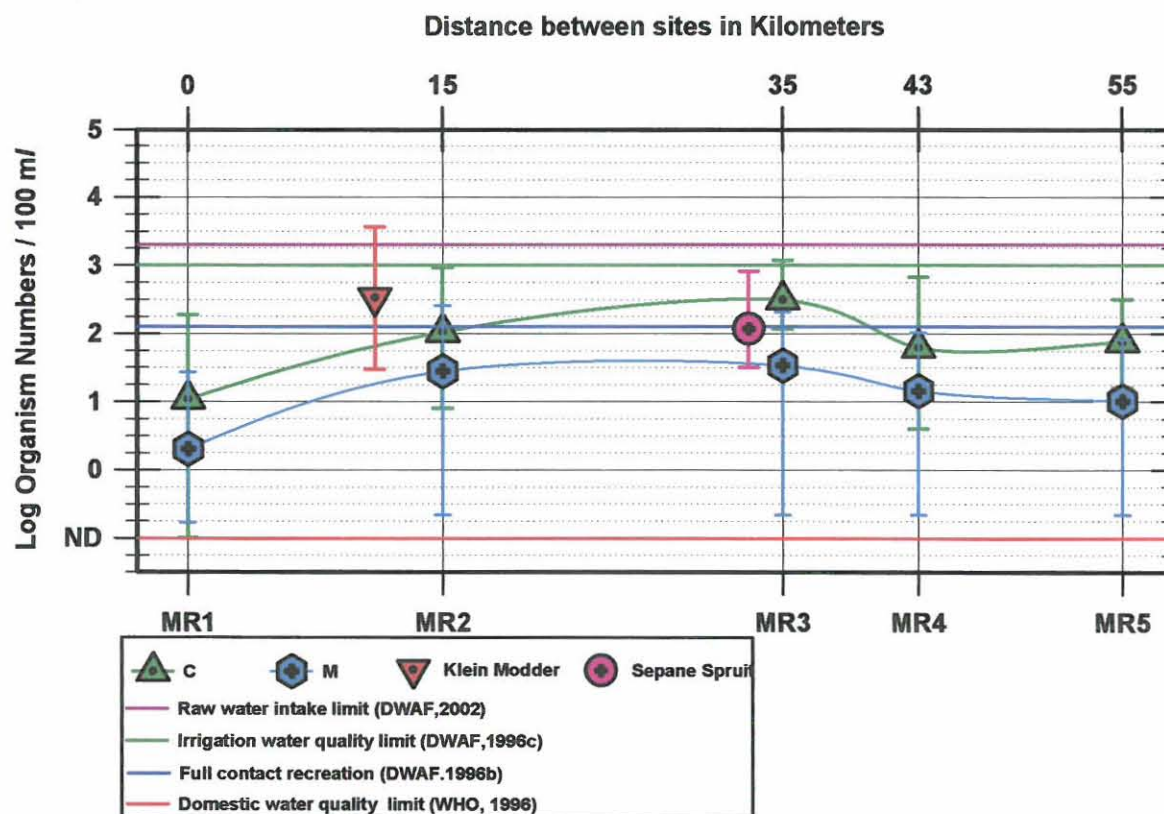


Figure 3.4 *E. coli* numbers in the Modder River detected by Colilert and the membrane-based methods

The C-method detected high *E. coli* numbers, especially after the impacts of the Klein Modder and Sepane Spruit, than the M-method. The C-method also indicated non-compliance with guidelines at more sites than did the M-method. This is discussed at a later stage (Section 3.2.2).

3.2.1 Using Colilert to determine impact points on the Modder River

The numbers of true positive *E. coli* detected in the Modder River, using the optimum C-method, are shown in Table 3.2. To quantify the impacts of the urban discharges, true positive *E. coli* numbers from MR1 to MR5 in the Modder River were statistically compared (Chapter 2, Section 2.6.2).

Table 3.2 Comparing Colilert-derived *E. coli* numbers detected at Modder River sample sites

<i>E. coli</i>	MR1	MR2	MR3	MR4	MR5	ANOVA
n	15	16	16	16	16	Significant difference
Mean of logs	0.95	1.96	2.39	1.68	1.87	$P \leq 0.001$
90 th percentile	1.76	2.70	2.67	2.66	2.28	H_0 rejected; MCT test

The zero hypothesis is rejected since the *E. coli* numbers detected from the various sites differed significantly. This implies that some of the surface urban run-offs (presumably) from the two cities (Botshabelo and Thaba 'Nchu) had a significant impact on the health-related microbiological water quality of the Modder River. However, while Table 3.2 shows statistically significant differences between the data sets, it was still to be confirmed which data set(s) differed from the other(s).

To identify the sets(s) that differ, the data sets of all five sites were subjected to Dunn's multiple comparison test (MCT) procedure described in Appendix F and the results shown in Table 3.3.

**Table 3.3** Determining over different sites in the Modder River

Comparison of sites	Dunn's MCT $P < 0.05$
MR1 vs MR2	Yes
MR2 vs MR3	No
MR1 vs MR3	Yes
MR3 vs MR4	Yes
MR3 vs MR5	Yes

Results in Table 3.2 indicated that *E. coli* numbers detected at MR2 and MR3 were not significantly different although the numbers at MR3 were significantly higher than those detected at MR1, MR4 and MR5. This implies that MR2 and MR3 were more polluted than other sites, indicating the impacts from the two upstream cities.

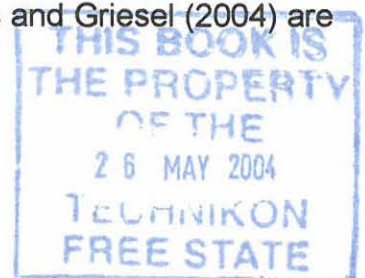
Contrary to this, the results of MR4 and MR5 shown in Table 3.4 did not differ significantly from each other yet both differed significantly from MR1. This means that when compared, MR4 and MR5 were more polluted than MR1, thus confirming that MR1 was the less polluted site in the Modder River.

Table 3.4 The significantly different sites of lesser polluted waters in the Modder River

Comparison of sites	Dunn's MCT ($P < 0.05$)
MR1 vs MR5	Yes
MR4 vs MR5	No
MR1 vs MR4	Yes

This also suggests that despite the impacts from MR2 and MR3, the river assimilated and reduced the pollution from MR3 to MR5.

Jagals (2004) also reported that the *E. coli* numbers at MR5 were not as high as would have been expected from the upstream pollution impacts that Jagals had reported in 1997 and 2000. The reason could probably be improved sanitation in the two cities by the Mangaung Local Municipality, as Jagals and Griesel (2004) are currently reporting.





3.2.2 The Colilert and Membrane Filtration methods to assess Modder River water for various uses

Waters in the Modder River are used for various purposes. To strengthen the recommendation to use the C-method to assess the suitability of these uses, Table 3.5 shows the discrepancies between the two methods. In Table 3.5, the red crosses (✖) give an indication that at the site the water was indicated by the particular method as NOT suitable to use for specific purpose. The black tick (✓) represents indications by the particular method that the water was suitable to use for the specific purpose. These indications are reported at the 90th percentile.

Table 3.5 Water use in the Modder River

Water use @ 90 th percentile	MR1		MR2		MR3		MR4		MR5	
	C	M	C	M	C	M	C	M	C	M
Domestic	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
Recreation	✖	✓	✖	✖	✖	✓	✖	✓	✖	✓
Agriculture	✓	✓	✓	✓	✖	✓	✓	✓	✓	✓
Abstraction	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

3.2.2.1 Water-use at MR1

The low *E. coli* numbers detected (Table 3.2) had shown that the health-related microbiological quality of water at MR1 was unpolluted. The water was not subject to any form of urban pollution and the site generally showed significantly lower pollution levels, since no surface water discharges affected the water quality. Studies by Griesel and Jagals (2002) in the Renoster Spruit (same catchment) and Jagals (2000) in this part of the Modder River have also shown similar sites not influenced by any polluted surface water discharges from urban developments. At the 90th percentile (Figure 3.4) results from both methods indicated that the water was not suitable for domestic use (drinking, food preparation, bathing at <1 *E. coli* / 100mℓ) as indicated by the red line.



