# INVESTIGATION OF FAECAL POLLUTION AND OCCURRENCE OF ANTIBIOTIC RESISTANT BACTERIA AS A FUNCTION OF A CHANGED ENVIRONMENT

by

**Monwabisi Jonathan Pantshwa (B.Sc)**

**Submitted in partial fulfilment of requirements for the degree of**

**MASTER OF ENVIRONMENTAL SCIENCE (M.ENV.SC),**

**School of Environmental Science and Development,**

**North-West University: Potchefstroom Campus**

**Supervisor**
Prof. C.C. Bezuidenhout
**Co-supervisor**
Mrs A.M. van der Walt

**Date Submitted**

**DECEMBER 2006**

B.Sc

Department of Life Sciences

Faculty of Science

University of South Africa

2003

# DECLARATION

I declare that the dissertation for the degree of Master of Environmental Science (M.Env.Sc) at the North-West University: Potchefstroom Campus hereby submitted, has not been submitted by me for a degree at this or another University, that it is my own work in design and execution, and that all material contained herein has been duly acknowledged.

.......................................                …………………………………………

**Monwabisi Jonathan Pantshwa**                **Date**

## ACKNOWLEDGEMENTS

I would like to express my appreciation and my sincere gratitude to the following people. This research work would not be possible without their help and support.

First and foremost God for making the research work possible and giving me the strength to complete it. My supervisor, Prof Carlos Bezuidenhout, thank you for your supervision, guidance and advice academically and socially during my research and compilation of this dissertation. My co-supervisor, Mrs AM. van der Walt for her motivation. All the personnel and my fellow post-graduate students in the School of Environmental Science and Development: Microbiology for their support and encouragement during the entire study period. Thank you to my best friend Tshiamo, for her support, friendship and encouragement. My mother, Nozizwe Nomvuyo for her love, and her "A!!! Jongihlanga", sister Cynthia and Nceba thank you for being there for me.

The National Research Foundation of South Africa and the North-West University for their financial support during this study, is also acknowledged. Finally I would like to say the words of appreciation to all these people quoting from the words of Sir Isaac Newton "If I have seen further than any man that is because I have stood on the shoulders of the Giants".

# ABSTRACT

Worldwide, rapid industrialization and urbanization results in excessive release of pollutants into the water resources and the decline in water quality of rivers passing through these urban areas is well documented. Few studies have been conducted to assess physico-chemical and microbial quality of fresh water resources passing through urban areas in South Africa. Currently, not enough is known about the physico-chemical and microbial quality of the water resources in the North-West Province. However, human disturbances resulting from increasing urbanization in this Province is causing faecal pollution of the aquatic environments and ultimately degradation of stream biological integrity. A motivation for this study was the increasing concern of the possible link between faecal pollution gradients due to urbanization and development of bacterial resistance to antimicrobial agents. Such a study has not been conducted before. The aim of this study was to investigate the levels of faecal pollution and occurrence of antibiotic resistant bacteria in the Mooi River system as a function of a changed environment. Defined urbanization gradients were used as focal points. Eight sites along the Mooi River system were selected and monitored monthly for 1 year. Three samples per site were collected from the pre-determined sites along the Mooi River system from the Klerkskraal Dam to the North (site1) and several points along Mooi River passing through Potchefstroom to points on the southern side of Potchefstroom before Mooi River enters the Vaal River. River water samples were subjected to physico-chemical analyses and faecal indicator bacterial levels were determined. Faecal coliforms to enteroccoci levels were used to determine the ratio between these groups. Results indicated seasonal and locational variation in most of the physico-chemical parameters and faecal indicators studied. Rainfall was an important factor which strongly influenced the characteristics of these parameters. Also temperature, pH and rainfall influenced the elevated levels of the microbiological indicators observed. High levels of the faecal indicator bacteria were observed in the Potchefstroom urban area when compared to upstream and downstream river segments. Levels of heterotrophic plate count bacteria were such that no marginal and log differences were observed or enumerated on media without and with ampicillin. Results of faecal coliform to enteroccoci ratio suggested that non-human sources contributed greater towards faecal pollution. River water isolates of faecal coliform and enteroccoci from the Potchefstroom sites exhibited resistance to multiple antibiotics. More than 60% of enteroccoci were resistant to at least 4 antibiotics and between 60-80% of the faecal coliform were resistance to 6 antibiotics. Some isolates were resistant to as many as 10 antibiotics. Among the 6-group MAR indices, highest indices were indicated for the Potchefstroom urban area (0.32 for faecal coliform and 0.28 for enteroccoci). Cluster diagrams based on antibiotic inhibition zone diameter data were constructed. The purpose was to establish whether there were isolates from different sites with similar antibiotic exposure histories. Faecal coliform cluster analysis revealed patterns of association between Potchefstroom, downstream and upstream isolates. Enteroccoci cluster analysis could not clearly resolve differences between samples from different sources. However, urban-rural gradients were recognized in terms of faecal indicator bacteria such total coliform, faecal coliforms and enterococci and also in terms of MAR index.

The antibiotic resistance technique used in this study proved a valuable tool to study impacts of urbanization on associated water resources. It is however advised that the study period be extended over a two year period in order to gain sufficient data, and also because microorganisms show seasonal fluctuations with respect to numbers and species.

# OPSOMMING

Die vinnige tempo van industrialisasie en verstedeliking het wêreldwyd tot gevolg dat oormatige hoeveelhede besoedeling stowwe in waterbronne vrygestel word en die agteruitgang van watergehalte van riviere wat deur sulke stedelike gebiede loop, is goed gedokumenteer. Min studies is in Suid Afrika uitgevoer om die fisies-chemiese en mikrobiologiese gehalte van varswater wat deur stedelike gebiede beweeg, te bepaal. Daar is huidig te min bekend oor die fisies-chemiese en mikrobiologiese gehalte van waterbronne in die Noordwes Provinsie. Menslike versteuring as gevolg van toenemende verstedeliking in hierdie provinsie veroorsaak egter fekale besoedeling van water omgewings en uiteindelik die degradering van stroom biologiese integriteit. 'n Motivering vir hierdie studie was toenemende besorgdheid oor 'n moontlike skakel tussen fekale besoedelingsgradiënte as gevolg van besoedeling en die ontwikkeling van bakteriële weerstandbiedendheid teen antimikrobiese middels. Sodanige studie is nog nie voorheen uitgevoer nie. Die doel van die studie om die vlakke van fekale besoedeling en voorkoms van antibiotikum weerstandbiedende bakterieë in die Mooirivier systeem te ondersoek as 'n funksie van 'n veranderde omgewing. Gedefinieerde verstedelikingsgradiënte is as fokale punte gebruik. Agt terreine is langs die Mooirivier sisteem gekies en vir een jaar maandeliks gemoniteer. Drie monsters per terrein is by elkeen van die voorafbepaalde terreine langs die Mooirivier geneem, vanaf Klerkskraaldam in die noordelike rigting (terrein 1) en verskeie punte al langs die Mooirivier waar die deur Potchefstroom gaan tot by punte aan die suidekant van Potchefstroom voordat die Mooirivier in die Vaalrivier inloop. Rivierwater monsters is aan fisies-chemiese ontleding onderwerp en vlakke van fekale indikator bakterieë bepaal. Vlakke van fekale kolivormige en enterokokke is gebruik om die verhouding tussen hierdie groepe te bepaal. Resultate het seisoen- en lokaliteitvariasie vir meeste van die fisies-chemiese veranderlikes en fekale indikatore wat bestudeer is, getoon. Reënval was 'n belangrike

faktor wat eienskappe van veranderlikes sterk beïnvloed het. Temperatuur, pH en temperatuur het ook die verhoogde vlakke van mikrobiologiese indikatore wat waargeneem is, beïnvloed. Hoë vlakke van fekale indikatorbakterië is in die Potchefstroom se stedelike gebied waargeneem in vergelyking met stroom-op en stroom-af riviersegmente. Vlakke van heterotrofe bakteriële plaattellings was sodanig dat geen marginale of logaritmiese verskille waargeneem en getel is op mediums met en sonder ampisillien nie. Die resultate van fekale kolivormige tot enterokokke verhouding dui op 'n groter bydrae vanaf nie-menslike bronne tot fekale besoedeling. Rivierwater isolate van fekale kolivormige en enterokokke vanaf Potchefstroom terreine het weerstandbiedendheid teen veelvoudige antibiotikums getoon. Meer as 60 % van die enterokokke was weerstandbiedend teen ten minste 4 antibiotikums en tussen 60-80 % van die fekale kolivormiges was weerstandbiedend teen 6 antibiotikums. Sommige isolate was weerstandbiedend teen soveel as 10 antibiotikums. Die hoogste van die 6-groep MAW indekse is aangedui vir die Potchefstroom stedelike gebied (0.32 vir fekale kolivormiges and 0.28 vir enterokokke). Bondeldiagramme gebaseer op data van antibiotikum inhibisie sone deursneë is gekonstrueer. Die doel hiervan was om vas te stel of isolate van verskillende terreine soortgelyke geskiedenis van antibiotikum blootstelling vertoon. Ontleding van fekale kolivormige bondeldiagramme het patrone van assosiasie tussen isolate van Potchefstroom, stroom-op en stroom-af aan die lig gebring. Ontleding van enterokokke bondeldiagramme kon nie verskille tussen monsters van verskillende bronne duidelik uitwys nie. Stedelik-landelike gradiënte is egter waargeneem in terme van fekale indikator bakterieë soos kolivormiges, fekale kolivormiges en enterokokke en ook in terme van die MAR indeks.

Die antibiotikumweerstandbiedenheidstegniek wat in hierdie studie gebruik is, blyk 'n waardevolle instrument te wees om impakte van verstedeliking op geasosieerde waterbronne te bestudeer. Daar word egter aanbeveel dat die studie periode verleng word na twee jaar ten einde voldoende data te bekom en ook omdat mikroörganismes seisonale fluktuasies ten opsigte van getalle en spesies samestelling vertoon.

# TABLE OF CONTENT


**DECLARATION** .......... iii

**ACKNOWLEDGEMENTS** .......... iv

**ABSTRACT** .......... v

**OPSOMMING** .......... vii

**TABLE OF CONTENT** .......... x

**LIST OF FIGURES** .......... xiv

**LIST OF TABLES** .......... xvi

**CHAPTER 1** .......... 1

**INTRODUCTION** .......... 1

1.1 GENERAL INTRODUCTION AND PROBLEM STATEMENT .......... 1

1.2 RESEARCH AIM AND OBJECTIVES .......... 3

**CHAPTER 2** .......... 4

**LITERATURE REVIEW** .......... 4

2.1 ECOSYSTEM STRUCTURE AND FUNCTION ALONG URBAN-RURAL GRADIENTS .......... 4

2.2 EFFECTS OF URBANIZATION ON WATER QUALITY .......... 5

2.3 SOURCES OF FAECAL POLLUTION IN URBAN AREAS .......... 9

2.3.1 Population growth .......... 9

2.3.2 Urban runoff and sewage overflows .......... 10

2.3.3 Local weather patterns .......... 10

2.4 SOURCES OF FAECAL POLLUTION IN RURAL AREAS .......... 11

2.5 REGULATIONS, POLICIES, SUSTAINABLE DEVELOPMENT TO MANAGE RIVER WATER QUALITY ........ 11
2.5.1 Source directed control measures ........ 12
2.6 MICROBIAL INDICATORS OF FAECAL POLLUTION ........ 13
2.6.1 Faecal pollution ........ 13
2.6.2 Microbial indicators ........ 15
(a) Heterotrophic bacteria ........ 16
(b) Total coliforms bacteria ........ 16
(c) Faecal coliform bacteria ........ 16
(d) Enterococci ........ 17
2.7 TOOLS FOR TRACKING FAECAL POLLUTION ........ 18
2.7.1 Differentiation of faecal pollution from human and animal origin ........ 18
2.7.3 Antibiotic resistance and multiple antibiotic resistance ........ 19
2.8 OTHER TECHNIQUES THAT CAN BE USED FOR MICROBIAL SOURCE TRACKING. ........ 20
2.9 SUMMARY ........ 22
**CHAPTER 3 ........ 23**
**MATERIALS AND METHODS ........ 23**
3.1 SAMPLING AREA ........ 23
3.2 SITE DESCRIPTION AND LAND USE ........ 25
3.3 SAMPLE COLLECTION STRATEGY and PHYSICO-CHEMICAL ANALYSIS . 29
3.3.1 Classifications of sites ........ 29
3.4 MICROBIOLOGICAL ANALYSIS ........ 30
3.4.1 Sampling media ........ 30

3.4.2 Assay for levels of bacterial faecal indicators ..... 30
(a) Heterotrophic plate count ..... 30
(b) Faecal indicator bacterial ..... 30
3.4.3 Purification of faecal indicator bacteria ..... 31
3.5 ANTIBIOTIC SUSCEPTIBILITY ..... 31
3.5.1 Interpretation of inhibition zone diameter ..... 32
3.5.2 Multiple antibiotic resistance (MAR) index ..... 32
3.6 STATISTICAL ANALYSIS ..... 33
**CHAPTER 4 ..... 34**
**RESULTS ..... 34**
4.1 PHYSICO-CHEMICAL PARAMETERS AND INFLUENCE OF LOCAL RAINFALL EVENTS ..... 34
4.1.1 Temperature and pH ..... 35
4.1.2 Total dissolved solids (TDS) and eletro-conductivity (EC) ..... 36
4.1.3 Dissolved oxygen (DO) and chemical oxygen demand (COD) ..... 37
4.1.4 Statistical analysis of the physico-chemical parameter relationships ..... 39
4.2 MICROBIOLOGICAL analysis of the water samples from the Mooi river system ... 39
4.2.1 Faecal coliforms and enteroccoci bacterial levels ..... 40
4.2.2 Overall heterotrophic plate count (HPC) and total coliforms (TC) bacteria ..... 43
4.2.3 Faecal coliform (FC)/faecal enteroccoci (FE) ratio ..... 47
4.2.4 Statistical analysis of the total coliforms and faecal coliform to enteroccoci ratio47
4.3 ANTIBIOTIC RESISTANCE ANALYSIS AMONG FAECAL COLIFORMS AND ENTEROCCOCI ISOLATES ..... 49
4.3.1 Antibiotic resistance patterns ..... 49