While the C-method indicates being not suitable for full-contact recreation, (limit ≤ 130 *E. coli* or faecal coliforms/100 ml indicated by the blue line), the M-methods indicated that it was. However, water would be suitable for recreation and would only be a problem at the level of pollution indicated by the C-method only be a problem if swallowed on a constant basis. Depending on the number of times this were to happen, a risk of infection may occur (Steyn, 2003). So even if non-suitability is indicated, it should generally not be a problem for water skiers, swimmers etc.

Both methods showed that the waters were suitable for the irrigation of health-sensitive crops, livelihood fishing, and livestock watering (indicated by the green line at $\leq 1,000$ faecal coliforms/100ml). This is also supported by reports from studies done by Griesel and Jagals (2002) in unpolluted waters of the Renoster Spruit, as well as by Jagals (2000).

The Rustfontein drinking water treatment facility extracts water to treat for drinking (Jagals, 2004). Both methods showed that the water can safely be extracted at MR1 for treatment since the *E. coli* numbers were within the limits stipulated by the NMMP (DWA, 2002) at $\leq 2,000$ faecal coliforms/100 ml. For agricultural purposes and extraction to be treated for drinking purposes, water was suitable as the 90th percentile was below the stipulated limit.

3.2.2.2 Water use at MR2 and MR3

Both sites represent waters with a typical urban run-off profile causing an increase in *E. coli* numbers as shown in Table 3.2 below the confluence with the Klein Modder River (KMR) (indicated in Figure 3.4) draining from Botshabelo and the Sepane Spruit from Thaba 'Nchu. The results are shown in Table 3 (Appendix G).



Koning and Roos (1999) as well as (Kienzie Lorentz and Schulze 1997) reported similar results at these sites. However, their results appeared to be higher than the results generated for this study. As indicated earlier, the reason could probably be because sanitation infrastructure is currently being improved in the upstream urban area, as Jagals and Griesel (2004) are currently discovering.

In other areas such as in the Western Cape (Wright *et al.* 1993), in the Natal area (Bezuidenhout *et al.* 2002; Kienzie Lorentz and Schulze 1997), in the Umtata catchment (Fatoki *et al.* 2001) and in the Jukskei River catchment (Davie 2002) the researchers reported high faecal coliform numbers because of peri-urban and urban run-offs from informal settlements and effluent discharge from inefficient sewage works.

At the 90th percentile, both methods showed that that water from KM2 and KM3 in the Klein Modder River would not have been suitable to use for domestic and recreational purposes. Water-related infections associated with the use of this water ranged from mild eye, skin and respiratory infections to diarrhoea, caused by organisms such as *Shigella* spp., *Salmonella* spp (Steyn, 2003; Jagals, 2000; Sinton, Finlay and Hannah, 1998 and DWAF, 1996 [b]).

If the water is to be used to irrigate crops and cultivate fish, care should be taken that the users, especially at KM3, are aware that these products should not be eaten raw or undercooked since a risk of infection may occur when used for such purposes. Both methods indicated that the water at these sites, although polluted, was suitable for extraction and treatment for drinking purposes. The surface water discharges from the Sepane Spruit contained human faeces due to poor sanitation infrastructure and inadequately treated sewage effluent, as well as cattle faeces

according to a study done by (Jagals, 2000). In a sub-catchment adjacent to this, Griesel and Jagals (2002) also found that the water of the Bloem Spruit, which drains storm water and other run-off from residential, business and industrial areas of Bloemfontein as well as final effluent from two wastewater treatment facilities, was not suitable for domestic, recreational or agricultural purposes.

3.2.2.4 Water use at MR4 and MR5

Both sites downstream in the Modder River presented water that had become less polluted due to the river assimilating pollution (Jagals, 2000; 1997; Koning and Roos, 1999). Although the water quality improved somewhat downstream from MR4 towards MR5, the C-method showed that the *E. coli* numbers still exceeded the proposed limits for domestic and recreational use as indicated in Chapter 1, Table 1.1.

The M-method, however, did not show this non-compliance, which implies that water users would not be aware of the microbiological health hazard that existed if they were to rely solely on data generated by the M-method. Steyn (2003), using the Chromocult method, also found only limited occasions of risk in these waters.

Both methods showed that water was suitable for agriculture irrigation and raw water extraction. This was suitable especially at MR5 where water is extracted to a drinking water treatment facility to treat water and to distribute it for drinking supply to residential areas of the city of Bloemfontein. Jagals (2004) also reported that the *E. coli* numbers at MR5 were not as high as Jagals (2000, 1997) had reported as a result of the upstream pollution impacts.

3.2.3 The Colilert and Membrane-based methods to assess Klein Modder River water for various uses

Waters in the Klein Modder River are also generally used for various purposes other than for formal raw water extraction. However, a number of households do use the water from the river and subject this to limited treatment at home (Jagals, 2000). While the NMMP uses $\leq 2,000$ faecal coliforms per 100 mL as an extraction maximum for full treatment i.e. sedimentation, filtration and disinfection, the limit is also used as a guideline in this part of the study for people who do home treatment in the area. Figure 3.5 illustrates the increase in the *E. coli* numbers as the water flows through the city down to sample point KM3. Table 3.6 shows the discrepancies between the two methods.

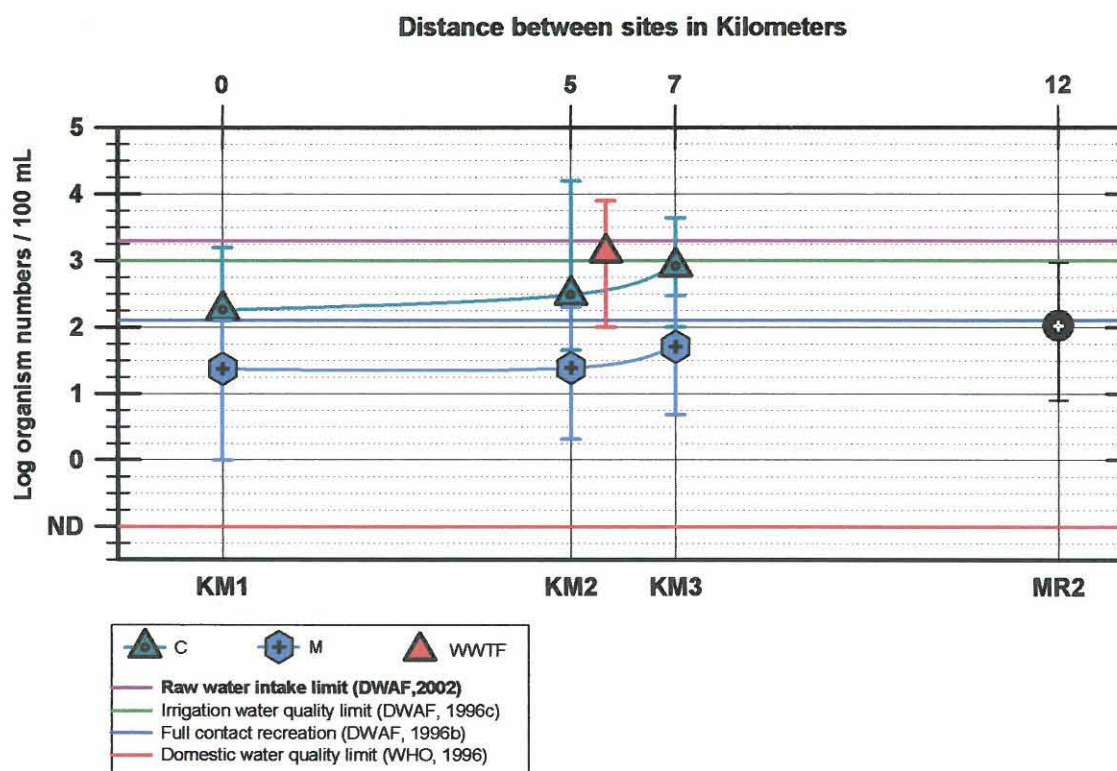


Figure 3.5 *E. coli* numbers in the Klein Modder River detected by Colilert and the membrane-based methods



The confluence of the Klei and the Modder River is situated immediately above site MR2. In the figure the black symbol represents MR2, a sampling point in the Modder River.

Table 3.6 General use of water in the Klein Modder River

Water use @ 90 th percentile	KM1		KM2		KM3	
	C	M	C	M	C	M
Domestic	x	x	x	x	x	x
Recreation	x	✓	x	x	x	x
Agriculture	x	✓	x	✓	x	✓
Abstraction	x	✓	x	✓	x	✓

Water uses at KM1, KM2 and KM3

Previous studies by Jagals (1994; 2000) and Jagals and Grabow (1996) in the area showed that the waters from the Klein Modder River were of exceptionally poor microbiological quality. Not only did this impact negatively on the quality of the water in the Modder River (Koning and Roos, 1999), but it also posed a risk of infection to the many people that used this little river for various purposes. The Klein Modder River is a popular playing area for the children of Botshabelo especially on hot summer days, since no public or privately-owned swimming pools have ever been built in the city. People use the water for laundry, body-washing, small-scale irrigation, livestock watering as well as livelihood fishing.

At the 90th percentile the C-method indicated that waters at the three sites in the Klein Modder River were generally unsuitable to be used for domestic, recreation, agriculture or for raw water extraction. Figure 3.5 illustrates this. Water at these sites should not be used for the above mentioned purposes as infection may occur in consumers. Environmental Health practitioners in the city should make people aware of the health hazards associated with using untreated water of the Klein Modder River.



If the results from the M-method were used (remember that the M-FC method was preferred for the NMMP), water will be judged unsuitable for use only for domestic purposes at KM1 as well as the other two sites (KM2 and KM3). At KM1, full-contact recreation would still appear to be in order, while the C-method in fact indicated that it was not.

The M-methods indicated that waters at all three sites were suitable to use for irrigation of health-sensitive crops, livestock watering, livelihood fishing and treatment for drinking water, when in fact the C-method showed the contrary. The M-method appeared to have grossly underestimated the *E. coli* numbers in these heavily polluted waters.

It is recommended that future data gathering for inclusion into the NMMP database be generated using the Colilert method, especially where heavily polluted surface waters are encountered.

3.3 GENERAL WATER QUALITY OF MODDER RIVER

In general, using the guideline for the Quality of Domestic Water Supplies (WRC, 1998), the overall standard of water quality in the Modder River at all monitoring sites was completely unacceptable for use without treatment. This indicates that the water is not fit for domestic use and may pose a serious risk of infection to communities along the river bank who rely on this water for primary domestic use. This is in agreement with findings by other researchers who have conducted similar studies in areas where surface water is used for purposes such as drinking (Steyn, 2003; Obi, Potgieter, Bessong and Matsaung, 2002; Nevondo and Cloete, 1999).



The implication is that drinki reated) and full-contact recreation in these waters should generally be discouraged, as an occasional risk of infection may be expected if people use this water for such purposes.

According to DWAF, (2002), water of the Modder River will be suitable to extract and fully treat for drinking water. The Colilert method will be used to detect *E. coli* numbers. All the results obtained were below the stipulated limit for raw water intake at $\leq 2,000$ faecal coliforms per 100ml.





CHAPTER 4: SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 SUMMARY

The reliability of various existing and recently-developed methodologies was evaluated for assessing the presence and levels of faecal coliforms and *E. coli*. For the selected indicator groups the following methods were compared:

- ① Faecal coliform detection with M-FC reagent medium and the membrane filtration technique (Standard Methods, 1998).
- ② *E. coli* detection with a chromogenic substrate reagent agar-based medium Chromocult Coliform Agar® and membrane filtration (Merck Corporation, 1996).
- ③ *E. coli* detection with a Most Probable Number Technique (MPN) using the Colilert reagent medium and its related QuantiTray method (IDEXX, 2000).

The aim of this study was to determine the optimum method for detecting health-related microbiological indicator organism numbers in untreated surface water of the Modder River catchment, situated in the South-Eastern Free State province, South Africa. The optimum method was determined by comparing the Chromocult, Colilert and M-Fc methods.

This aim was achieved by:

- ① Assessing the true positive occurrence of the selected microbiological indicator organisms at various monitoring sites within the catchment using these three methods. For this, the Colilert method performed best.



- ② Adjusting the original data towards to assume the true values. The M-FC method required the most adjustment followed by the Chromocult method. Data for the Colilert method did not require adjustment.
- ③ By comparing the adjusted results, the optimal method turned out to be the Colilert method, especially in heavily polluted waters.
- ④ The results from the Colilert method described higher risks of infection to exposed water users while the membrane-based methods underestimated the *E. coli* numbers in the waters.

4.2. CONCLUSION

According to the study results obtained, the Colilert method turned out to be the optimum method since it generated the highest true positive indicator organism numbers in unpolluted and polluted water resources.

Using the optimum method, water quality within the Modder River catchment, generally did exceed recommended limits with regards to purposes such as drinking, preparation of food, bathing and laundry (WRC, 1998; WHO, 1996) and also those for recreational purposes (DWAF, 1996). These findings indicated that the water constituted a risk of infection if used for the above mentioned purposes. The water should therefore be treated, if it is to be used for these purposes. However, the water quality generally did not exceed the recommended limits for agriculture, which includes irrigation of crops, livelihood fishing and livestock watering (Jagals and Steyn 2002; DWAF, 1996c). Water of the Modder River was also suitable to be extracted to treat fully for drinking water as stipulated by the DWAF (2002) at $\leq 2,000$ faecal coliforms per 100ml.



The findings indicate that water samples should therefore utilise the Colilert method for detecting indicator organisms in water. The method proved to yield the highest indicator organism numbers when used in unpolluted and polluted waters. This in turn will save analysts analysis time as the method is quick, easy and reliable to use.

4.3. RECOMMENDATIONS FOR FUTURE WORK

A few recommendations for future work that could be done to reduce uncertainties when detecting indicator organisms in water are provided below:

- ① Research should be done on more polluted-type water resources using these methods (Chromocult[®], Colilert and M-Fc) to refine our knowledge on their detection capabilities.
- ② More research should be conducted to compare the performance of the BioRad[®] (previously Sanofi[®]) and the Colilert methods in the waters of the Modder River. Both methods use the Most Probable Number approach and each has its own distinct advantages. This will provide more options for smaller laboratories to monitor surface water resources effectively for its health-related microbiological water quality.
- ③ More research workshops should be conducted to educate small water laboratories on newly developed methods, especially in the study area, since there is lack of information on these new methods.
- ④ Environmental health practitioners should communicate the risk of infection in study area while using the optimum method, in order to manage the prevention of infections.

REFERENCE LIST

- Alonso JL, Soriaono A, Carbajo O, Amoros I and Garelick H. (1999).** Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41°C AND 44, 5 °C. *Appl. And Environ. Microbiol.* **65** (8) pp 3746-3749.
- Bezuidenhout CC, Mthembu N, Puckree T and Lin J. (2002).** Microbiological evaluation of the Mhlathuze River, KwaZulu-Natal (RSA). *Water SA.* **28** (3) pp 281-286.
- Biolab Catalogue. (1997).** Version 2 Merck.
- bioMérieux. (1998).** API 20E – Identification systems for *Enterobacteriaceae* and other Gram-negative rods. Catalogue No. 07584 B – 04/98.
- Blumenthal UJ, Peasey A, Ruiz-Palacois G and Mara DD. (1999).** Guidelines for wastewater reuse in agriculture and aquaculture: Recommended revisions based on new research evidence. Water and Environmental Health at London and Loughborough (WELL) study. June, Task No. **68** (Part 1).
- Bokako TC. (2000).** Health risk related to water supply and consumption in a marginalised urban area. *MTech: Environmental Health Dissertation.* Published. Technikon Free State, Bloemfontein.
- Brenner KP, Rankin CC, Roybal YR, Stelma GN, Scarpino PV and Dufour AP. (1993).** New medium for the simultaneous detection of total coliforms and *Escherichia coli* in waters. *Appl. And Environ. Microbiol.* **50**: pp 3534-3544.