4.3.2 MAR phenotypes of faecal coliforms and enteroccoci from the river water ........ 50

4.3.3 Cluster analysis ........ 54

(a) Faecal coliform cluster analysis ........ 54

(b) Enteroccoci cluster analysis ........ 57

4.3.4 Multiple antibiotic resistance (MAR) index ........ 58

4.4 SUMMARY OF RESULTS ........ 59

**CHAPTER 5 ........ 61**

**DISCUSSION AND CONCLUSIONS ........ 61**

5.1 INTRODUCTION ........ 61

5.2 LEVELS OF PHYSICO-CHEMICAL PARAMETERS ........ 62

5.3 MICROBIOLOGICAL OBSERVATIONS ........ 66

5.3.1 Faecal indicator bacterial levels ........ 67

5.3.2 Faecal coliform to faecal enteroccoci ratio ........ 69

5.3.3 Antibiotic resistance and multiple resistance among faecal coliforms and enteroccoci isolates ........ 70

5.3.4 River health categorization ........ 72

5.4 CONCLUSION ........ 74

5.5 RECOMMENDATIONS FOR FURTHER STUDY ........ 75

**REFERENCES ........ 77**

**APPENDIX A ........ 90**

**APPENDIX B ........ 108**

**APPENDIX C ........ 115**

**APPENDIX D ........ 139**

# LIST OF FIGURES

**Figure 2.1:** A composite, integrated model illustrating the effects of urbanization on ecological phenomena (after Pickett *et al.*, 1997)........ 5

**Figure 2.2:** The Mooi River system (North-West Province, South Africa) indicating the twelve sampling sites used in the study (De la Rey *et al.*, 2004). ........ 6

**Figure 2.3:** Species and relationship among indicator organisms (after Kim *et al.*, 2005)........ 15

**Figure 3.1:** A map of the Mooi River catchment, indicating the eight sampling sites (shown by green and red dots), three reservoirs and associated towns and cities (IWQS and Kempster, 1999)........ 24

**Figure 4.1:** Relationship between average rainfall, temperature and pH data collected from April 2005 to March 06........ 35

**Figure 4.2:** Relationship between rainfall, TDS and electro-conductivity values for wet and dry season. ........ 37

**Figure 4.3:** The total monthly rainfall, average dissolved oxygen and average chemical oxygen demand in the Mooi River system........ 38

**Figure 4.4 (a):** Seasonal concentrations of faecal coliform bacteria collected in the Mooi River during the dry and wet season. ........ 40

**Figure 4.4 (b):** Seasonal concentrations of faecal coliform bacteria collected in the Mooi River during the dry and wet season. ........ 41

**Figure 4.5:** Seasonal concentrations with and without antibiotic of enteroccoci bacteria data collected in the Mooi River during the dry and wet season. ........ 42

**Figure 4.6:** Examples showing the diversity of ampicillin resistant heterotrophic plate count bacteria isolated from the Mooi River........ 44

**Figure 4.7:** Antibiotic resistant patterns of faecal coliform (a) and enteroccoci (b) isolates from segments upstream, Potchefstroom and downstream. ........................................................ 49

**Figure 4.8:** Dendograms showing relatedness of faecal coliforms isolated from the Mooi river system (upstream, Potchefstroom and downstream segments). ........................................... 55

**Figure 4.9:** Dendograms showing relatedness of enteroccoci isolated from the Mooi river system (upstream, Potchefstroom and downstream segments). ....................................................... 57

# LIST OF TABLES

**Table 2.1:** Physico-chemical variables for different sites (De la Rey *et al.*, 2004) ....................... 8

**Table 2.2:** Ecological river health categorization and water use of the sampled sites in the Mooi River (DWAF, 1999) ....................... 9

**Table 3.1:** Site, monitoring point names with positional data, land use intensity and ecological descriptions. The latter were done using the criteria of IWQS (1999), Kempster (1999) and De la Rey *et al.* (2004) ....................... 26

**Table 3.2:** A table indicating the details of antibiotics that were used in this study. The concentration used as well as the inhibition zone measurements (in mm) that were considered resistant (R); intermediate resistant (I) and susceptible (S) are shown and were according to NCCLS (1999). The abbreviations (abbrev.) were according to the 2005 instructions to the authors for the Journal of Clinical Microbiology (http://jcm.asm.org/misc/itoa.pdf) ....................... 32

**Table 4.1:** Seasonal levels of heterotrophic plate count and total coliform bacteria collected in the Mooi River during the dry and wet season (April 2005 to March 2006). Values are averages of triplicates and E+20 = $10^{20}$ ....................... 46

**Table 4.2:** Faecal coliform/faecal enteroccoci ratio without ampicillin over a one year period (April 2005 to March 2006) ....................... 48

**Table 4.3**: Faecal coliform/faecal enteroccoci ratio on ampicillin containing plates over a one year period ....................... 48

**Table 4.4:** Most prevalent antibiotic resistance patterns for individual faecal coliform isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number

of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source. 52

**Table 4.5:** Most prevalent antibiotic resistance patterns for individual enteroccoci isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source. 53

**Table 4.6:** Table indicating results of analysis of clusters from Figure 4.9. The number (N) and the percentage (%) of faecal coliform isolates from all the sites are indicated. 56

**Table 4.7:** Table indicating results of cluster analysis from Figure 4.9. The number (N) and the Percentage (%) of enteroccoci isolates from all the sites are indicated 58

**Table 4.8:** Multiple antibiotic resistance (MAR) indices for faecal coliform and enteroccoci isolates per river segment. 58

**ble 2.1:** Physico-chemical variables for different sites (De la Rey *et al.*, 2004) 8

**Table 2.2:** Ecological river health categorization and water use of the sampled sites in the Mooi River (DWAF, 1999) 9

**Table 3.1:** Site, monitoring point names with positional data, land use intensity and ecological descriptions. The latter were done using the criteria of IWQS, 1999; Kempster, 1999; De la Rey *et al.*, 2004. 26

**Table 3.2:** A table indicating the details of antibiotics that were used in this study. The concentration used as well as the inhibition zone measurements (in mm) that were considered resistant (R); intermediate resistant (I) and susceptible (S) are shown and were according to NCCLS (1999). The abbreviations (abbrev.) were according to the 2005 instructions to the authors for the Journal of Clinical Microbiology (http://jcm.asm.org/misc/itoa.pdf) 32

**Table 4.1:** Seasonal levels of heterotrophic plate count and total coliform bacteria collected in the Mooi River during the dry and wet season (April 2005 to March 2006). Values are averages of triplicates and E+20 = $10^{20}$ ........ 46

**Table 4.2:** Faecal coliform/faecal enteroccoci ratio without ampicillin over a one year period (April 2005 to March 2006) ........ 48

**Table 4.3**: Faecal coliform/faecal enteroccoci ratio on ampicillin containing plates over a one year period ........ 48

**Table 4.4:** Most prevalent antibiotic resistance patterns for individual faecal coliform isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source. ........ 52

**Table 4.5:** Most prevalent antibiotic resistance patterns for individual enteroccoci isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source. ........ 53

**Table 4.6:** Table indicating results of analysis of clusters from Figure 4.9. The number (N) and the percentage (%) of faecal coliform isolates from all the sites are indicated. ........ 56

**Table 4.7:** Table indicating results of cluster analysis from Figure 4.9. The number (N) and the Percentage (%) of enteroccoci isolates from all the sites are indicated ........ 58

**Table 4.8:** Multiple antibiotic resistance (MAR) indices for faecal coliform and enteroccoci isolates per river segment. ........ 58

# CHAPTER 1

# INTRODUCTION

## 1.1 GENERAL INTRODUCTION AND PROBLEM STATEMENT

Anthropogenic activities resulting from increased urbanization elevates degradation of stream environmental quality and ultimately biological integrity (Holland *et al.*, 2004). Development in urban areas worldwide has caused increased point and non-point runoff pollution in many watersheds. Pollution as a consequence of urbanization, therefore, has important implications for ecosystem dynamics (Choi *et al.*, 2003). In the urban environment, sustainable development should involve management strategies that will reduce potential for environmental degradation of aquatic environments (Coombes, 2006). Development approval agencies should thus consider both the economic viability on the one hand as well as the ecological base-line data and potential ecological impacts, on the other hand.

Although rivers are resilient to moderate changes, extreme conditions may threaten the ability of a river to support aquatic and riparian life (Coombes, 2006). Worldwide, rapid industrialization and urbanization results in excessive release of pollutants into the waterways and there is a well documented decline in water quality of rivers passing through these urban areas (Cheung *et al.*, 2003). The Mooi River passes through the Potchefstroom urban area and increased development in the vicinity of the river may negatively impact the water quality, but also the self-purification capacity of the river. Some physico-chemical base-line data for the Mooi River system is available (De la Rey *et al.*, 2004). According to the previous findings based on data for ecological and physico-chemical parameters, generally the water quality of the Mooi River is poor and the water can only be used for agricultural and recreational purposes but not suitable for domestic purposes (DWAF, 1999; De la Rey *et al.*, 2004). However, a study on the Mooi River system in the context of a changing urban environment, focussing on faecal pollution has not been conducted. Such base-line data may be valuable in future environmental impact studies.

Faecal pollution from non-human (pets, livestock and wildlife) and human sources is often one of the major factors contributing to the degradation of water quality in developing as well as developed countries (Harwood *et al.*, 2000). Contamination of soil and surface waters with faecal material enhances the risk of human exposure to pathogenic enteric bacteria of intestinal origin (Shehane and Harwood, 2005). Water pollution causes several diseases like typhoid, cholera, bacterial and amoebic dysentery, enteritis, poliomyelitis, infectious hepatitis (jaundice) and schistosomiasis (Mallon *et al.*, 2002).

Therefore, water quality monitoring and assessments are of paramount importance to identify the river confluence vulnerable to the pollution impacts of urbanization. Enterococci, faecal coliform and total coliform counts are used as indices for measuring the quality of surface water (Holland *et al.*, 2004). However, in recent years antibiotic resistant bacteria have become invaluable as tools tracking and detecting the source of faecal pollution (Choi *et al.*, 2003). A technique, called antibiotic resistance analysis (ARA) is frequently used in aquatic studies to evaluate water quality as well as tracking pollution sources (Sankaramakrishnan and Guo, 2005). ARA is based on the premise that the differential exposure of bacteria to antimicrobial chemicals may lead to differential tolerance of bacterial populations. Bacteria from different antibiotic exposure histories will thus have different antibiotic resistance/susceptibility patterns and these could be grouped using clustering methods (Schwarz *et al.*, 2003). Consequently, the question posed is, to what extent has urbanization made an impact in the development of antibiotic resistance along the Mooi River continuum.

## 1.2 RESEARCH AIM AND OBJECTIVES

The aim of this study was to investigate the levels of faecal pollution and occurrence of antibiotic resistant bacteria in the Mooi River system as a function of a changed environment. Defined urbanization gradients will be used as focal points.

Objectives were:

(i) To determine the physico-chemical characteristics and levels of faecal indicator bacteria at various points in the Mooi River system, over a one year period.

(ii) To differentiate and compare the levels of faecal pollution at urban and rural sampling points.

(iii) To determine the associated antibiotic resistance/susceptibility profiles of these isolates.

(iv) To use bacterial counts and antibiotic resistant patterns to determine if the faecal pollution gradient exist along the Mooi River system.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 ECOSYSTEM STRUCTURE AND FUNCTION ALONG URBAN-RURAL GRADIENTS

The use of urban-rural gradients has proven to be an excellent tool for studying emergent human ecological activities across urbanizing landscapes (Hans and McDonnell, 2006). Anthropogenic activities change natural ecosystem characteristics along an aquatic continuum from urban (almost entirely human-made) to rural ecosystem types (those with the least human modification) (Kaye *et al.*, 2006). Urban expansion can be viewed as a complex environmental gradient with land use change in space determining, in part, the steepiness of the gradient in aquatic system structure and function. Interactions within the aquatic systems and between the environmental gradient and the aquatic systems affect the distribution and the behaviour of systems along this gradient (Tang *et al.*, 2005).

An integrated model framework of investigation of faecal pollution due to human activities along urban-rural gradients can be designed that accounts for the integral components of urbanization, and the resultant effects on ecosystem functioning (Ticehurst *et al.*, 2006) as shown in Figure 2.1 below. According to the integrated model, the component factors of urbanization, the biotic as well as aquatic environmental effects are divisible into: (i) physical structure such as impervious urban surfaces, (ii) demographic variables, such as density of people and (iii) landscape measures, such as mean patch size or fractal dimension. The physical and chemical environment and the dynamics of demographic structure, such as density of people and communities determine the levels of faecal pollution caused by urbanization (Theobald, 2005).

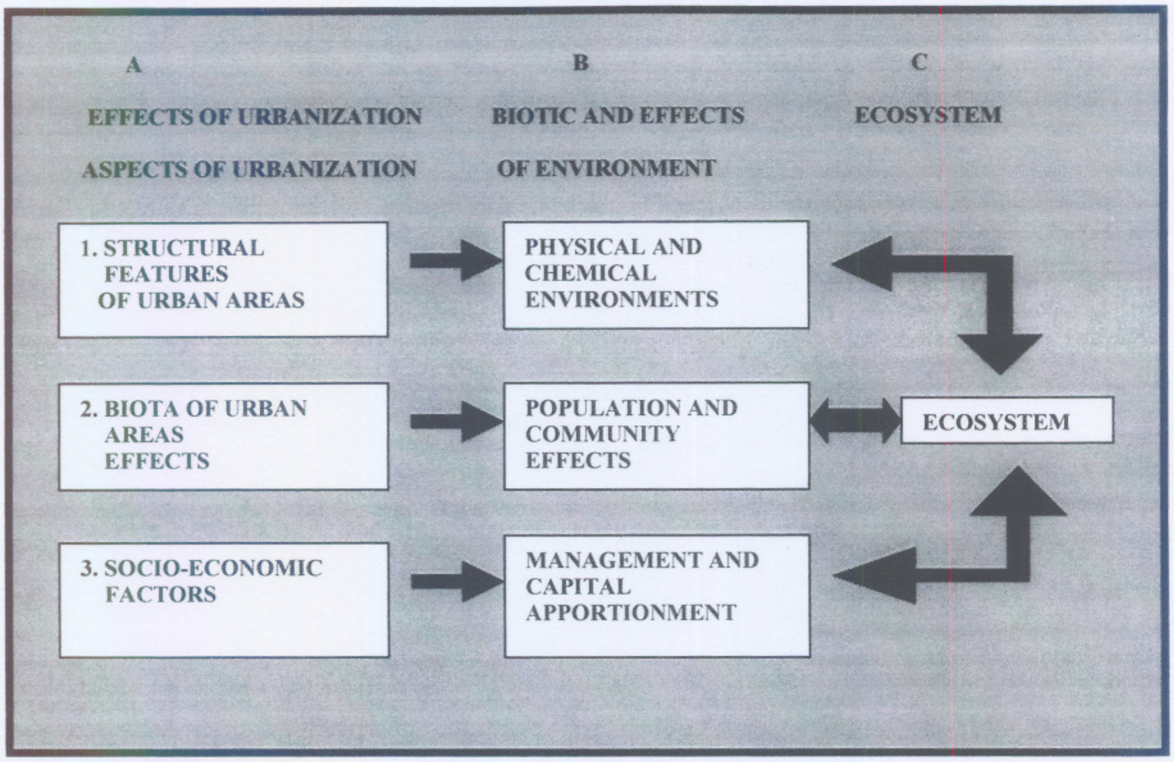



**Figure 2.1:** A composite, integrated model illustrating the effects of urbanization on ecological phenomena (after Pickett *et al.*, 1997).

## 2.2 EFFECTS OF URBANIZATION ON WATER QUALITY

Because ecological processes are interrelated with the landscape, the various elements resulting from urbanization have significant implications for the aquatic ecosystem functioning. The transformation of land cover favours microorganisms that are more capable of colonization and adaptation to the new conditions (Blair, 1996). Many ecological changes caused by the cities on their immediate aquatic environments are obvious and extreme (Coombes, 2006). Although ecological impacts of urban development often seem to be local, urbanization also causes environmental changes at larger scales (Marzluff *et al.,* 2001). Urbanization is the driving force altering local and regional hydrology and increasing non-point source pollution (Tang *et al.,* 2005).

The urban expansion rarely occurred homogeneous across the entire Mooi River system. In a study conducted in the Mooi River during May 2003 with the aim of determining the possible application value of diatoms as indicators of general water quality, the lowest water quality was observed in the Wasgoed Spruit (De la Rey *et al.*, 2004). In that study twelve sampling sites were selected and considered to represent a range of water quality and the impact of some tributaries entering the Mooi River similar to the present research study. The predetermine sites (Figure 2.2) extended from below Klerkskraal Dam to Potchefstroom.

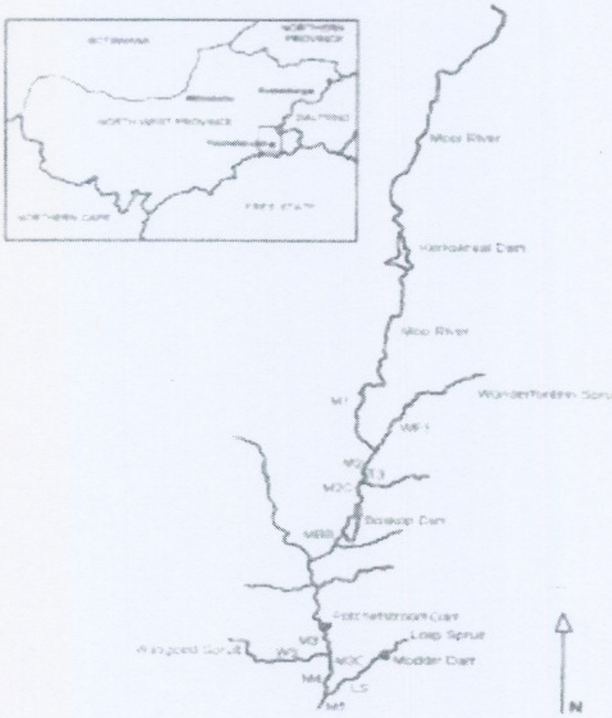



**Figure 2.2:** The Mooi River system (North-West Province, South Africa) indicating the twelve sampling sites used in the study (De la Rey *et al.*, 2004). .

Table 2.1 presents the data of the general water quality variables for different sites which were selected and sampled in the Mooi River system at the same period and some at the same sites as the present study (De la Rey *et al.*, 2004). For comparison purposes, results of physico-chemical parameters of the upstream and downstream sites in Table 2.1 of this previous study will be compared to physico-chemical parameters of the present study in the discussion of results in Chapter 5 of the present study. The comparison of these results is important for the evaluation of levels of faecal pollution and also in detecting the changing conditions of the river with time which are described in terms of ecological health categorization as shown in Table 2.2.

**Table 2.1:** Physico-chemical variables for different sites (De la Rey *et al.*, 2004)

| Site code | Temp | EC | pH | Turbidity | Total P | COD |
|---|---|---|---|---|---|---|
| **M1** | 16.12 | 408 | 7.67 | 3.2 | 0.18 | 6 |
| **WFS** | 12.11 | 617 | 7.19 | 2 | 0.14 | 46 |
| **M2** | 13.1 | 538 | 7.42 | 1.4 | 0.23 | 43 |
| **T3** | 20.94 | 668 | 7.3 | 0.6 | 0.22 | 6 |
| **M2C** | 13.41 | 537 | 7.33 | 1.5 | 0.23 | 25 |
| **MBB** | 12.49 | 506 | 7.34 | 2.2 | 0.17 | 104 |
| **M3** | 15.25 | 518 | 7.37 | 4.87 | 0.22 | 27 |
| **Average Upstream** | 14.77 | 541.7 | 7.37 | 2.252 | 0.1985 | 36.71 |
| | | | | | | |
| **WS** | 12.65 | 1678 | 6.78 | 8.08 | 0.35 | 37 |
| **M3C** | 15.53 | 540 | 7.74 | 4.8 | 0.26 | 6 |
| **M4** | 15.43 | 541 | 7 | 12.4 | 0.15 | 6 |
| **LS** | 14.16 | 568 | 7.55 | 20.2 | 0.28 | 31 |
| **M5** | 16.2 | 554 | 7.23 | 20.2 | 0.17 | 6 |
| **Average Potch** | 14.794 | 776.2 | 7.26 | 13.136 | 0.242 | 17.2 |

**M1** = Klerkskraal dam, **WFS**= Wonderfontein Spruit, **T3** = an unnamed tributary near Boskop Dam. **WS**= Wasgoed Spruit. **LS** = Loop Spruit , **M5**= Downstream the Potchefstroom Prozesky Bird Sanctuary

Ecological categorization of the state of the river system is described in terms of a health category ranging between good and poor water quality, as described in Table 2.2.

**Table 2.2:** Ecological river health categorization and water use of the sampled sites in the Mooi River (DWAF, 1999)

| RIVER HEALTH CATEGORIZATION | | | |
|---|---|---|---|
| **Site no's & river segment** | **Category** | **Description** | **Water Use** |
| **Upstream Potchefstroom** | Good Water Quality | Ecosystem essentially in good state, biodiversity largely intact. | Agricultural and recreational use |
| **Potchefstroom** | Poor Water Quality | Mainly tolerant species present or alien's species invasion, disrupted population dynamics, species are often diseased. | Agricultural further downstream |

## 2.3 SOURCES OF FAECAL POLLUTION IN URBAN AREAS

The key issues that relate water quality to urban development are population growth factors that can cause urban runoff and sewage overflows. These must be taken into consideration because are major point sources of faecal pollution in urban areas (Parveen, *et al.*, 1997).

### 2.3.1 Population growth

South Africa has a fairly evenly distributed urban to rural population, with 53,7% of its population estimated to be living within an urban environment. However, 34,9% of people in the North-West Province are urban dwellers with most of the population (65,1%) living in the rural areas (Statistics South Africa, 2001). Due to poor access to basic needs and services, more people migrate to urban areas. This implies an increased requirement for support facilities: housing developments, roads, shopping areas, and commercial and industrial facilities. The increase in the impervious surfaces in urban areas could lead to the degradation of natural water resources, which in turn make it less able to support human needs (USGS, 2006). Rapid urbanization is expected in Potchefstroom and elsewhere in this Province in future (Cilliers *et al.*, 2003).

### 2.3.2 Urban runoff and sewage overflows

Excessive urban runoff can contain high levels of contaminants, such as oil and waste material, which often goes directly into streams. Many sewer lines are constructed next to streams to take advantage of the continuous natural gradual slopes of stream valleys. Blockages, inadequate carrying capacity, leaking pipes, and power outages at pumping stations often lead to sewage overflows into nearby streams. These blockages frequently occur and are not attended to in poor settlements of urban areas. The inadequate sanitation due the lack of political will, shortage of trained staff, financial considerations results in the degradation of the environment. In the absence of adequate and affordable shelter; safe and affordable drinking water and appropriate management systems for domestic and industrial waste, human settlements become environmentally unsustainable (USGS, 2006). Sanitary sewage overflow is a common problem that causes water pollution in urban areas. Sanitary sewer overflows occur when sewer pipes clog or pumping stations break down. Raw sewage overflows from manholes and leaking pipes into nearby streams rather than backing up into homes and businesses (USGS, 2006). Combined sewers carry a combination of raw sewage and storm water runoff into the stream.

### 2.3.3 Local weather patterns

Changing seasonal patterns can exacerbate water quality aspects in urban areas. Local weather patterns, including storms, can facilitate delivery of bacteria, pesticides and viruses, into natural aquatic system, leading to deterioration of water quality (Jeng *et al.*, 2005). The storm event may also negatively impact on the rehabilitation and self- purification capacity of the river (Elmanama *et al.*, 2005).

## 2.4 SOURCES OF FAECAL POLLUTION IN RURAL AREAS

Faecal pollution in rural establishments is usually low, but tends to be high in areas with high levels of agricultural activity. Faecal pollution occurs when dairy shed, piggery effluent or manure produced by intensive livestock breeding is spread on land and is transported down to the water resource by percolating rainfall (Gannon *et al*, 2005).

## 2.5 REGULATIONS, POLICIES, SUSTAINABLE DEVELOPMENT TO MANAGE RIVER WATER QUALITY

With recognition of the ecological, economic, social and cultural significance of rivers and their sensitivity to anthropogenic activities, it is essential that these river systems be managed in a sustainable manner (Newham *et al.*, 2004). To fullfill these aims, there need to be an understanding of the processes and pressures affecting rivers to establish specific catchment management strategies by following an integrated approach in the use, planning and management of urban, sub-urban and peri-urban areas by understanding the nature of improving the sustainability (Ticehurst *et al.*, 2006). To achieve imbalances conservation, planning and management issues must operate successfully in the arena of both poverty and privilege. Therefore management strategies must truly function as an integral component of urban development to balance human activities and their environmental effects. Measures such as land reformation, provision of basic infrastructure, housing and targeted rural assistance (including extension services), and the maintenance of food security should ultimately reduce pressure on the natural aquatic environment (Cilliers *et al.*, 2003).

The Reconstruction and Development Program (RDP) of the Government of South Africa (1994) stressed that sustainable urbanization must be part of the process of post-apartheid-reconstruction. The mayor, town manager, development approval agencies and local government of Potchefstroom must seek to meet the social and economic needs of urban residents. In doing so local, regional and natural aquatic systems must be respected. Solving also the feacal pollution problems at the source and where possible using available quantitative and qualitative ecological data, microbial data including antimicrobial base-line data available, rather than shifting them to spatial locations or passing them on to other locations (Coombes, 2006)

### 2.5.1 Source directed control measures

There is a need to control, monitor and audit all point sources in the Mooi River catchment more effectively. The method used is to instruct all direct impactors to complete a strategic water management plan to ensure their effective management of the activities of total water balance. The water quality management plans should include, measures in order to minimize pollution at the source (N.W. Province-SOTE, 2002). The fundamental principle is to prevent, inhibit, retard or stop the hydrological, chemical, microbiological, radioactive or thermodynamic processes, which result in the contamination of the water environment.

If the water/waste water problems cannot be solved by the above water quality management strategies at source. Water/waste water recycling and minimization measures could be implemented. This would include the prevention of the inflow of ground and surface water into the industry and mining related activities. If the water/waste water problems cannot be solved by reuse and minimization measures, then water/waste water treatment applications should be implemented.

It should be appreciated that all of the above entails intensive negotiations between the relevant role players including catchment forums, consultants and specialists where necessary. This ensures participation, collaboration and transparency in decision making (N.W. Province-SOTE, 2002).

## 2.6 MICROBIAL INDICATORS OF FAECAL POLLUTION

### 2.6.1 Faecal pollution

Faecal pollution of the aquatic environment is a function of lifestyle and living standards, both of which show considerable variations. These variations are between the social extremes represented in rural settlements with poor or non-existent sanitary facilities, and developed urban communities where sophisticated sewerage systems and water treatment plants are in place. Faecal pollution from human sources is often one of the major factors associated with urbanization that contribute to the degradation of water quality in developing as well as developed countries (Webster *et al.*, 2004).

There are major water quality problems encountered in South Africa such as over-utilisation of riparian zones in rivers, water deficit where the demand for water exceeds its availability (Holland *et al.*, 2004). The extent of river water pollution varies according to the quantity and quality of the pollutant. Pollution presents a major health risk for recreational and domestic use of water. There is also strong evidence that the quality of aquatic life is influenced by river pollution

(Elmanama *et al.*, 2005). Contamination of soil and surface waters with faecal material enhances the risk of human exposure to pathogenic enteric bacteria of intestinal origin (Shehane and Harwood, 2005). Water pollution from sewage pathogens causes several diseases like some typhoid, cholera, bacterial and amoebic dysentery, enteritis, poliomyelitis, infectious hepatitis (jaundice), schistosomiasis and gastroenteritis (Mallon and Corkill, 2002).

Catastrophical impacts of faecal pollution in the developing countries worldwide are highlighted in the two following paragraphs. A child dies every fifteen seconds from a disease caused by lack of access to safe drinking water, inadequate sanitation and poor hygiene. Around four million people die every year from water-related diseases. More than a billion people around the world lack a basic water supply. In the past ten years diarrhoea has killed more children than all the people lost to armed conflict since World War II. At any time, 1.5 billion people suffer from parasitic worm infections stemming from human excreta and solid wastes in the environment (Red Cross International, 2006).

In Africa, 30 % of the rural water supplies are not functioning at any one time. In Asia, and Latin America and the Caribbean, the percentages are respectively 17 % and 4 %. Health is one of the most important reasons for investing in water, sanitation and hygiene. Experience shows that the provision of water and sanitation technology alone (without changes in hygiene behaviour through health education) will usually achieve little health improvement in the longer term. Hygiene related-illness cost developing countries five billion working days per year. Half of the world's developing hospital beds are occupied by victims of unsafe water and sanitation. Malaria is one of the most critical disease problems of today in Africa and elsewhere. Approximately

7000 people die every day from malaria. Improved sanitation and vector control can break this trend (Red Cross International, 2006).