Budnick GE, Howard RT a

Central University of
Technology, Free State

(1996).

Evaluation of enterolert for enumeration of enterococci in recreational waters. *Appl. And Environ. Microbiol.* **62** (10): pp 3881-3884.

Byamukama D, Knasiime F, Mach RL and Farnleitner H. (2000). Determination of *Escherichia coli* contamination with chromocult coliform agar showed a high level of discrimination efficiency for differing faecal pollution levels in tropical waters of Kampala, Uganda. *Appl. and Environ. Microbiol.* **66** (2) pp 864-868.

Davie L. (2002). Zooming in on the dirty water hotspots. City of Johannesburg [web:] <http://www.joburg.org.za>. December 2003

Day D. (1996). How Australia social policy neglects water environments. *Australian Journal of Soil and Water Conservation.* **9.** pp 3-9

Department of Water Affairs and Forestry (DWAF). (1996 [a]). South African Water Quality Guidelines. Volume. 1 (Domestic use). Pretoria.

Department of Water Affairs and Forestry. (1996 [b]). South African Water Quality Guidelines. Volume. 2 (Recreational use). Pretoria.

Department of Water Affairs and Forestry. (1996 [c]). South African Water Quality Guidelines. Volume. 4 (Irrigation use). Pretoria.

Department of Water Affairs and Forestry. (2002). National Microbial Monitoring Programme Implementation Manual for Surface Waters. WRC Report No K5/1118/0/1. Pretoria.

Eckner, K. (1998). Comparison of Membrane Filtration and Multiple-Tube Fermentation by the Colilert and Enterolert Methods for Detection of Waterborne

Water Quality Monitoring in Southern Sweden

Elmund GK, Allen MJ, and Rice EW. (1999). Comparison of *Escherichia coli*, total coliform, and faecal coliform populations as indicators of wastewater treatment efficiency. *Water Environmental Research*. **71**(3). pp 332-339.

Environmental Protection Agency (EPA). (1999). In National Primary Drinking Water Regulations. *U.S Code of Federal Regulations*, 40 CFR, Part 141, Subpart C, Section 141.21, Washington, D.C.

Environmental Protection Agency. (2001). Guidelines establishing test procedures for the analysis of pollutants; analytical methods for biological pollutants in ambient water. **66**. No 169.

Fatoki OS, Morrison G, Persson L and Ekberg A. (2001). Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment on the Keiskamma River. *Water SA*. **27** (4). pp 475-480.

Fatoki OS, Muyima, NYO and Lujiza, N. (2001). Situation analysis of water quality in the Umtata River catchment. *Water SA*. **27** (4). pp 467-473.

Fatoki OS, Gogwana P, and Ogunfowokan AO. (2003). Pollution assessment in the Keiskamma River and in the impoundment downstream. *Water SA*. **29**(2). pp183-187

Fisher DW and Endale DM. (1999). Total Coliform, *E. coli* and Enterococci bacteria in grazed and wooded watersheds of the Southern Piedmont.

Glantz SA. (1997). Primer of Biostatistics. (4th Ed.). McGraw-Hill, New York.





Grabow WOK. (1990). Mic nking water treatment: Reclaimed waste water. In: Drinking Water Microbiological – Progress and Recent Developments. McFeters (Ed). Springer Verlag, New York. pp185-203.

Grabow WOK. (1996). Waterborne diseases: Update on water quality assessment and control. *Water SA*. **22**(2). pp193-202.

Grant MA. (1997). A new membrane filtration medium for simultaneous detection and enumeration of *Escherichia coli* and Total coliforms. *Appl. And Environ Microbio*. **63**(9): pp3526-3530.

Gregory MB and Frick EA. (2000). Indicator-bacteria concentrations in streams of the Chattahoochee River national recreation area.

Griesel M and 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). pp227-234.

Haile RW, Witte JS, Gold M, Cressey R, Cressey C, McGee RC, Millikan A, Glasser N, Harawa C, Ervin, P, Harmon, J, Harper J, Dermand J, Alamillo K, Barrett M, Nides and Wang G. (1999). The health effects of swimming in ocean water contaminated by storm drain run-off, *Epidemiol*. **10**. pp355-363

Hanko E. (2000). Colilert: What is it all about? 2000 papers for 63rd Annual Water Industry Engineers and Operators. Victoria.

Helsel DR and Hirsch RM. (1995). Statistical Methods in Water Resources. Elsevier Science B.V. Amsterdam, The Netherlands.

IDEXX. (2000). Coliforms and *E. coli* 24 hours or Less Quantified Results-Manual. Colilert from IDEXX Laboratories.



ISO. (1994 [a]). Water Quality - Detection of *E. coli* in Surface and Waste Water: A Miniaturised Method by Inoculation in Liquid Media (MPN). Document ISO/TC147/SC4/WG2-WD9308-3. International Organisation for Standardisation, Geneva.

ISO. (1995 [a]). Water Quality - Detection of Coliforms Organisms, and *Escherichia coli* –Part 1: Membrane Filtration Method. ISO DIS 93081. International Organisation for Standardisation, Geneva.

Jagals C. (2004). Applying an abbreviated hazard analysis and critical control point programme to evaluate the effectiveness of two potable water treatment systems to remove health-related contaminants. Unpublished *MTech Dissertation*. Bloemfontein: Technikon Free State.

Jagals P. (1994). The effect of diffuse effluents on the quality of water in the Modder River. Published *MTech Dissertation*. Bloemfontein: Technikon Free State.

Jagals P. (1997). Stormwater runoff from typical developed and developing South African urban developments: Definitely not for swimming. *Water Science & Technology*. **35** (11-20), pp133 -140.

Jagals P. (2000). The impacts of polluted urban run-off on the Modder River catchment: A microbiological perspective. *DTech Environmental Health thesis*. Technikon Free State, Bloemfontein.

Jagals P and Grabow WOK. (1996). An evaluation of sorbitol-fermenting bifidobacteria as specific indicators of human faecal pollution of environmental water. *Water SA*. **22** (3). pp235-238.



Jagals P and Griesel M. (2004). Effects of sanitation management in expanding urban areas on the microbiological water quality of the Modder River - what does ten years of monitoring tell us?. Draft manuscript to WISA 2004 Biennial Conference Cape Town 2-6 May.

Jagals P and Koekoe L. (2002). Selection of Modder River sites - Personal discussions and arrangements.

Jagals P and Steyn M. (2002). A Review of the Applicability of the South African Guide for the Permissible Utilisation and Disposal of Treated Sewage Effluent in Agriculture and Aquaculture. Interim report to the Water Research Commission: 1039/1/02. November.

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

Kienzie SW, Lorentz ZA and Schulze RE. (1997). Hydrology and Water Quality of the Mgeni Catchment. Department of Agricultural Engineering, University of Natal, Pietermaritzburg. Water Research Commission. WRC Report No. TT87/97. ACRU Report 45/97. pp72.

Koning N and Roos, JC. (1999). The continued influence of organic pollution on the water quality of the turbid Modder River. University of the Free State, Bloemfontein.

Landre JB, Gavriel AA and Lamb AJ. (1998). False-positive coliform reaction mediate by *Aeromonas* in the colilert defined substrate technology system. *Appl. Environ Microbio.* **26**. pp352-354.



of the Novel ColiPlate™ Kit and the standard membrane filter technique for enumerating total coliforms and *Escherichia coli* bacteria in water.

Merck Corporation. (1996). Coliforms show their true colours. Catalogue. No. 1.10426. Merck KgaA, 64271 Darmstadt, Germany.

Millipore Corporation. (1992). Water Microbiology. Laboratory and Field Procedures. Catalogue No. AD323 Re.3/92 92-217. Bedford, Massachusetts.

Morrison G, Fatoki OS, Persson L and Ekberg A. (2001). Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment plant on the Keiskamma River. *Water SA*. **27** (4): pp475-480.