### 2.6.2 Microbial indicators

A combination of indicator organisms are more useful as a tool than any one individual indicator *per se* to identify the contaminant sources and predict the environmental impact of land use activities (Whitlock *et al.*, 2002). Indicators are generally used for assessing the microbiological safety of domestic and recreational water and also to distinguish between faecal pollution of human and animal origin during wet and dry weather (Sankaramakrishnan and Guo, 2005). Among indicator organisms, heterotrophic bacteria, total coliforms, faecal coliforms and enterococci bacteria are used as indices for measuring surface water quality, chosen for easier isolation and identification of contamination within 48 hours (Kim *et al.*, 2005). Figure 2.3 shows the species of indicator microorganisms and their relationship (after Kim *et al.*, 2005).

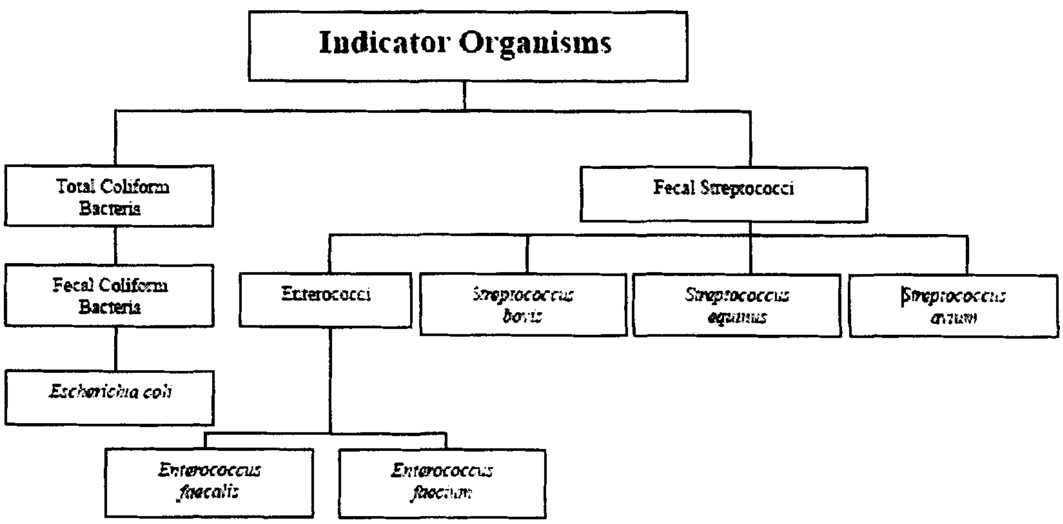



**Figure 2.3:** Species and relationship among indicator organisms (after Kim *et al.*, 2005)

(a) Heterotrophic bacteria

Heterotrophic bacterial plate count, expressed as colony-forming units per millilitre of sample (cfu/ml), is used in standard procedures for microbial water quality testing but does not represent the total bacterial population present. They are used to test the bacterial content of surface and drinking water, assess efficiency of water treatment and disinfection processes, to test the integrity of distribution systems for resulting growth and to determine the quality of water used in industrial processes (Jeena *et al.*, 2006)

(b) Total coliforms bacteria

The total coliform group consists of bacteria that ferment lactose with gas and acid formation within 48h at 35°C and are primarily used as a practical indicator of the general hygienic quality of water, mainly used in routine monitoring of drinking supplies. Total coliforms alone are not a good indicator of faecal contamination as many strains included in this group originate from the environment and not from faeces (Bezuidenhout *et al.*, 2002).

(c) Faecal coliform bacteria

The faecal coliform bacteria live in the intestines of warm-blooded animals and are facultative anaerobic, Gram-negative, non-spore forming, rod-shaped bacteria that grow and produce gas in tryptone broth at 44.5°C within 24hrs. They also live in the waste material or feaces excreted from the intestinal tract (Evanson and Ambrose, 2006).

When faecal coliform bacteria are present in high numbers in a water sample, it means that the water may have received faecal matter from one source or another. Although not necessarily agents of disease, faecal coliform bacteria may indicate the potential presence of pathogenic organisms, which live in the same environment as the faecal coliform bacteria. This means that their presence in water is an indication of potential faecal pollution and the possible presence of enteric pathogenic organisms in aquatic environments (Noble and Furman, 2001).

*Escherichia coli* is one species of the faecal coliform bacterial group used as a specific indicator of faecal pollution which originates from humans and warm-blooded animals and are present at concentrations much higher than the pathogens they predict (Crowther *et al.*, 2002). Faecal coliforms in aquatic environments peak after a rainfall event; thereafter they decrease or disappear from the water column with time through death or sedimentation processes that can concentrate them in sediments at high densities (Chigbu and Strange, 2005). *E. coli* may not be a reliable indicator in tropical and subtropical environments due to its ability to replicate in contaminated soils (Scott, *et al* 2003).

(d) Enterococci

The presence of enteric indicator organisms does not necessarily indicate human contamination, as livestock and wildlife are also sources. Resolving urban (human) and rural (animal) inputs has presented a significant challenge to traditional indicator systems. One strategy that was pursued to overcome these limitations was the evaluation of alternate microbial indicators, such as Enterococcus which is relatively specific of faecal pollution and tends to survive longer in the environments than coliform bacteria (Desmarais *et al.*, 2003).

Enterococci are Gram-positive, facultative anaerobic organisms which prefer anaerobic conditions and are found in the gastrointestinal tract of humans and warm blooded animals (Kayser *et al.*, 2003). They are differentiated from other streptococci by their ability to grow in

6.5% NaCl, high pH (9.6) and temperature (45°C). Enterococci have been used successfully as alternative microbial indicators of point and non-point sources of faecal pollution and are especially reliable as indicators of the increased health risk of acquiring an infection in aquatic environments and recreational waters. It is known, however, that environmental reservoirs of enterococci exist and that regrowth of these organisms may be possible once they are introduced into the environment (Desmarais *et al.*, 2003). However, like other currently recognized faecal indicators, enterococci are consistently found in faeces of all warm-blooded animals and therefore share the drawback of host non-specificity with the faecal and total coliforms (Kim *et al.*, 2005).

## 2.7 TOOLS FOR TRACKING FAECAL POLLUTION

### 2.7.1 Differentiation of faecal pollution from human and animal origin

Traditional methods used to measure faecal pollution levels, such as faecal coliform detection methods including the faecal streptococci to faecal coliform ratio and cluster analysis, do not discriminate among different source species. Identification of sources of faecal pollution using general faecal coliforms to faecal enteroccoci ratio is based on the premise that a ratio of $\geq$4.0 would indicate human pollution and a ratio of $\leq$0.6 would indicate non-human pollution (Gildreich and Kenner, 1969). Bacteria from different antibiotic exposure histories will thus have different antibiotic resistance/susceptibility patterns and these could be grouped using clustering methods (Schwartz *et al.*, 2003)

Consequently, source identification has been the subject of much research, leading to the development of methods such as antibiotic resistance patterns of faecal bacteria and *E. coli* ribotyping to identify human and non-human sources of faecal pollution. However, these methods ultimately rely on culturing faecal bacteria from the environment, and the extent to which survival of faecal bacteria affects these results has not been addressed. Molecular diagnostic pulsed field gel electrophoresis tools are an alternative to traditional culture-based methods, thus circumventing potential culture biases (Webster *et al.*, 2004).

It is very important to know whether a pollution source is human or animal. This will indicate to environmental managers entirely different methods for risk management (e.g. environmental engineering solutions to reduce human pollution inputs versus wildlife management options to reduce loadings from wildlife species). By identifying sources of contamination in water samples collected over a large area of the basin, potential problem areas can be located and management strategies can be developed to reduce or eliminate the sources (Bernhard *et al.*, 2003)

### 2.7.3 Antibiotic resistance and multiple antibiotic resistance

The overuse of antibiotics, chemicals such as disinfectants, antiseptics, pesticides together and the practice of raw sewage discharge into receiving waters, has resulted in a significant increase of antibiotic resistant bacteria in aquatic environments. These antimicrobial agents are washed off into streams and rivers during rainfall events resulting in development and spread of antibiotic resistant bacteria (Schwartz *et al.*, 2003). Bacteria may be defined as resistant when they are not susceptible to a concentration of antimicrobial agent such as antibiotics and this is indicative of the selection pressure exerted on bacteria (Cloete, 2003).

In recent years antibiotic resistant and multiple antibiotic resistant bacteria have become invaluable as tools tracking, detecting and differentiating human and animal faecal pollution sources (Choi *et al.*, 2003). A technique, called antibiotic resistance analysis (ARA) is frequently used in aquatic studies to evaluate water quality as well as tracking pollution sources (Sankaramakrishnan and Guo, 2005). ARA is based on the premise that bacteria from wildlife species are generally lacking in antibiotic resistance, while strains from humans will exhibit MAR and strains from domestic animals will be somewhat intermediate in MAR (Schwartz *et al.*, 2003). This multiple antibiotic resistance can used to assess resistance to antibiotics that are commonly associated with human and animal therapy, as well as animal feed (Krumperman, 1983).

The advantage of using faecal indicators and MAR is their ability to provide rapid results, indicators are nonpathogenic, easily enumerated, have survival characteristics that are similar to those of the pathogens of concern and can be strongly associated with the presence of pathogenic microorganisms (Evanson and Ambrose, 2006). MAR can be used to discriminate isolates from multiple animal sources. However, these techniques have their limitations due to variable survival rates of enteroccoci. Faecal coliform (*E. coli*) may not be a reliable indicator in tropical and subtropical environments due to its ability to replicate in contaminated soils (Desmarais *et al.*, 2003). MAR requires reference database; may be geographically specific; isolates that show no antibiotic resistance cannot be typed.

## 2.8 OTHER TECHNIQUES THAT CAN BE USED FOR MICROBIAL SOURCE TRACKING.

Current techniques used for microbial source tracking include *Bifidobacterium sp*, *B. fragilis* HSP40 bacteriophage, F+ RNA bacteriophage, ribotyping, human enteric virus, bacteroides-prevotella molecular marker, caffeine, faecal sterols and/or stanols also have their share of advantages and disadvantages (Wiggins, 1996). Overall, there is no single method that is capable of identifying specific sources of faecal pollution in the environment with absolute certainty. Also host-specific differences in fatty acid methyl ester (FAME) profiles of faecal coliforms have proven to have potential to be used as a phenotypic microbial source tracking tool (Duran *et al.*, 2006). Therefore, the usefulness of the microbial indicators as tools for risk assessment can be significantly enhanced by the development of testing methods and analysis of the techniques that can define specific sources of these organisms (Scott *et al.*, 2003).

Future prospectives should address issues such as relationships between the survival characteristics of indicator organisms with regard to those of the pathogens they are designed to predict. Furthermore, epidemiological studies should be implemented in multiple source tracking techniques so that assessments of risk can be more closely associated with the results produced by a given technique (Duran *et al.*, 2006).

A human integrated model of investigation of faecal pollution due to urbanization can be designed that accounts for ecosystem structure and function along urban-rural gradients of the water resource. The urban and rural sources of faecal pollution must be taken into consideration in sustainable management of water resources. Microbiological indicators such as faecal indicator bacteria including antibiotic resistance bacteria, can be used as a tool to assess the levels of faecal pollution and predict the environmental impact of land use activities. The usefulness of the microbial indicators as tools for risk assessment, can be significantly enhanced by the development of testing methods and analysis of the techniques that can define specific sources of faecal pollution.

## 2.9 SUMMARY

It can be concluded that there may be association between faecal pollution due to urbanization and occurrence of antibiotic resistance. Increased development in Potchefstroom urban area which is in the vicinity of the Mooi River may negatively impact on the water quality but also the recovery, rehabilitation and self-purification capacity of the river. Faecal pollution causes several waterborne diseases and is seen as major threat to both human health especially children, aquatic life and economy. A tool called antibiotic resistance analysis is used to assist with faecal contamination source tracking. The question posed by this study was whether antibiotic resistant bacteria were present in the urban-rural aquatic system, and to what extent has urbanization made an impact in the development of this resistance along the River system.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 SAMPLING AREA

Potchefstroom is located in the south eastern part of the North-West Province (Figure 2.2) with the climate typical of the South African Highveld and annual rainfall in excess of 150 mm (N.W. Province-SOTE, 2002). The Mooi River passes through the magisterial district of Potchefstroom and includes rural upstream and downstream segments with a city segment shaped by decades of urbanization followed by population growth. There are various dams situated in the Mooi River and Potchefstroom municipality abstract domestic water for the city from the Boskop Dam (Figure 3.1). The Mooi River is further used for angling and general recreational purposes. Industrial use of water from the Mooi River is concentrated in and around Potchefstroom. Water is abstracted by farmers along the upper and lower reaches of the river for agricultural purposes and domestic supplies. The Mooi River and its tributaries receive contamination from a wide variety of point and diffuse sources, including agricultural and industrial effluents. The river system is strongly influenced by the rainfall from October to March. The dry season is from April to September. Rainfall may be highly variable, both in space and time, often resulting in severe droughts or flooding (N.W. Province-SOTE, 2002).

The climate in Potchefstroom is warm to hot during the summer months (September to April). Summer day time temperatures could range from ±10°C in the morning to more than 32°C at midday. Winter months (May-August) are cold to mild and dry. Temperatures could vary from -4°C in the morning to more than 25°C in the midday.