Nel E. (2002). An overview of current economic development policy and strategies in South Africa's nine metropolises and key secondary cities. Grahamstown Rhodes University.

Nevondo TS and Cloete TE. (1999). Bacterial and chemical quality of water supply in the Dertig village settlement. *Water SA*. **25** (2): pp215-220.

Noble RT, Moore DF, Leecaster MK, McGee CD and Weisberg SB. (2002). Comparison of total coliform, faecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research* 37:1637-1643.

Obi CL, Potgieter N, Bessong PO and Matsaung G. (2002). Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA*. **28** (3): pp287-292.

Palintest. (2001). The breakthrough in Colilert testing-Manual. Palintest, Ltd.



Pegram GC, Rollins N and I). Estimating the costs of diarrhoea and epidemic dysentery in KwaZulu-Natal and South Africa

Pourcher AM, Devriese LA, Hernandes JF and Delattre. (1991). Enumeration by a miniaturised method of *Escherichia coli*, *Streptococcus bovis* and enterococci as indicators of the origin of faecal pollution of waters. *Journal. of Applied Bacteriology.* **70.** pp525-530.

Pretorius E. (1996). An investigation into the effects of various levels of sanitation on surface water quality in a typical developing community. *MTech Dissertation.* Technikon Free State, Bloemfontein.

Ralikontsane KF. (2000). The Implementation of secure residential tenure in the rural areas of Thaba Nchu. Mangaung Local Municipality.

SABS. (1984). South African Standard Specification for Water for domestic supplies. SABS Method 241-1984. The Council of the South African Bureau of Standards, Pretoria. 15pp.

SABS. (1987). Bacteriological quality of water by the membrane filter method. SABS Method 221. South African Bureau of Standards, Pretoria. 6 pp.

Sartory DP and Watkins J. (1999). Convectional culture for water quality assessment: Is there a future? *Journal. Appl. Microbiol. Symp. Suppl.* **85** 225S-233S.

Sigmaplot® (2001). Exact graphs for exact science: Users Manual. SPSS Science Inc. Chicago.

Sigmastat® (1997). Statistical Software: Users Manual. SPSS Science Inc. Chicago.



faecal contamination in water: A review. *New Zealand Journal of Marine and Freshwater Research*. **32** pp323-348.

Solo-Gabriele HM, Wolfert MA, Desmarais TR and Palmer CJ. (2000). Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. and Environ. Microbiol.* **66** (1) 230-237.

Standard Methods. (1998). Standard Methods for the Examination of Water and Wastewater. (20th Ed.). Andrew D Eaton. Lenore S Clesceri and Arnold E Greenberg. American Public Health Association, Washington DC.

Steyn M. (2003). An assessment of the human health risk posed by pathogens. *MTech Dissertation*. Technikon Free State, Bloemfontein.

Tamagnini LM and Gonzalez RD. (2001). Evaluation of two media and different plating techniques for enumeration of thermotolerant coliforms in river water. **43** (2): pp84-87.

Van Niekerk H, Jagals P, Molapo A and Koekoe L. (2001). Selection of NMMP sites in Botshabelo - Personal discussions and arrangements.

Van Niekerk H, Kuhn A, Du Preez M and Murray K. (2002). National Microbial Monitoring Programme Implementation Manual for Surface Water. WRC Report No. K5/1118/0/1. Pretoria

Van Pouke SO and Nelis HJ. (2000). Rapid detection of fluorescent and chemiluminescent total coliforms and *E. coli* on membrane filters. *Journal of Microbiological Methods*. **42**: pp233-244.



Venter SN. (2001). Microbia n the 21st century. *SA Waterbulletin* 27 (1).

Venter SN, Steynberg MC.,Du Plessis G, De Wet CME, Hohls D, Rodda N. and Kfir R. (1996). Tools for Microbiological Water Quality Assessment of South African Rivers, Report to the Water Research Commission. Division of Water, Environment and Forestry Technology with Rand Water. WRC Report No. 380/1/96. Pretoria.

Water Research Commission. (1998). Quality of Domestic Water Supplies. (1): Assessment Guide (2nd Ed). Department of Water Affairs and Forestry, Department of Health, Pretoria.

World Gazette. (2003). [web:] <http://www.world-gazette.com> January 2004

World Health Organisation. (1996). Guidelines for Drinking Water. (3): Surveillance and control of community water supplies. 2nd Ed. Geneva.

Wright A, Kloppers W and Fricke A. (1993). A hydrological investigation of storm water run-off from the Khayelitsha urban catchment in the False Bay area, South Western Cape. Water Research Commission. WRC Report No. 323/1/93. pp106

Yakub GP, Castric DA, Stadterman-Knauer KL,Tobin MJ, Blazina M, Heineman TN, Yee GY and Frazier L. (2002). Evaluation of colilert and enterolert defined substrate methodology for wastewater applications.

APPENDIX A

MEMBRANE FILTRATION TECHNIQUE

Traditionally the membrane filtration technique is the most frequently employed technique used for the detection and enumeration of microbiological indicator organisms in water. The technique was introduced to water analysts in 1951.

This technique makes it possible for large volumes of water samples to be examined thus increasing sensitivity and reliability while allowing reduction of time, labour, equipment, space and material. The technique involves primary recognition of a presumptive positive result followed by a multi-step process of verification of colonies (Hanko, 2000). Unfortunately, there are several drawbacks associated with this technique, in particular the time taken to confirm positive results which usually takes from two to five days, according to the Environmental Protection Agency (EPA) 2001.

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

The membrane filtration system:

The membrane filtration machine is assembled from the following equipment:

- ◆ Glass funnels which are about 250ml in capacity.
- ◆ Three Millipore® three-place PVC manifolds.
- ◆ Two EDWARDS® 1.5 Two-STAGE 220/240 V 50/60 Hz vacuum/pressure pumps.



- Two one-litre vacuum funnels for trapping moisture before vacuum pumping.
- Clamps to secure funnel on base after loading the filter membrane.
- Silicone rubber tubes for connecting the machine.

Membrane filters

Sterile Millipore® 0.45-µm pore size filter membranes were used. The filter membranes were 47mm in diameter, white and grid marked so that colony counting is facilitated.

Sterilisation

All equipment to be used for filtration was steam-sterilised in an autoclave at 121°C for 30 minutes. Glassware was autoclaved separately, covered in foil. Pipette points were also autoclaved for sterility. To indicate that the autoclave had reached an acceptable temperature of sterility, autoclave tape was used. The tape turned black thus giving an indication that the appropriate temperature for sterility had been reached.

Dry sterilisation for filtering funnels was done in an oven at 180°C for 10-15 minutes. This was also done between each water sample filtered so that cross contamination was avoided during filtration.

Forceps were used for picking up and placing filtered membranes on the selective growth media. The forceps were decontaminated using an open flame for about a minute before every filter membrane handling.

Appendices

Phosphate buffer

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

Sterile working solutions of buffer were made up by adding 1.25mℓ of phosphate solution (34g KH_2PO_4 / 500mℓ distilled water) and 5 millilitres of magnesium chloride solution (81.1g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ / 1000 mℓ distilled water) to 1 litre of reagent grade water.

Dilutions

Dilutions were made up to achieve colony counts of between 20 to 80 colonies per plate as stipulated in Standard Methods (1998). All the water samples were filtered by using three filter membranes per dilution. Samples that were not diluted ranged from 1mℓ, 10mℓ and 100mℓ. When rainy seasons occurred dilutions of up to 10^{-3} were used.

Dilutions were achieved the following manner:

- ◆ An empty covered test tube was autoclaved and sterilised.
- ◆ 9mℓ of sterile phosphate buffer was poured into the sterile test tube. The test tube was flamed before the buffer was poured into it.
- ◆ 1mℓ of the water sample was extracted aseptically with a sterile pipette point and transferred into the 9mℓ of buffer to give a 10^{-1} dilution.
- ◆ 1mℓ from the 10^{-1} dilution was transferred into another 9mℓ test tube of phosphate buffer, thus giving a 10^{-2} dilution.
- ◆ This dilution process was used for making the remainder of the dilutions.