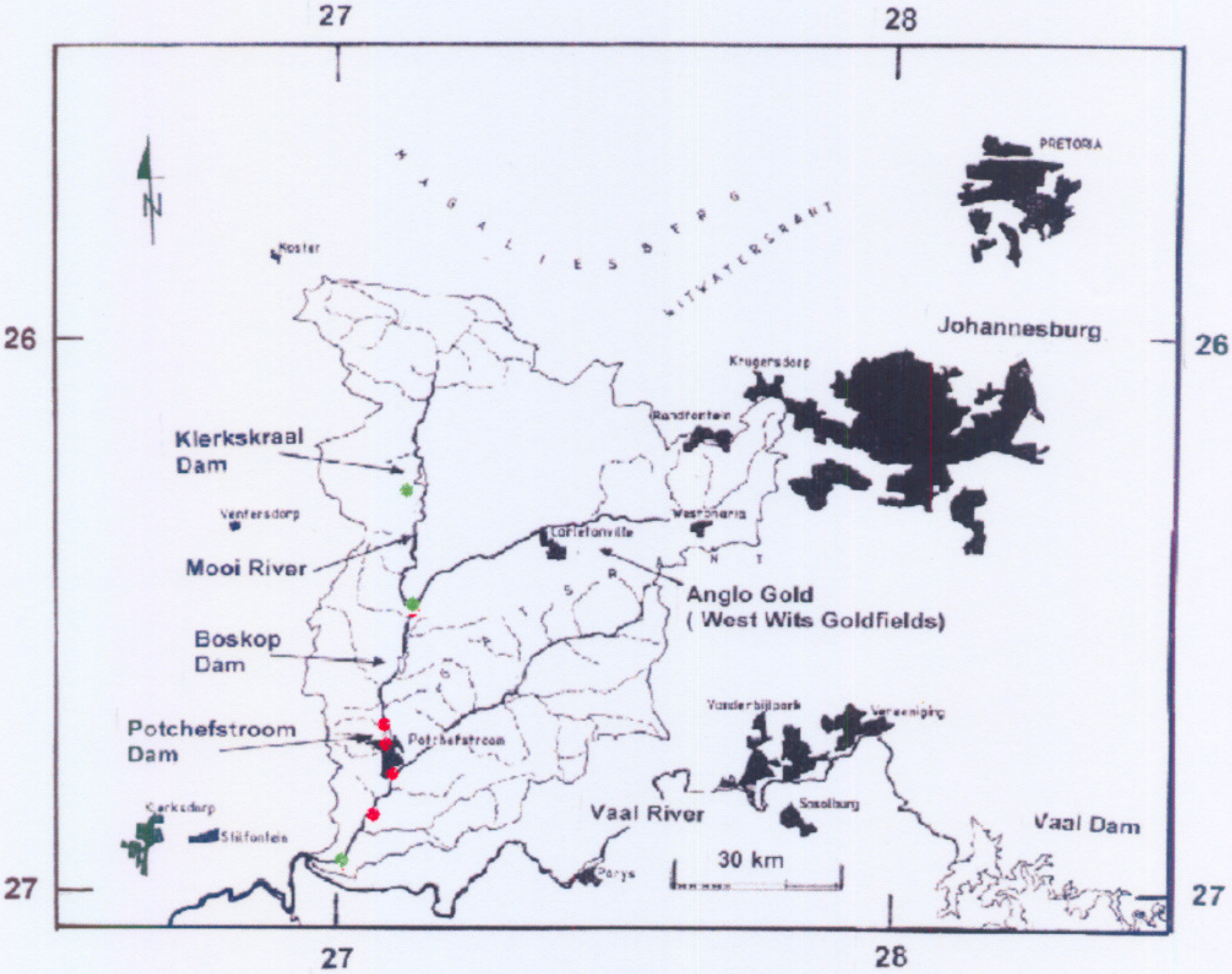



**Figure 3.1:** A map of the Mooi River catchment, indicating the eight sampling sites (shown by green and red dots), three reservoirs and associated towns and cities (IWQS and Kempster, 1999).

## 3.2 SITE DESCRIPTION AND LAND USE

Eight sites along the Mooi river system were selected and monitored for 1 year (monthly). Factors taken into account in the selection of the sites were according to IWQS and Kempster, (1999) and included the following:

(i) The potential for large-scale agricultural and recreational water use.

(ii) The identification of significant point and diffuse source discharges before and after the rainfall period from upper and lower reaches of the Mooi River.

(iii) The identification of the effects of Potchefstroom urbanization in quality of source waters of the Mooi River

(iv) The need to establish, as far as possible, natural background levels.

Table 3.1 summarizes the sampling site information, land use change and identifies the location of the sites using global positioning system (GPS) coordinates and satellite images (http://www.maplandia.com/South-africa/north-west/potchefstroom/Potchefstroom/).The satellite pictures are enlarged in Appendix A (Figure A.1) showing the exact location of the sampling sites.

**Table 3.1:** Site, monitoring point names with positional data, land use intensity and ecological descriptions. The latter were done using the criteria of IWQS (1999), Kempster (1999) and De la Rey *et al.* (2004).

| **Site No & site satellite Picture** | **Monitoring point and GPS coordinates** | **Land use intensity and ecological description** |
| --- | --- | --- |
|  1 | Klerkskraal dam<br><br>Latitude: 260.15.159`<br>Longitude: 270.06.432` | Resource conditions are slightly to moderately altered from natural class due to human activity and water use.<br><br>Recreation activities - Angling. Agricultural activities up stream and around the dam. Eco-system essentially in good state, biodiversity largely intact. |
|  2 | Muiskraal bridge<br><br>Latitude: $26^0$.26.704`<br>Longitude: $27^0$.07.100` | Human activity has caused minimal changes to the historical natural structure.<br><br>Agricultural and ecosystem essentially in good state, biodiversity largely intact. |
|  3 | Potchefstroom Dam weir<br><br>Latitude: $26^0$.40.418`<br>Longitude: $27^0$.05.782` | Resource conditions are slightly to moderately altered from natural class due to human activity and water use.<br><br>Recreational uses including boating, campgrounds and parks and fish habitat and ecosystem essentially in good state. |

| | | |
|---|---|---|
| 4  | Wasgoed Spruit tributary<br><br>Latitude: $26^0$.42.159\`<br>Longitude: $27^0$.06.432\` | Urban runoff from urban surfaces (industrial effluents and Potchefstroom storm-water drains).<br><br>Water resource that is ecologically unsustainable due to pollution. |
| 5  | Police Rugby field<br><br>Latitude: $26^0$.42.452\`<br>Longitude: $27^0$.06.337\` | The water resource is heavily impacted by human activity and hydrological characteristics, banks and channel of the resource altered.<br><br>Urban- people squatting near the banks of the river, storm-water drains from Potchefstroom also enter here. |
| 6  | Opposite River Walk<br><br>Latitude: $26^0$.42.808\`<br>Longitude: $27^0$.06.318\` | Urban (downstream site 3), human settlements- River walk shopping mall, truck parking, (now being developed for a shopping mall)<br><br>Biological communities and chemical concentrations are significantly changed and water cannot even be used for agricultural purposes. |

| | | |
|---|---|---|
| 7  | Upstream from the Sewage Treatment Plant on the bridge opposite Potchefstroom prison<br><br>Latitude: 26⁰.45.153`<br>Longitude: 27⁰.06.017` | Cow grazing upstream. Close to Prozesky bird sanctuary, agricultural activities further downstream. Functioning of biological communities and chemical concentrations are slightly altered. |
| 8  | Mooi River Mouth (on the Scandinavia river drift bridge)<br><br>Latitude: 26⁰.52.825`<br>Longitude: 26⁰.57.825` | Maize fields near the river bank, cattle grazing, and green algal blooms observed during the entire sampling period. |

## 3.3 SAMPLE COLLECTION STRATEGY AND PHYSICO-CHEMICAL ANALYSIS

Three samples per site were collected from the pre-determine sites along the Mooi River system from: Klerkskraal dam to the North (site1) and several points along Mooi River passing through Potchefstroom to points on the southern side of Potchefstroom (before Mooi River enters Vaal River (site 8)) as shown in Figure 3.1 and Table 3.1. These samples were collected into sterile sample bottles and stored on ice in a cooler box and analyzed within 6 hours of collection. The sampling frequency was monthly. Sample collection lasted from April 2005 to March 2006. The surface water was analyzed on site for physicochemical parameters such as temperature, pH, total dissolved solids (TDS), dissolved oxygen, as well as conductivity, using a transportable multi-meter (Multi 350i Universal multimeter, WTW™, Germany). Chemical oxygen demand (COD) was analyzed in the laboratory using Merck spectroquant kits (Merck™ Germany). The Rainfall data was provided by the South African Weather Services courtesy of Me. C. de Villiers (www.weathersa.co.za).

### 3.3.1 Classifications of sites

The sites were then divided into three groups based on their origin. One group included samples upstream from Potchefstroom, (Site1- Klerkskraal dam, Site 2- Muiskraal Bridge, Site 3- Potchefstroom Dam weir). The second group included samples from Potchefstroom urban origin (Site 4- Wasgoed Spruit tributary, Site 5 – Opposite Police rugby field, Site 6- Opposite River Walk). The third group included samples from downstream origin (Site 7 - upstream from the Sewage Treatment Plant on the bridge opposite Potchefstroom prison and Site 8 - Mooi River Mouth -on the Scandinavia river drift bridge).

## 3.4 MICROBIOLOGICAL ANALYSIS

### 3.4.1 Sampling media

Sampling media consisted of plate count, m-Endo, mFc and m-Enterococci agar plates supplemented to contain either 50µg/ml ampicillin or 50µg/ml kanamycin (Mast Diagnostics, UK). Antibiotic solutions were added to cooled, autoclaved agar. Petri dish were filled with ±15ml media and allowed to dry. All the media were from Biolab (Merck, South Africa).

### 3.4.2 Assay for levels of bacterial faecal indicators

(a) Heterotrophic plate count

Series of tenfold dilutions of the water samples were prepared for enumeration of heterotrophic bacterial contents using plate counts. Hundred microliters of diluted water sample was spread on the surface of a plate count medium without and with ampicillin or kanamycin incubated at $37^{0}$C. The number of different types of visible distinct colonies that developed after 24hrs were counted in duplicate based on morphology and colour.

(b) Faecal indicator bacterial

Water samples were also assayed in triplicates for bacterial indicators by filtering 100ml through 47mm diameter membrane filters (0.45 µm pore size). Faecal coliform bacteria were enumerated by standard methods on mFC agar. Enumeration was done on media without any antibiotic as well as media that contained ampicillin or kanamycin. Plates were incubated at 44.5°C for 48hrs. Total coliforms and enterococci were enumerated on m-Endo agar and enterococcus agar, respectively and were incubated at 35°C for 48hrs. The plating strategy for total coliforms and enteroccoci was similar as for faecal coliforms. The addition of ampicillin or kanamycin to media was used to indicate the levels of antibiotic resistant indicator bacteria.

### 3.4.3 Purification of faecal indicator bacteria

Ampicillin or kanamycin resistant isolates of faecal coliforms and enterococci were purified by successive streaking of selected single colonies onto appropriate selective media. Gram stain, according to standard procedures (Prescott *et al.*, 2002), was used to confirm cell morphology and whether the bacteria were Gram-negative or Gram-positive.

The ratio of faecal coliforms to faecal enterococci was determined, where a ratio of $\geq$4.0 would indicate human pollution sources and a ratio of $\leq$0.6 would indicate non-human pollution sources. The rationale behind the use of this method was the observation that human feaces contain higher faecal coliform counts, while animal feaces contain higher levels of faecal enterococci (Geldreich and Kenner, 1969).

## 3.5 ANTIBIOTIC SUSCEPTIBILITY

These purified isolates were tested for susceptibility to different antibiotics using the Kirby-Bauer method (Bauer *et al.,* 1966). This technique is standardized procedure for determining antibiotic susceptibility on Mueller-Hinton agar. Cultures were grown in 5ml nutrient broth and incubated at 37°C for 24hrs. One hundred microliters of the nutrient broth culture was transferred onto Mueller-Hinton plates. A flamed-sterilized spreader was used to inoculate the plates evenly. The plates were left for 15 minutes to dry. Disks impregnated with antibiotic (Mast Diagnostics, UK supplied by Davies-Diagnostics, SA) were dispensed onto the agar by means of an automatic disk dispenser (Mast Diagnostics, UK supplied by Davies-Diagnostics, SA). The dispenser has six slots into which tubes containing antibiotic disks can be fitted. When the dispenser is pressed, six equally spaced antibiotic disks are dispensed directly onto the media. Plates were incubated at 37°C for 24hrs. The ten antibiotics used are indicated in Table 3.2. These antibiotics were chosen because: (i) all have been used in the treatment of human and animal illnesses, as livestock supplements, or both; and (ii) have been used in previous surveys of antibiotic resistance in aqueous environments (Krumperman, 1983).

**Table 3.2:** A table indicating the details of antibiotics that were used in this study. The concentration used as well as the inhibition zone measurements (in mm) that were considered resistant (R); intermediate resistant (I) and susceptible (S) are shown and were according to NCCLS (1999). The abbreviations (abbrev.) were according to the 2005 instructions to the authors for the Journal of Clinical Microbiology (http://jcm.asm.org/misc/itoa.pdf).

| Antibiotic Class | Antibiotic | Abbrev. | Conc. | R | I | S |
|---|---|---|---|---|---|---|
| **B-lactams** | Ampicillin<br>Amoxicillin | AMP<br>AMO | 10μg<br>10 μg | ≤16<br>≤16 | -<br>- | ≥17<br>≥17 |
| **Aminoglycosides** | Kanamycin<br>Neomycin | KAN<br>NEO | 30 μg<br>30 μg | ≤13<br>≤13 | 14-17<br>14-16 | ≥18<br>≥17 |
| **Tetracycline** | Ox tetracycline | OXY-TET | 30 μg | ≤14 | 15-18 | ≥19 |
| **Chloramphenicol** | Chloramphenicol | CHL | 30 μg | ≤12 | 13-17 | ≥18 |
| **Glycopeptides** | Vancomycin | VAN | 30 μg | ≤14 | 15-16 | ≥17 |
| **Quinolones** | Ciproflaxin | CIP | 5 μg | ≤15 | 16-20 | ≥21 |
| **Cephems** | Cephalothin | CEP | 30 μg | ≤14 | 15-17 | ≥18 |
| **Macrolides** | Erythromycin | ERY | 15 μg | ≤13 | 14-22 | ≤23 |

### 3.5.1 Interpretation of inhibition zone diameter

A clear zone around the antibiotic disk indicated inhibition of microbial growth. The inhibition zone diameter was measured to the nearest millimetre using a ruler and interpreted using NCCLS (1999) guidelines (Table 3.2). Antibiotic resistance profiles were compiled for each isolate.

### 3.5.2 Multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance (MAR) index of each isolate, as well as for MAR per site were determined by using the following formula:

$$MAR = \frac{\text{Number of isolates resistant to all antibiotics at specific site}}{\text{(number of antibiotics tested) x (total number of organisms in sample)}}$$

## 3.6 STATISTICAL ANALYSIS

Average and standard deviations of the levels of the indicator organisms, rainfall, temperature and pH, total dissolved solids (TDS), electro-conductivity, total dissolved oxygen (DO) and chemical oxygen demand (COD) were calculated and represented graphically using histograms and other appropriate graphical representations. The inhibition zone diameters of all antibiotics used were measured and percentage of organisms resistant to various antibiotics determined. The antibiotic resistant patterns of different microorganism were also shown graphically using percentage resistance per site. Geometric mean of microbiological organisms, rainfall, temperature and pH analysis data were used to present monthly values for these parameters. Pearson's correlation (a measure of linear coefficient (r) association) was used to show correlation between microbiological data on the one hand and rainfall and surface water temperature on the other. The Student's t-test was used to determine the statistical significance. Probability was set at $p<0.05$. Multivariate exploratory techniques using Ward's clustering method and Euclidean distances were used to generate dendrograms of the inhibition zone data (Berge *et al.*, 2003).

# CHAPTER 4

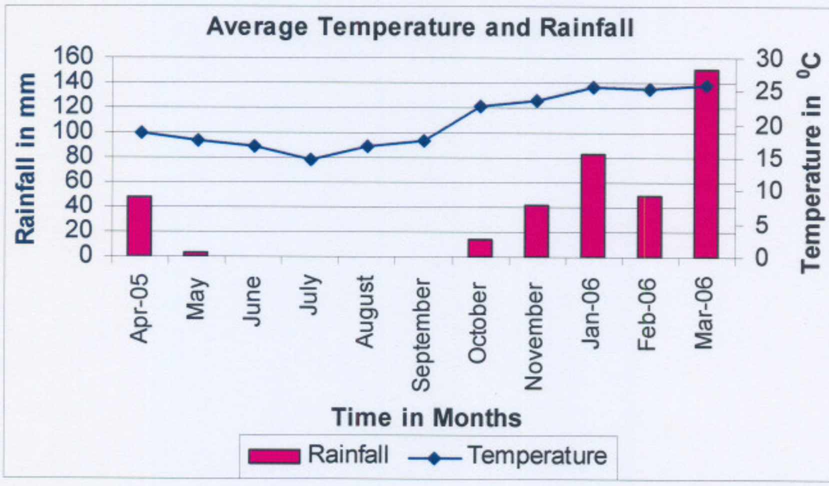
# RESULTS

## 4.1 PHYSICO-CHEMICAL PARAMETERS AND INFLUENCE OF LOCAL RAINFALL EVENTS

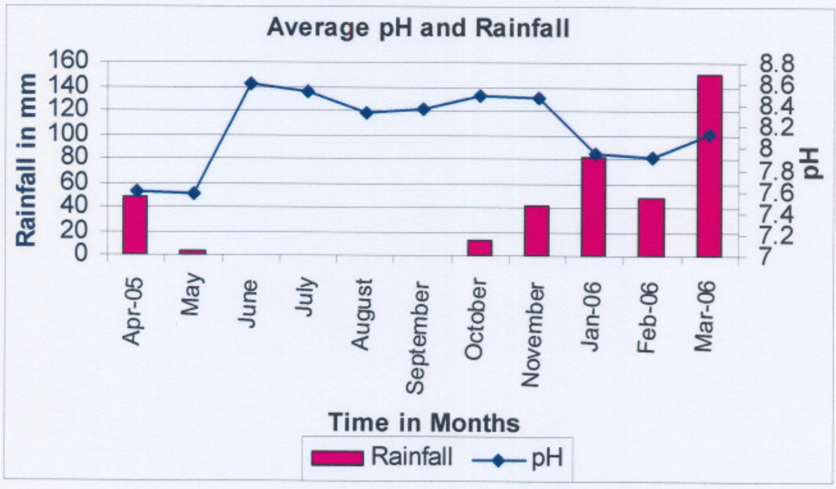
A number of physico-chemical parameters of the Mooi river water were determined during the dry and wet season over a one year period (April 2005 to March 2006) as described in Section 3.3. Results are presented in Figures 4.1 to 4.4 and also Appendix A (Table A1 to A.6). Data of the sites were then divided into three segments Potchefstroom, upstream and downstream from Potchefstroom based on their origin as described in Section 3.4. Overall results of the physico-chemical parameters are presented in Appendix A (Figure A.2 to A.7). Monthly rainfall during the wet season (October 2005 to March 2006) ranged from 13.2 mm to 150.8 mm and during the dry season (May 2005 to September 2005) ranged from 0 mm to 3 mm (SA Weather Services, 2006). During the study period the highest average rainfall was measured during March 2006 (Figure 4.1). The pattern of increasing rainfall events throughout the summer period that peaks in early autumn is normal in Potchefstroom (SA Weather Services, 2006). Statistical analysis of the results of the physico-chemical parameters was performed using the t-test and Pearson's correlation to establish significance and linear correlations between various paired locations of the Mooi River system as presented in Appendix B (Table B.5, Table B.6 and Table B.7).

### 4.1.1 Temperature and pH

Average temperature of the river water during the entire study period varied between 15 $^{0}$C and 25 $^{0}$C as shown in Figure 4.1(a). It was higher during the summer period (between 20-25 $^{0}$C) than during the winter period (between 15-20 $^{0}$C). The mean monthly pH values of the sites were within an acceptable range (7.6- 8.6; DWAF, 1996) during the entire monitoring period. In the winter period there was a slight increase in pH (Figure 4.1(b)). The overall pH values for all the grouped sites were more or less constant. Trends in water temperature and pH after each rain event were lower at the sites studied as shown in Figure 4.1 (a) and (b).




(a)

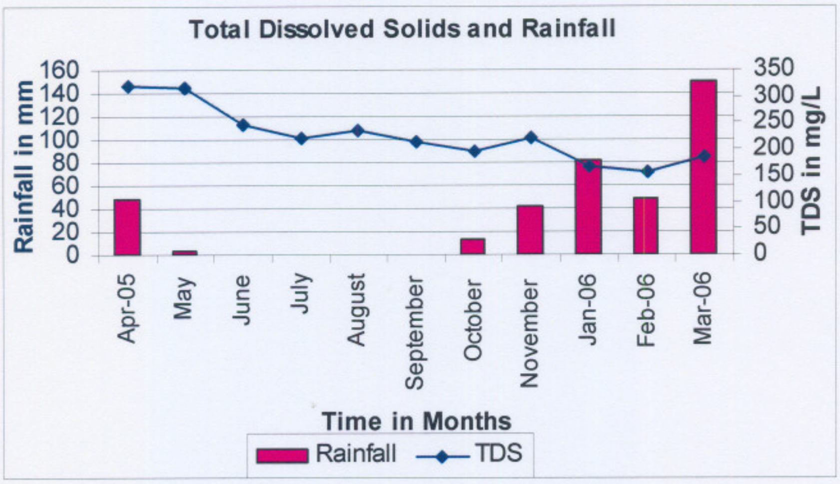



(b)

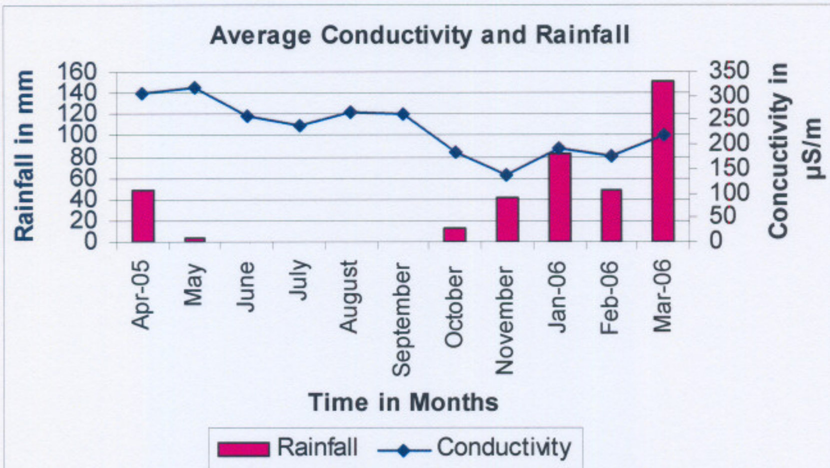
**Figure 4.1:** Relationship between average rainfall, temperature and pH data collected from April 2005 to March 06.

### 4.1.2 Total dissolved solids (TDS) and eletro-conductivity (EC)

The results of the TDS and EC varied from 156.5 to 321.1 mg/L and from 138.25 to 319.87 μS/m respectively and are presented in Figure 4.2(a) and (b). Electro-conductivity values were generally equivalent and directly proportional to TDS values as expected (Figure 4.2(a) and (b)). TDS and electro-conductivity values increased steadily throughout the rainy season and reached the highest peak at the end the season. These values dropped to ± 200 mg/L (and μS/m respectively) during the dry season, and thereafter remained constant until the end of the season. Elevated overall Potchefstroom TDS and conductivity levels were observed during entire monitoring period and upstream values were lower but higher than the downstream values as illustrated in Appendix A (Table A.5). However, in winter there was a slight decrease in electro-conductivity that might be related to the drop in pH as shown in Figure 4.1(b) and 4.2(b).




(a)




(b)

**Figure 4.2:** Relationship between rainfall, TDS and electro-conductivity values for wet and dry season.

### 4.1.3 Dissolved oxygen (DO) and chemical oxygen demand (COD)

Figures 4.3(a) and (b) illustrate the seasonal variation in both DO and COD at the sampling sites. According to the data colleted during the dry and wet season, DO was inversely proportional to COD. With rainfall, as the dissolved oxygen demand increased per site, the chemical oxygen demand decreased. Relatively high DO levels (between 4 and 6 mg/l) were measured during the period April 2005 to September 2005 (non-rainy season). Dissolved oxygen decreased to 3 mg/l at almost all sites during the rainy season (October 2005 to March 2006). The average was 3.2

mg/l which did not vary greatly as indicated in Figure 4.3 and the highest levels of dissolved oxygen were found in downstream sites.

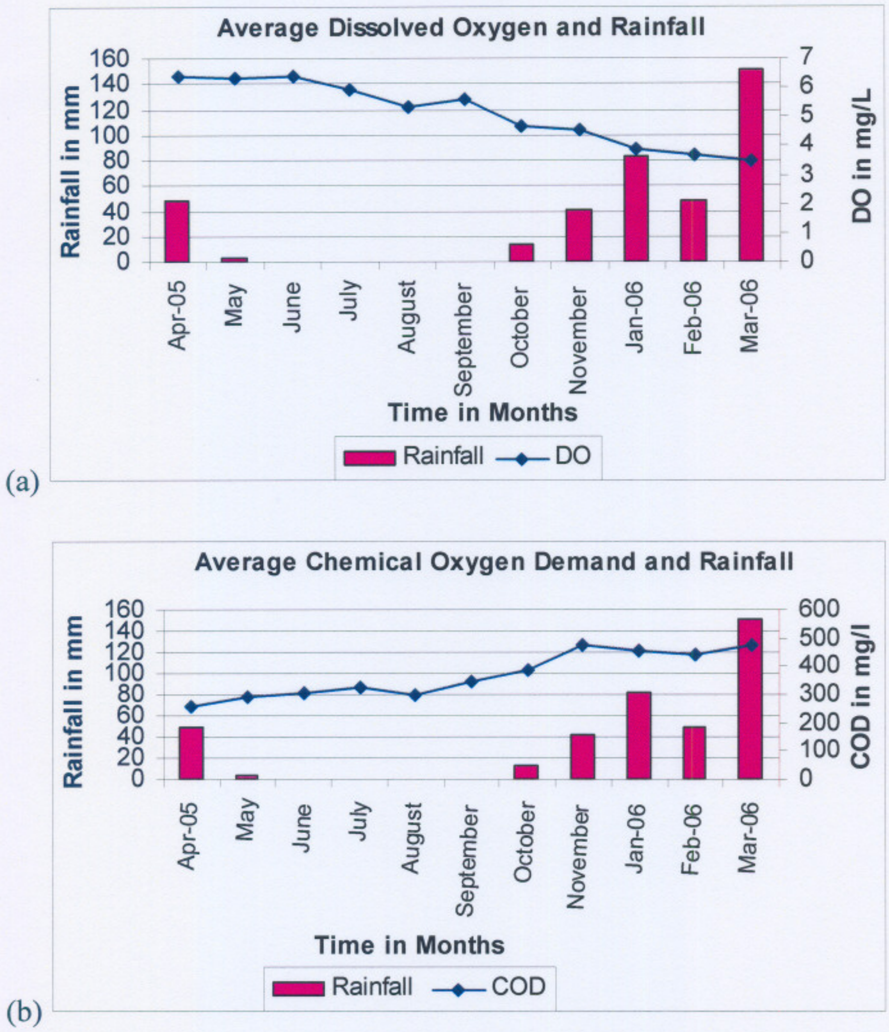



**Figure 4.3:** The total monthly rainfall, average dissolved oxygen and average chemical oxygen demand in the Mooi River system.

Although there were trends in all the results of the physico-chemical parameters determined, the pollution gradient did not exist along the Mooi River as demonstrated by these results. Most of the physical and chemical values obtained were according to the South African and international guidelines in the ranges for recreational and agricultural use (DWAF, 1998; DOH, 1998; WHO, 2004).

#### 4.1.4 Statistical analysis of the physico-chemical parameter relationships

Paired t tests were used to detect variations in the measured parameters with location in the study area. Pearson's correlation was used to detect linear correlations between various locations. Appendix B (Table B.5, Table B.6 and Table B.7) summarizes the paired t test results and the Pearson correlations of physico-chemical parameter relationships in Mooi River water samples of the study area.

There was insignificant ($p>0.05$) strong ($r = 0.78$) correlation between increase in surface water temperature and increase rainfall from winter to summer periods as shown in Appendix B (Table B.5). The overall pH values for all the grouped sites were more or less constant, insignificant ($p>0.05$) negative correlation between pH and rainfall are shown in Appendix B (Table B.5). Electro-conductivity and pH showed significant ($p<0.05$) weak ($r = -0.43$) correlation among the various locations as shown in Appendix Table B.5. Dissolved oxygen (DO) correlated insignificantly with rainfall in most locations. However the relationship between dissolved oxygen (DO) and chemical oxygen demand (COD) was significant with strong correlation in all the locations observed as shown in Appendix B (Table B.5).

## 4.2 MICROBIOLOGICAL ANALYSIS OF THE WATER SAMPLES FROM THE MOOI RIVER SYSTEM

The river water was tested for microbial indicators and results showed typical seasonal but also locational variations. Faecal coliforms, enteroccoci, total coliforms and heterotrophic bacteria were the microbial indicators which were used for this study. Results are indicated in Figures 4.4 to 4.7 and Table 4.2 to 4.3.