Appendices

- ◆ All the glassware and pipette points that were used for filtration were steam sterilised by being autoclaved at 121°C for 30 minutes.
- ◆ The membrane filters were placed with grid side up, onto the glass support of funnel holder with a sterile forceps.
- ◆ The funnel was thereafter clamped onto the filter base.
- ◆ The sample in the Whirlpack[®] was shaken for a few seconds. About 30 ml of sterile phosphate buffer was poured into the glass funnel and a volume of water sample was then poured into the same funnel.
- ◆ Filtering of the sample was then applied.
- ◆ The glass funnel and clamp were then removed and the filtered membrane filter was lifted with a sterile forceps and placed grid side up onto a petri dish containing the selective growth media.
- ◆ The petri dishes were marked and incubated invertedly Standard Methods, (1998) at the selected incubation temperatures at which microbiological indicator organisms grow (Hanko, 2000), as described in Appendices C and D.

Counting

After incubation was completed for the appropriate period of time, colonies were counted according to prescriptions for each microbiological indicator organism grown on the selective media. Colonies of faecal coliforms and *Escherichia coli* were counted under a microscope.

To achieve reliable statistical quantification, the final count per 100 ml of sample was calculated as follows (Standard Methods, 1998):



Sum of three filters / 3 X

sample size and then by sample

dilute.

The formula was programmed in Microsoft Excel® 2000 spreadsheets and entered as follows:

1. The count from each of the three membrane filters on the plate.
2. The sample size - maximum 1ml for dilutes samples.
3. The dilutions were expressed as 0.1; 0.01; etc.

From this, counts of faecal coliforms and *Escherichia coli* were calculated and expressed as colony-forming units (CFU) per 100 ml.

APPENDIX B

MOST PROBABLE NUMBER: QUANTI-TRAY® - / 2000 COLILERT 18 METHOD

The Quanti-Tray®-Colilert 18 manufactured by IDEXX Laboratories using the Most Probable Number, has recently been gaining increasing popularity amongst water analysts for microbiological indicator organism number detection in water resources (Elmund *et al.*, 1999). This method is less expensive and requires less formal training than historically used methods (Noble *et al.*, 2003). Multiple tubes or wells, with Most Probable Number approach are used for detecting the presence of microbiological indicator organisms like total coliforms and *Escherichia coli* (*E. coli*) in water.

The equipment

- ◆ Sterile plastic Quanti-Tray® consisting of 97 wells
- ◆ Quanti-Tray® heat sealer
- ◆ Incubator 35°C
- ◆ Marker pen
- ◆ Long wavelength UV (366 nm) light viewer
- ◆ Quanti-Tray® / Colilert 18 reference comparator.
- ◆ 100ml measuring cylinders
- ◆ Colilert medium consisting of various salts
- ◆ Orange Quanti-Tray® rubber pad consisting of 97 wells
- ◆ Most probable number (MPN / 100 ml) table

Sterilisation

All equipment to be used was steam-sterilised in an autoclave at 121°C for 30 minutes. Glassware was covered in foil. As an indication that the autoclave had reached the acceptable temperature, an autoclave tape was used. The tape turned black thus giving an indication that the autoclave had reached the acceptable temperature.

Culture media and reagents

Sachets of Quanti-Tray[®] Colilert medium consisting of salts were used for mixing with the water sample.

Diluting

Dilutions were made up to detect colony counts that exceeded 2420 organisms per QuantiTray (IDEXX, 2000). The following dilution series used were: 50/50; 25/75 and 1/99

Dilutions were made up in the following manner:

- 💧 50/50 50 ml of sample and 50 ml of diluent (sterile distilled water) were combined to achieve 100ml which is the limit for using the method.
- 💧 25/75 25 ml of sample and 75 ml of diluent (sterile distilled water) were combined to achieve 100ml which is the limit for using the method.
- 💧 1/99 1ml of sample and 99ml of diluent (sterile distilled water) were combined to achieve 100ml which is the limit for using the method.

Appendices

Analytical method

- At least 20 minutes before processing of water samples the Quanti-Tray® heat sealer was turned on to warm up.
- Sterile measuring cylinders were placed on the table and 100 ml of water sample was poured into them.
- Colilert sachets consisting of the dehydrated reagent medium were then tapped and snapped to open them.
- The reagent medium was then added to the 100 ml of water sample and shaken rigorously for 5 minutes to dissolve the medium. The cylinder was aseptically sealed with petrifilm.
- The Quanti-Tray® was then opened by making sure that the plastic was facing the palm of the hand with the top uppermost.
- The contents of the cylinder were gently poured into the sterile Quanti-Tray® with wells for enumeration of bacteria. Care was taken not to touch the lips of the sterile tray with hands.
- The Quanti-Tray® was then placed onto the orange Quanti-Tray® rubber pad and then mechanically sealed with Quanti-Tray® heat sealer to distribute the sample mixture into all of the wells.
- The sealed Quanti-Tray® was labelled on the back with sample identification and date and then placed in the incubator for the appropriate incubation period time.

Counting

After at least 18 hours of incubation, all positive wells in the retrieved tray were counted using the UV light. The tray was placed under the light and the fluorescing

Appendices



wells counted as positive for which are pale blue in colour when viewed under the UV light.

Calculation of results

The numbers of *Escherichia coli* counted were determined from the Most Probable Number (MPN 100 ml) table provided by the IDEXX manufacturer.

Confirmation

Confirmation was not done on the positive wells produced by the Quanti-Tray® Colilert 18 method. Studies done by Poucher *et al* (1991), who also used other miniaturised liquid MPN techniques based on chromogenic substrates, have shown that the fluorescence, produced by the enzyme reaction in the selective medium used inside the wells, is highly specific for *E. coli*, therefore minimising the possibility of misinterpretation. This was also assumed valid for the Colilert method.

APPENDIX C

M-FC Reagent Medium for detecting faecal coliforms

Faecal coliforms were enumerated by means of the membrane filtration technique (Appendix A), M-Faecal Coliform Agar. Enumeration was done in triplicate on 90 mm petri dishes.

Preparation of M-FC Agar

- ◆ 50 g of the powder was suspended in 1 litre of distilled water.
- ◆ The mixture was shaken to dissolve the powder and boiled until the powder had totally dissolved with no crystals remained.
- ◆ The prepared media was cooled to 50°C and then poured into 90 mm petri dishes.
- ◆ The media does not require autoclaving.
- ◆ Fresh plates, sealed in plastic bags for moisture retention, were stored in the dark and refrigerated at <8°C.

Incubation

The plates were inverted and incubated at 44, 5°C for 24 hours, ± 2 hours.

Identification and Confirmation

Faecal coliform colonies appeared in shades of dark blue (Merck Corporation, 1996). Cultured isolates were confirmed on API[®] 20E (bioMerieux, 1998) as described in Appendix E.

APPENDIX D

CHROMOCULT® COLIFORMEN AGAR for detecting *Escherichia coli*

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

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

- ◆ 26.5 g of the powder was suspended in 1 litre of distilled water.
- ◆ The mixture was boiled in a microwave and gently stirred until the powder was totally dissolved. No crystals or undissolved media were visible. The media does not require autoclaving.
- ◆ The prepared media was cooled to 40- 50°C and the Cefsulodin solution (10 mg in 2 ml of distilled water), an antibiotic, was added to the 1 litre of medium by gently shaking to homogenise. Cefsulodin solution was added to eliminate accompanying flora, especially *Pseudomonas* spp. and *Aeromonas* spp.
- ◆ The prepared media was then poured into 90 mm petri dishes.
- ◆ Fresh plates, sealed in plastic bags for moisture retention, were stored in the dark and refrigerated at <8°C.

Incubation

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

Identification and Confirmation

E. coli colonies appeared in various shades of dark blue to violet (Merck Corporation, 1996). Colonies were confirmed on API® 20E (Appendix E).