### 4.2.1 Faecal coliforms and enteroccoci bacterial levels

Figure 4.4 and 4.5 shows the seasonal variations of faecal coliform bacteria and enteroccoci from all eight sites collected during this study period. The sites were divided into Potchefstroom sites, upstream and downstream from Potchefstroom sites based on their origin as explained in Section 3.4. The graph in Figure 4.4(a) represents the levels of faecal colifoms that were enumerated on mFc media without ampicillin and the graph in Figure 4.4 (b) on media containing ampicillin. Faecal coliform and enteroccocal bacteria levels were highest during January 2006 but decreased during February and March 2006 (Figure 4.4 and Figure 4.5).

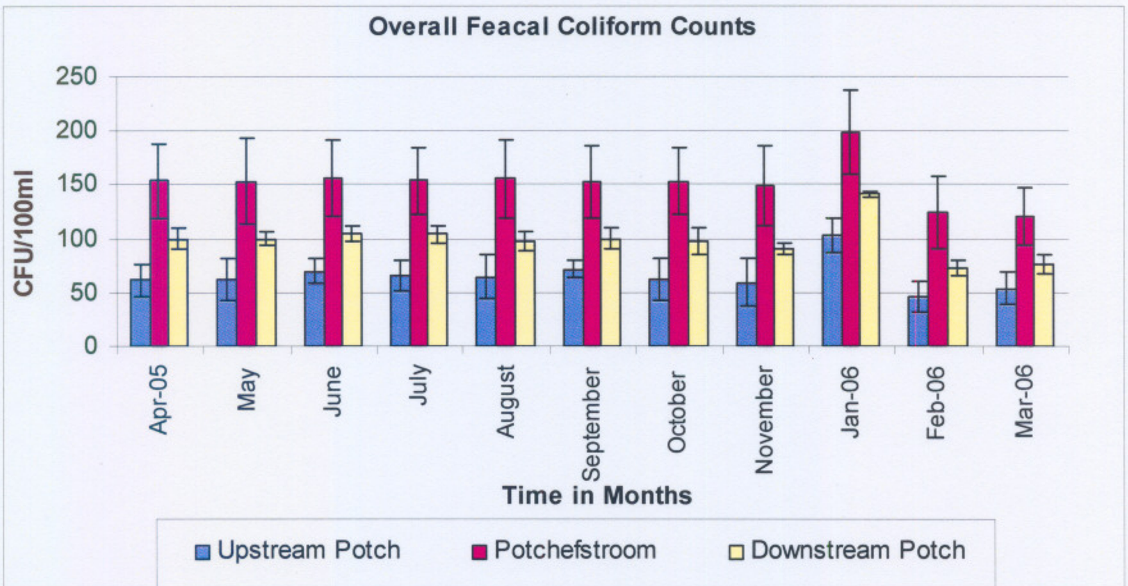



**Figure 4.4 (a):** Seasonal concentrations of faecal coliform bacteria collected in the Mooi River during the dry and wet season.

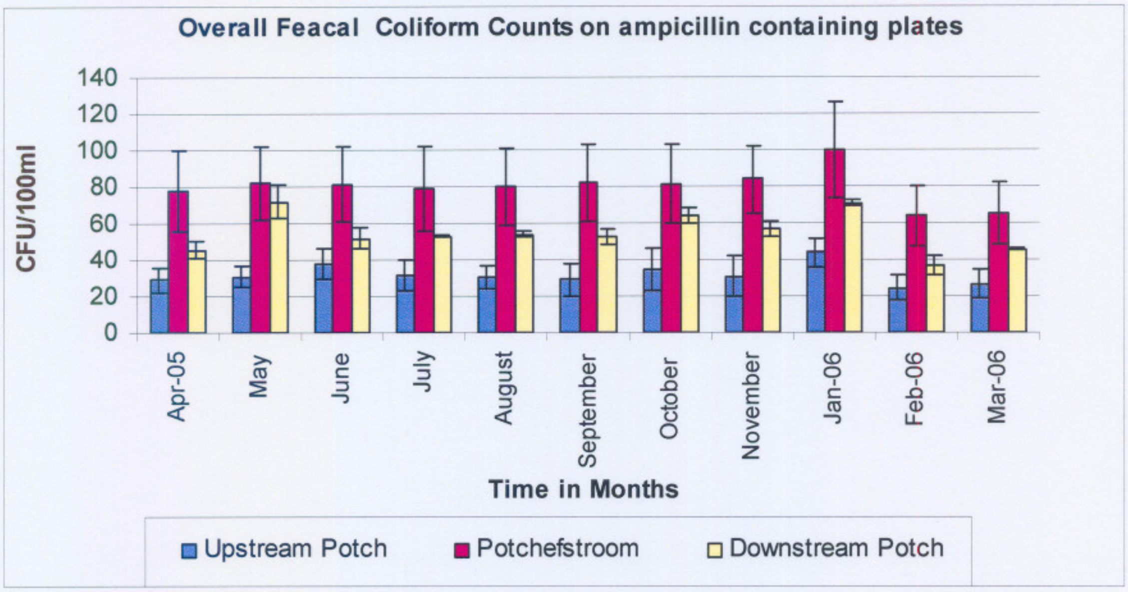
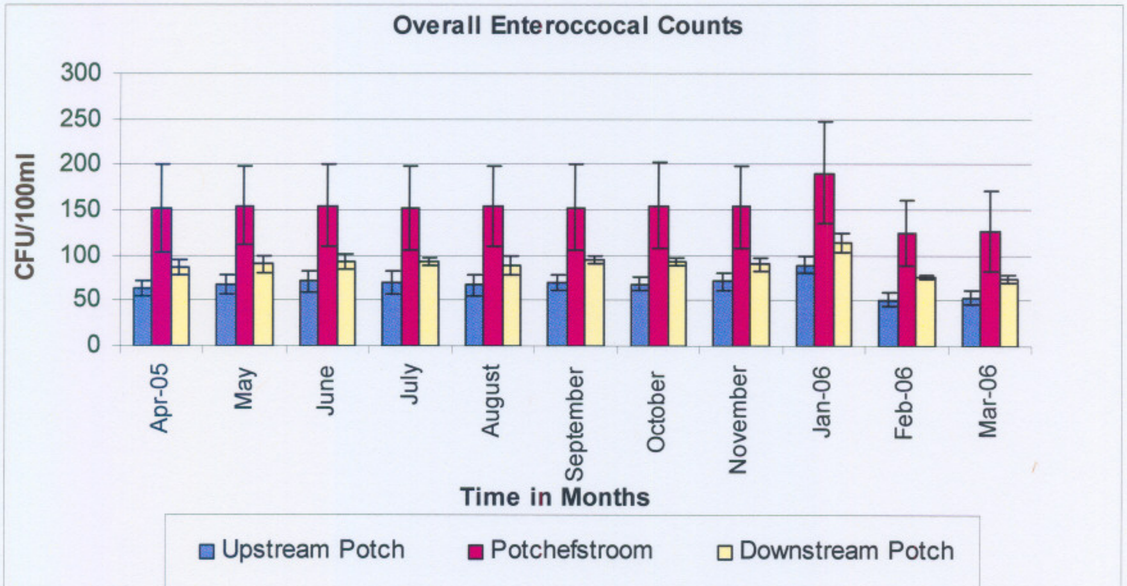



**Figure 4.4 (b):** Seasonal concentrations of faecal coliform bacteria collected in the Mooi River during the dry and wet season.

Relatively large numbers of faecal coliforms and enteroccoci per 100ml sample (cfu's/100ml) were detected during all seasons as depicted in Figure 4.4 and 4.5. The results also indicate the higher levels of faecal coliform bacteria and enteroccoci at certain urban sampling sites compared to upstream and downstream sites. Location 4, 5 and 6 (Potchefstroom sites) may be associated with urban runoff discharges from Potchefstroom to the river, had highest faecal indicator levels. These sites are downstream from the Wasgoed Spuit Tributary.



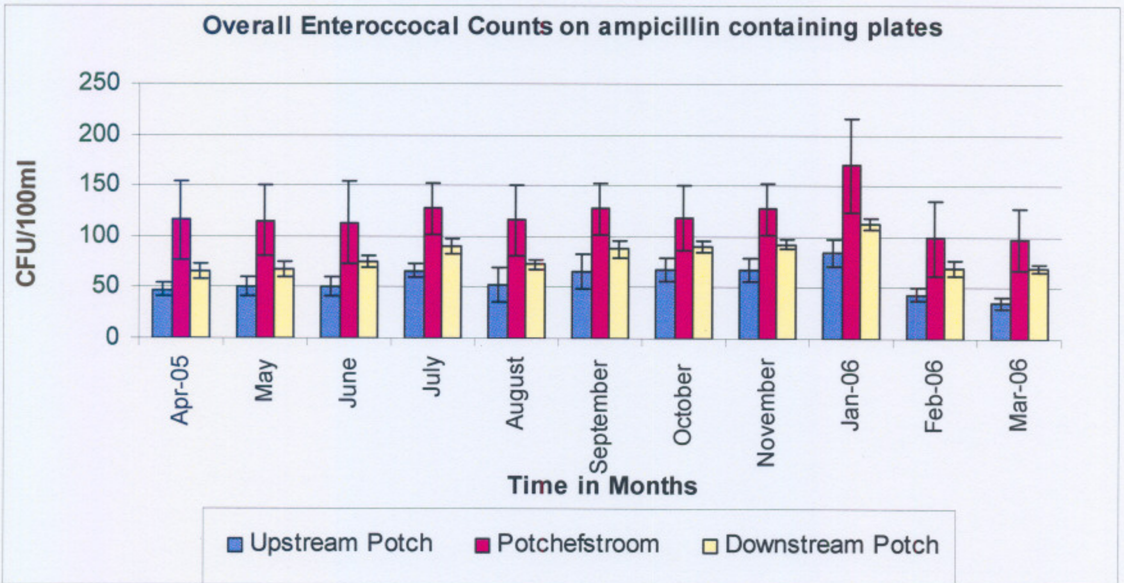



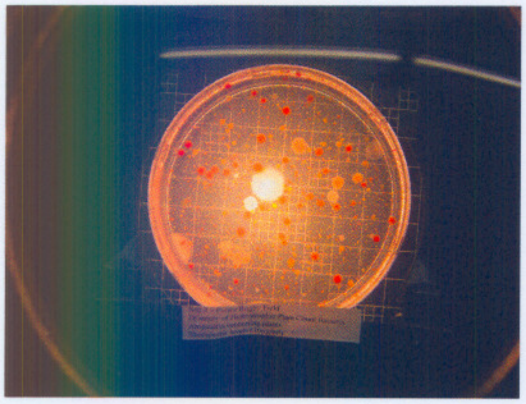

**Figure 4.5:** Seasonal concentrations with and without antibiotic of enteroccoci bacteria data collected in the Mooi River during the dry and wet season.

There was a significant positive correlation ($r$ = 0.933; $p$<0.05) of the lower faecal coliform levels in the upstream and downstream segment of the Mooi River when compared to the higher levels in the Potchefstroom urban segment of the river (Appendix Table B.6). Similar trends were observed for enteroccoci levels.

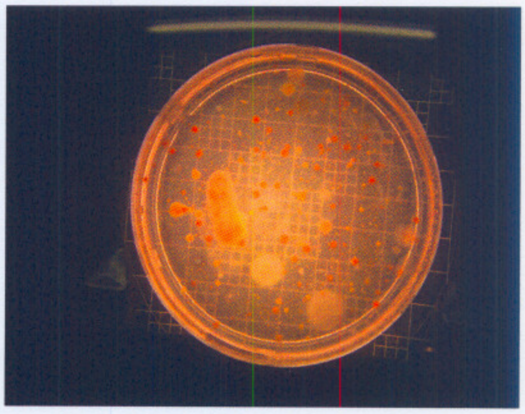
Most of the faecal coliforms and enteroccoci values obtained were according to the South African and international guidelines in the ranges for recreational and agricultural use (DWAF, 1998; DOH, 1998; WHO, 2004). Site-specific as well as cumulative inputs from a variety of non-point sources are likely to be responsible for the lower upstream, high Potchefstroom urban area and elevated downstream levels of the indicator bacteria measured in this water system (Figures 4.4 and 4.5). These results demonstrate the potential existence of the faecal pollution gradient along the Mooi River system in terms of faecal coliform and enteroccoci levels enumerated.

### 4.2.2 Overall heterotrophic plate count (HPC) and total coliforms (TC) bacteria

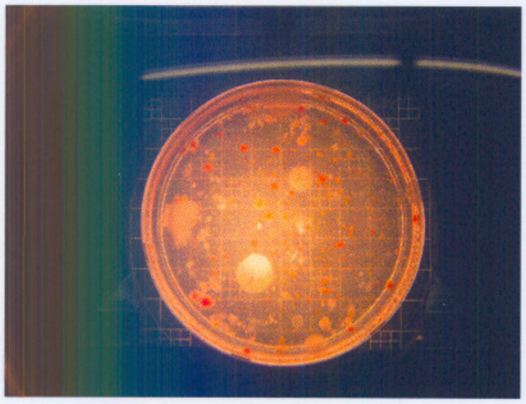
Diverse groups of heterotrophic bacteria were resistant to ampicillin as shown by the representative site pictures in Figure 4.6. The changes detected in heterotrophic bacterial counts were similar to those of other indicator micro-organisms (Table 4.1). There were no marginal and log differences in HPC enumerated on media without and with ampicillin. Therefore, there was no pollution gradients observed in terms of heterotrophic bacteria.