APPENDIX E

CONFIRMING COLONIES

Colony verification

False positives are a problem for this type of assessment routine (Jagals, 2000). According to Standard Methods (1998), false positives are non-indicator organisms that are capable of growing on the selective growth medium within the same colour range normally used to identify the true indicator organisms. This often leads to substantial misinterpretation of the actual indicator organism numbers, especially when using the membrane filtration technique. For this study, estimating the false positive faecal coliform and *Escherichia coli* colonies grown on the filter membranes was done by using an analytical profile index procedure and API® 20E identification kits by bioMérieux® (APPENDIX E). Between 12% and 40% of the entire target colonies cultured on various selective growth media were randomly selected for verification (Standard Methods, 1998). This was done by streaking out and growing single colonies from the selective media on plate count agar (Standard Methods, 1998; APPENDIX E). Verification of the selected colonies was not carried out, because there was doubt about the manufacturer's interpretation of single step identification, but rather because there was doubt about the colour interpretation amongst the analysts.

Verifications were not done on the positive wells produced by the QuantiTray® Colilert 18 method. Studies done by Poucher *et al* (1991), who also used other miniaturised liquid MPN techniques based on chromogenic substrates, have shown that the fluorescence, produced by the enzyme reaction in the selective medium used inside the wells, is highly specific for *E. coli*, therefore minimising misinterpretation. This was also assumed to be valid for the Colilert method.

Appendices

API® 20E is a standardised identification system for *Enterobacteriaceae* and other non-fastidious gram-negative rods. The system uses 12 and 20 miniaturised biochemical tests (respectively) with a related database. This system is used to identify a substantial number of species that includes the most important species used in this study.

The plate count agar was used to streak colonies that have been stripped of their colour to be used in the confirmation of colonies.

Preparation of Plate Count Agar

- ◆ 23 g of the powder was suspended in 1 litre of distilled water.
- ◆ The mixture was autoclaved at 121°C for 15 minutes.
- ◆ The prepared media was cooled to 40-50°C.
- ◆ The prepared media was then poured into 90 mm petri dishes.
- ◆ Fresh plates, sealed in plastic bags for moisture retention, were stored in the dark and refrigerated at <8°C.

Incubation

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

Identification

Colourless colonies appeared (Merck Corporation, 1996)

Confirmation

Cultured isolates on the selective growth media were confirmed on API® 20E (bioMerieux, 1998)

Homogenous bacterial suspensions of the selected (and purified) colonies were made according to the prescriptions contained in the manual provided with the commercial identification kit (bioMerieux, 1998).

Inoculation of the strips

The micro-tubes on the prepared strips were filled according to prescription and incubated for 18-24 hours 35-37°C.

Reading the strips

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

Identification

The pattern of each of the reactions obtained was hand-coded, according to the results, into a numerical profile. These numerical profiles were read into the analytical profile index as a number. The index then provided the name of the strips that matched the code.

Quality control (QC)

Several QC tests were done on the various batches of strips acquired. The stock cultures used were obtained from pathological laboratories. The reference organisms used were *Klebsiella pneumoniae pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.

APPENDIX F

STATISTICAL ANALYSES

Characteristics of water resources data

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

1. A lower bound of zero - no negative values are possible.
2. Presence of outliers. These are observations considerably higher or lower than most of the data. This occurs infrequently but regularly. Outliers on the high side are more common in water resources data.
3. Positive skewness, due to 1 and 2. Skewness can be expected when outlying values occur only in one direction. For positive skewness, the mean exceeds more than 50% of data.
4. Non-normal distribution of data, due to items 1-3 above. While many statistical tests assume that data follow a normal distribution, water resources data often do not.
5. Data reported only below or above some threshold (censored data).
6. Seasonal patterns. Values tend to be higher or lower in certain seasons of the year.
7. Dependence on other uncontrolled variables.

Mean of the logs (geometric mean)

The geometric mean is the mean of the logarithms, transformed back to the original unit. In this study, the format of central location was used. The geometric mean is the preferred best estimate of central tendencies of the transformed microbiological data (Standard Methods, 1998).

MEASURE OF SPREAD

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

THE 90TH PERCENTILE

The 90th percentile is used when the analyst wants to make certain predictions about the data. The 90th percentile is an estimate of the range of values within which the sample parameter may be expected to lie. For this study, this compliance level (90th percentile) was selected to indicate what level of risk of infection people are exposed to by the water investigated, by comparing the results obtained to the stipulated water quality guideline limit.

OUTLIERS

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

Outliers generally have one of

- A measurement or recording error.
- An observation from a population not similar to most of the data.
- A rare event from a single population that is quite skewed.

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

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

SAMPLE SIZE

According to Helsel and Hirsch (1995), the data about which a statement or summary is to be made, are called the population or, sometimes, the target population. It is known that it may be physically or financially impossible to collect all the data of concern and therefore a subset of the data, called the sample, is used to represent the entire population. The minimum sample sizes for statistical significant differences were determined before each series of experiments commenced at the various levels and approaches of this study.

One should determine approximately how large the sample size has to be in order to detect an impact or statistical difference at a specified level. The larger the sample size, the greater the power of the relevant test applied (SigmaStat[®] v3.04, 1997; Helsel and Hirsch, 1995). The statistical programme SigmaStat[®] v3.04, (1997) was used to calculate the sample size needed for statistical significance.

Appendices

Equal variance test results display whether or not the data have passed or failed the test of the assumption that the samples were drawn from populations with the same variance (SigmaStat[®] v3.04, 1997). The classic technique for this comparison of data is the analysis of variance (ANOVA) (Helsel and Hirsch, 1995). ANOVA includes a series of parametric tests done under the assumption that the data concerned are normally distributed around the mean with similar variance. For this study, the instances where the data did not pass the test for normality, were by far the majority. Non-parametric testing for variance was employed throughout the study.

Non-parametric tests

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

- ◆ **Kruskal-Wallis ANOVA on Ranks** (based on rank transformation) (Helsel and Hirsch, 1995). This test was selected when three or more groups were compared (Helsel and Hirsch, 1995; Glantz, 1997; SigmaStat[®] v3.04, 1997). It compares results from several different experimental groups that may be affected by a single factor. Rank transformation of data implies that the original data are replaced by ranks, which omits substantial variance and error from the multiple comparison procedure (Helsel and Hirsch, 1995)

The *P value* is the probability of being wrong in concluding that there is a true difference between the groups. This implies falsely rejecting the zero

Appendices



hypotheses. The smaller (0.05), the greater the probability that the results from the samples in the selected data sets are significantly different.

- Multiple comparison tests (MCTs). When more than two methods are compared, the interest is not only whether the methods determined at each point to detect indicator organisms have differed. Therefore, multiple comparison tests (MCTs) were applied where significant differences were encountered. The MCTs, however, are only applied after the H_0 had been rejected (Helsel and Hirsch, 1995). The following MCT's were used:

The Dunn multiple comparison test uses the following parameters:

- P value indicates the difference in the ranks of the group means (median values) being compared. Group means are ranked in order from the highest to the lowest and P is the number of means spanned in the comparisons.
- If the P value is greater than 0.05, it cannot be confidently concluded that there is a statistically significant difference between the means of the two groups compared.

Data presentation

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

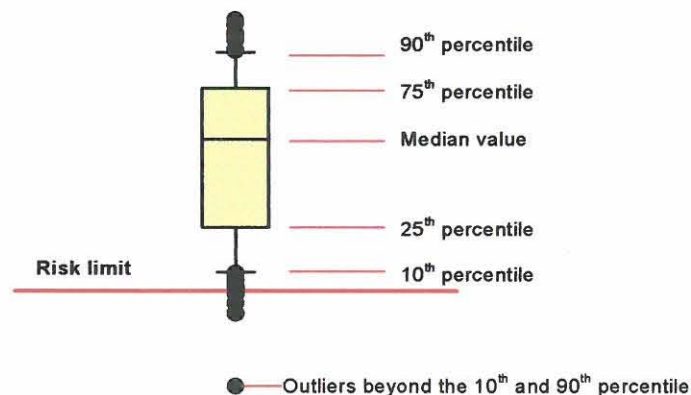
- to provide insight for the analyst into the data under scrutiny; and
- to illustrate important concepts when presenting the results

Methods for detecting indicator organisms at various sampling points were illustrated on the graph types indicated below:

Appendices

Boxplots are used because of their ability to represent certain statistical values in graphic form. According to Helsel and Hirsch (1995), boxplots provide most clearly the visual summaries of the following:

- The centre of the data - (the geometric mean, the yellow line, is the preferred measure of central tendency for the data in the result sections. The median, the black line centre line in the box, is also resistant to the effects of the outliers, and would therefore tend to indicate the more realistic central point in the data).
- The variation or spread (interquartile range (IQR): the box height indicates the spread of the data between the 25th and the 75th percentile). The closer the data are clustered to the median within the IQR, the less variation (more stable) the data have.



- The skewness. This is also referred to as the quartile skew and is represented by the relative size of the box halves. The smaller the upper quartile skews, the



more positive the data a characteristic of water resources data (Helsel and Hirsch, 1995).

- The presence (or absence) of unusual values. The whiskers on the lines protruding above and below the box indicate the 90th and the 10th percentiles. The dot symbols beyond the last percentiles indicate outliers beyond the 90th and 10th percentiles.

APPENDIX G

Table 1 Faecal coliforms on M-FC Agar

Organism species	Unpolluted river water	Polluted urban run-off
Number of colonies selected for confirmation	31	44
True faecal coliforms	25	37
False faecal coliforms	6	7
% POSITIVE Faecal coliforms	81%	84%
%FALSE POSITIVES Faecal coliforms	19%	16%
Confirmed <i>Escherichia coli</i>	24	29
% <i>E coli</i> confirmed of FC	96%	78%
% <i>E coli</i> of counted FC	77%	66%
OTHER FAECAL COLIFORMS Thermotolerant at 41°C - 44.5°C		
<i>Citrobacter koseri</i> (diversus)		1
<i>Klebsiella pneumonia</i>	12	2
<i>Klebsiella oxytoca</i>		3
<i>Pantoea</i> (<i>Enterobacter</i>) <i>agglomerans</i>		1
<i>Klebsiella ornithinolytica</i>		1
Total Faecal coliforms	25	37
FALSE POSITIVES OTHER COLIFORMS Non-thermotolerant(<41°C)		
<i>Escherichia fergusonii</i>	1	
<i>Enterobacter aerogenes</i>	1	
<i>Serratia odorifera</i>	1	3
<i>Serratia liquefaciens</i>		1
Total non-thermotolerants	3	4
OTHER NON-COLIFORMS		
<i>Aeromonas hydrophila</i>	1	
<i>Burkholderia cepacia</i>	1	
<i>Hafnia alvei</i>	1	3
Total non-coliforms	3	3

Table 2 *Escherichia Coli* on Chromocult Coliform Agar

Organism species	Unpolluted river water	Polluted urban run-off
Number of colonies selected for confirmation	8	23
Total <i>E. coli</i>	4	18
Total other than <i>E coli</i>	4	5
% POSITIVE <i>Escherichia coli</i>	50%	78%
% FALSE POSITIVE	50%	22%
OTHER COLIFORMS (Non-<i>E coli</i>)		
<i>Pantoea agglomerans</i>	2	
<i>Entobacter aerogenes</i>		1
<i>Serratia marcescens</i>		1
<i>Serratia liquefaciens</i>		1
Total non- <i>E.coli</i> coliforms	2	3
OTHER NON-COLIFORM GENERA		
<i>Kluyvera</i> spp.		1
<i>Past pneumo</i> / <i>haemol</i>		1
<i>Yersinia perstis</i>	2	
Total other non-coliforms	2	2

Table 3 Indicator organism numbers detected at monitoring sites in the Modder River

	Chromocult	Colilert	<i>E. coli</i> in M-FC	Kruskal Wallis ANOVA on Ranks
MR1	n= 33	n= 15	n= 33	Significant difference (P=0.002)
	Mean of logs= 0.24	Mean of logs= 0.95	Mean of logs= 0.37	H ₀ rejected
	90 th percentile= 1.17	90 th percentile= 1.76	90 th percentile= 1.23	Dunn Multiple comparison test Table 6
MR2	n= 34	n= 16	n=34	Significant difference (P=0.028)
	Mean of logs=1.71	Mean of logs= 1.96	Mean of logs=1.44	H ₀ rejected
	90 th percentile=2.62	90 th percentile= 2.70	90 th percentile=2.39	Dunn Multiple comparison test Table 6
MR3	n= 34	n= 16	n=34	Significant difference (P≤0.001)
	Mean of logs=1.86	Mean of logs= 2.39	Mean of logs=1.60	H ₀ rejected
	90 th percentile=2.64	90 th percentile= 2.67	90 th percentile=2.26	Dunn Multiple comparison test Table 6
MR4	n= 34	n= 16	n=34	Significant difference (P=0.011)
	Mean of logs=1.42	Mean of logs= 1.68	Mean of logs=1.23	H ₀ rejected
	90 th percentile=2.35	90 th percentile= 2.66	90 th percentile=2.00	Dunn Multiple comparison test Table 6
MR5	n= 34	n= 16	n=34	Significant difference (P≤0.001)
	Mean of logs=1.20	Mean of logs= 1.87	Mean of logs=0.99	H ₀ rejected
	90 th percentile=1.88	90 th percentile= 2.28	90 th percentile=1.87	Dunn Multiple comparison test Table 6

Table 4 Indicator organism numbers detected at monitoring sites in the Klein Modder River

	Chromocult	Colilert	<i>E. coli</i> in M-FC	Kruskal Wallis ANOVA on Ranks
KM1	n= 16	n=10	n=16	Significant difference (P=0.013)
	Mean of logs=1.45	Mean of logs= 2.23	Mean of logs=1.42	H ₀ rejected
	90 th percentile=2.33	90 th percentile= 3.10	90 th percentile=2.04	Dunn Multiple comparison test Table 7
KM2	n= 16	n=10	n=16	Significant difference (P=0.025)
	Mean of logs=1.61	Mean of logs= 2.06	Mean of logs=1.32	H ₀ rejected
	90 th percentile=2.83	90 th percentile= 4.06	90 th percentile=2.28	Dunn Multiple comparison test Table 7
KM3	n= 16	n=10	n=16	Significant difference (P=0.002)
	Mean of logs=1.74	Mean of logs= 3.02	Mean of logs=1.70	H ₀ rejected
	90 th percentile=2.81	90 th percentile= 1.70	90 th percentile=2.45	Dunn Multiple comparison test Table 7

Table 5 Indicator organism numbers detected at monitoring site of the Sepane Spruit

	Chromocult	Colilert	<i>E. coli</i> in M-FC	Kruskal Wallis ANOVA on Ranks
TB9	n= 16	n= 8	n= 16	Significant difference (P=0.007)
	Mean of logs= 1.76	Mean of logs= 2.00	Mean of logs= 1.39	H ₀ rejected
	90 th percentile= 2.56	90 th percentile= 2.87	90 th percentile= 2.23	Dunn Multiple comparison test Table 8

Table 6 Multiple comparison test results for monitoring sites in the Modder River

Points	Comparison	MCT P<0.05
MR1	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	Yes
	M-FC vs Chromocult	No
MR2	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	No
	M-FC vs Chromocult	No
MR3	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	Yes
	M-FC vs Chromocult	No
MR4	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	No
	M-FC vs Chromocult	No
MR5	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	Yes
	M-FC vs Chromocult	No

Table 7 Multiple comparison test results for monitoring sites in the Klein Modder River

Points	Comparison	MCT P<0.05
KM1	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	Yes
	M-FC vs Chromocult	No
KM2	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	No
	M-FC vs Chromocult	No
KM3	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	Yes
	M-FC vs Chromocult	No

Table 8 Multiple comparison test results for monitoring site in the Sepane Spruit

Points	Comparison	MCT P<0.05
TB9	Colilert (MPN) vs M-FC	Yes
	Colilert (MPN) vs Chromocult	No
	M-FC vs Chromocult	No

Appendices