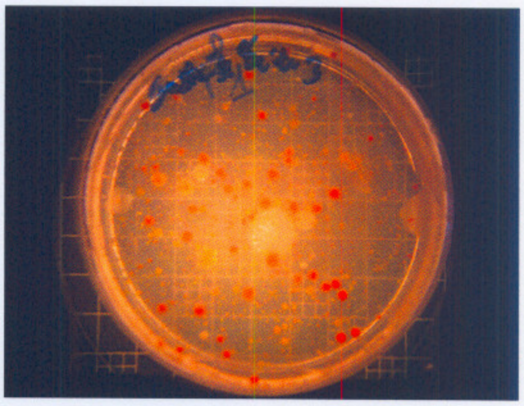
Site 5 – Opposite police rugby field



Site 6 – Opposite River Walk mall



Site 4 – Downstream Wasgoed Spuit



Site 3 – at the Potch dam weir

**Figure 4.6:** Examples showing the diversity of ampicillin resistant heterotrophic plate count bacteria isolated from the Mooi River

The proportion of ampicillin resistant total coliforms was ± 50% of the overall total coliform population (Table 4.1). However the levels of total colifoms in the Potchefstroom urban area as well as downstream from Potchefstroom demonstrated a different trend. The total coliforms enumerated on ampicillin containing plates were 60 % of the overall levels of total coliforms enumerated on non- antibiotic containing plates as shown in Table 4.1. Therefore, according to these results the may be potential existence of the faecal pollution gradient along the Mooi River system in terms of total coliforms enumerated.

**Table 4.1:** Seasonal levels of heterotrophic plate count and total coliform bacteria collected in the Mooi River during the dry and wet season (April 2005 to March 2006). Values are averages of triplicates and E+20 = $10^{20}$.

| | **Overall heterotrophic plate count bacteria (HPC) cfu/ml** | | | | | | **Overall total coliform (TC) cfu/100ml** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **HPC overall** | | | **HPC on ampicillin containing media** | | | **TC without ampicillin** | | | **TC on media containing ampicillin** | | |
| **DATE** | **Up** | **Potch** | **Down** | **Up** | **Potch** | **Down** | **Up** | **Potch** | **Down** | **Up** | **Potch** | **Down** |
| **Apr-2005** | 2.84E+20 | 6.09E+20 | 3.85E+20 | 2.13E+20 | 5.00E+20 | 3.00E+20 | 80.7 | 158 | 106 | 40.3 | 101 | 66.5 |
| **May** | 3.25E+20 | 6.78E+20 | 4.75E+20 | 2.30E+20 | 4.80E+20 | 3.00E+20 | 104 | 164 | 136 | 49.3 | 104 | 68.3 |
| **June** | 3.36E+20 | 7.67E+20 | 4.80E+20 | 2.30E+20 | 4.91E+20 | 3.06E+20 | 101 | 164 | 133 | 50.3 | 101 | 68.2 |
| **July** | 3.87E+20 | 7.40E+20 | 4.89E+20 | 2.13E+20 | 5.01E+20 | 3.11E+20 | 96.7 | 166 | 132 | 41.3 | 107 | 64.4 |
| **August** | 3.41E+20 | 7.32E+20 | 4.89E+20 | 2.39E+20 | 5.08E+20 | 3.37E+20 | 97.3 | 165 | 120 | 50.1 | 108 | 71.5 |
| **September** | 4.04E+20 | 6.98E+20 | 5.01E+20 | 2.16E+20 | 5.18E+20 | 3.37E+20 | 93.7 | 174 | 130 | 50.0 | 101 | 71.1 |
| **October** | 4.13E+20 | 6.74E+20 | 4.89E+20 | 2.52E+20 | 5.08E+20 | 3.53E+20 | 92.7 | 171 | 125 | 44.0 | 103 | 60.4 |
| **November** | 3.95E+20 | 7.23E+20 | 5.00E+20 | 2.28E+20 | 4.99E+20 | 3.40E+20 | 87.7 | 153 | 128 | 34.3 | 97.5 | 51.3 |
| **Jan-06** | 5.07E+20 | 8.33E+20 | 6.99E+20 | 2.97E+20 | 6.33E+20 | 4.27E+20 | 95.7 | 190 | 132 | 60.2 | 133 | 86.2 |
| **Feb-06** | 2.83E+20 | 4.34E+20 | 3.80E+20 | 1.28E+20 | 3.85E+20 | 1.67E+20 | 59.7 | 137 | 100 | 26.3 | 72.8 | 48.4 |
| **Mar-06** | 2.12E+20 | 4.51E+20 | 2.87E+20 | 1.28E+20 | 3.67E+20 | 1.95E+20 | 55.3 | 135 | 90.3 | 18.3 | 66.5 | 36.3 |

### 4.2.3 Faecal coliform (FC)/faecal enteroccoci (FE) ratio

Identification of sources of faecal pollution using general faecal coliforms to faecal enteroccoci ratio is based on the premise that a ratio of ≥4.0 would indicate human pollution and a ratio of ≤0.6 would indicate nonhuman pollution (Gildreich and Kenner, 1969). Table 4.2 and 4.3 shows that the faecal coliform /faecal enteroccoci ratio without ampicillin ranged from 0.829 to 1.15, these values are between 0.6 and 4.0. However, when the ratios of ampicillin resistant faecal coliform to enteroccoci were analyzed values were generally 0.6 or smaller (Table 4.3). Both these sets of results suggest that non-human sources contributed greater towards faecal pollution.

### 4.2.4 Statistical analysis of the total coliforms and faecal coliform to enteroccoci ratio

The statistical analysis of the various locations, revealed that the total coliforms showed significant ($p<0.05$) strong ($r=0.882$) correlation among upstream- upstream, Potch-Potch, downstream-downstream paired segments as shown Appendix-Table B.7. The faecal coliform to enteroccoci ratio showed that there was insignificant ($p>0.05$) strong ($r>0.882$) correlation among the upstream- Potchefstroom paired segments, whereas faecal/enteroccoci ratio on ampicillin contaning plates indicated significant strong correlation in Potch-downstream paired segment of the Mooi River. The faecal/enteroccoci ratio with/without ampicillin showed strong significance and positive correlation in Potch-Downstream segments as presented in Appendix B (Table B.7).

**Table 4.2:** Faecal coliform/faecal enteroccoci ratio without ampicillin over a one year period (April 2005 to March 2006).

| **Time** | **Overall faecal coliform (FC) counts (cfu/100ml)** | | | **Overall enteroccoci counts (cfu/100ml)** | | | **faecal coliform/enteroccci** | | |
|---|---|---|---|---|---|---|---|---|---|
| **DATE** | **Up** | **Potch** | **Down** | **Up** | **Potch** | **Down** | **Up** | **Potch** | **Down** |
| **Apr-2005** | 61.3 | 153 | 100 | 63.7 | 152 | 87.2 | 0.962 | 1.010 | 1.151 |
| **May** | 62.4 | 153 | 100 | 67.7 | 155 | 90.4 | 0.922 | 0.987 | 1.112 |
| **June** | 69.7 | 156 | 104 | 71.3 | 154 | 93.1 | 0.977 | 1.010 | 1.120 |
| **July** | 65.3 | 153 | 104 | 69.3 | 152 | 93.5 | 0.942 | 1.010 | 1.110 |
| **August** | 64.7 | 156 | 98.5 | 67.3 | 154 | 89.6 | 0.961 | 1.010 | 1.100 |
| **September** | 71.6 | 153 | 100 | 70.3 | 153 | 95.3 | 1.020 | 1.000 | 1.201 |
| **October** | 61.3 | 153 | 98.3 | 68.3 | 155 | 93.8 | 0.897 | 0.987 | 1.201 |
| **November** | 59.3 | 149 | 90.3 | 71.5 | 153 | 90.6 | 0.829 | 0.973 | 0.997 |
| **Jan-06** | 102 | 198 | 141.5 | 89.6 | 191 | 115 | 1.140 | 1.040 | 1.230 |
| **Feb-06** | 46.4 | 124 | 73.8 | 51.6 | 125 | 76.3 | 0.899 | 0.992 | 0.967 |
| **Mar-06** | 54.5 | 120 | 76.0 | 53.3 | 127 | 73.7 | 1.020 | 0.944 | 1.030 |

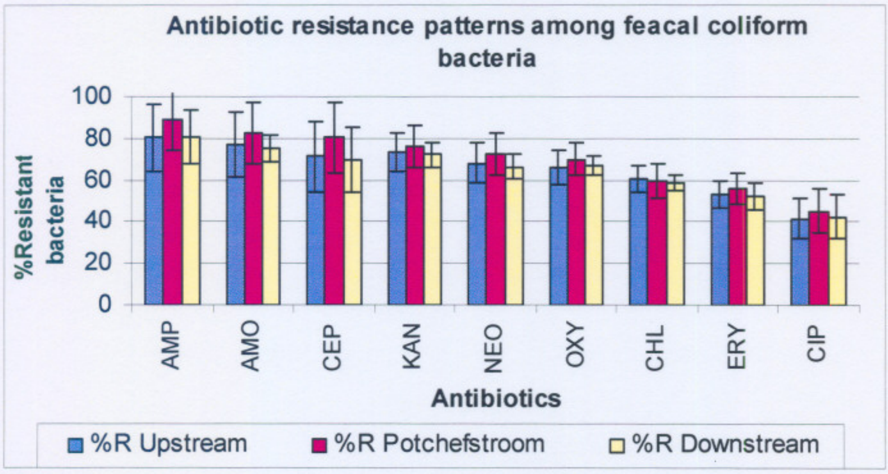
**Table 4.3**: Faecal coliform/faecal enteroccoci ratio on ampicillin containing plates over a one year period.

| **Time** | **Faecal coliform (Fc) on ampicillin containing media (cfu/100ml)** | | | **Enteroccoci(Ent) on ampicillin containing media (cfu/100ml)** | | | **Faecal coliform/Enteroccci ratio on ampicillin containing media** | | |
|---|---|---|---|---|---|---|---|---|---|
| **DATE** | Up | Potch | Down | Up | Potch | Down | Up | Potch | Down |
| **Apr-2005** | 29.4 | 77.5 | 45.7 | 47.7 | 116 | 66.6 | 0.616 | 0.668 | 0.686 |
| **May** | 31.0 | 81.8 | 72.4 | 50.3 | 115 | 68.8 | 0.616 | 0.711 | 1.201 |
| **June** | 38.0 | 81.5 | 52.6 | 51.3 | 113 | 76.5 | 0.740 | 0.721 | 0.688 |
| **July** | 31.6 | 78.6 | 53.8 | 66.7 | 127 | 90.3 | 0.474 | 0.619 | 0.596 |
| **August** | 30.7 | 79.7 | 54.6 | 53.3 | 115 | 73.1 | 0.576 | 0.693 | 0.747 |
| **September** | 29.0 | 82.0 | 52.5 | 65.7 | 127 | 88.3 | 0.441 | 0.645 | 0.595 |
| **October** | 35.0 | 81.6 | 64.8 | 67.6 | 119 | 90.2 | 0.518 | 0.686 | 0.718 |
| **November** | 30.7 | 84.5 | 57.7 | 67.7 | 127 | 93.4 | 0.453 | 0.920 | 0.618 |
| **Jan-06** | 43.7 | 100 | 71.4 | 85.5 | 171 | 112 | 0.511 | 0.585 | 0.637 |
| **Feb-06** | 24.6 | 63.7 | 36.9 | 43.6 | 98.7 | 70.8 | 0.564 | 0.645 | 0.521 |
| **Mar-06** | 26.7 | 65.0 | 45.7 | 35.6 | 97.5 | 70.1 | 0.750 | 0.667 | 0.652 |

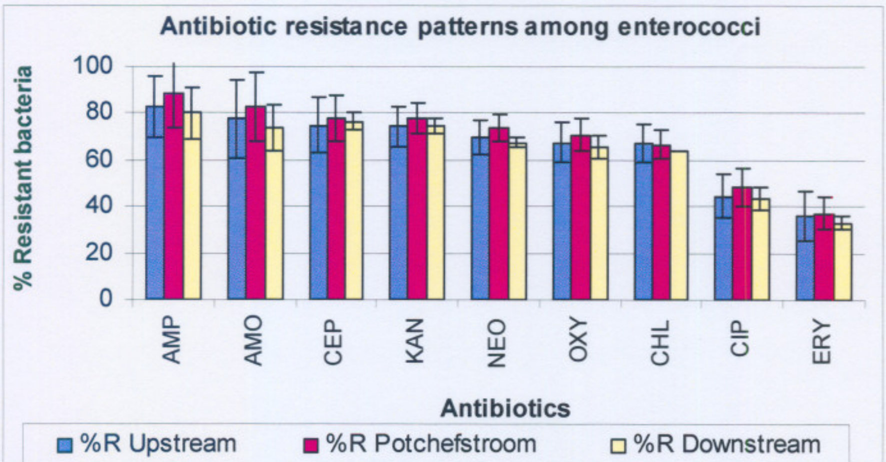
## 4.3 ANTIBIOTIC RESISTANCE ANALYSIS AMONG FAECAL COLIFORMS AND ENTEROCCOCI ISOLATES

### 4.3.1 Antibiotic resistance patterns

The antibiotic resistance profile of each isolate was determined and this was used to calculate the percentage of faecal coliforms and enteroccoci that were resistant to each antibiotic.




(a)




(b)

**Figure 4.7:** Antibiotic resistant patterns of faecal coliform (a) and enteroccoci (b) isolates from segments upstream, Potchefstroom and downstream.

Results for faecal coliforms and enteroccoci isolates from upstream, Potchefstroom, and downstream that were resistant are represented in Figure 4.8. Antibiotic resistance profiles of the isolates are presented in Appendix C (Table C.4 & Table C.5). Variation in the percentage resistant between isolates of the various sites would give an indication of antibiotics or other antimicrobial usage in the vicinity of site. There were large numbers (+80%) of bacterial resistant to ß-lactam antibiotics. This was expected if one considers results in the previous sections (Section 4.1 and 4.2). More than 60% of enteroccoci were resistant to vancomycin, oxy-tetracycline, neomycin, chloramphenicol and between 40 and 50 % were resistant to erythromycin, and ciproflaxin. Between 60-80 % of the faecal coliform were resistance to kanamycin, cephalothin, neomycin, oxy-tetracycline, ciproflaxin, and 20 to 40 % resistant to chloramphenicol and erythromycin.

### 4.3.2 MAR phenotypes of faecal coliforms and enteroccoci from the river water

A total of 63 out of 171 enteroccoci isolates and 50 out of 182 faecal coliform isolates were resistant to more than 4 antibiotics (Table 4.4). The highest levels of resistant bacteria were observed for the Potchefstroom urban area compared to upstream and downstream levels (Table 4.4 and 4.5). The most predominant faecal coliform and enteroccoci resistance phenotypes Amp-Amo-Cep- Kan and Amp-Amo-Cep respectively. The latter formed the basis formed the basis of all the phenotypes obtained. The most common multiple antibiotic resistance pattern for an individual faecal coliform and enteroccoci isolates was Amp-Amo-Cep-Kan-Neo-Oxy and Amp-Amo-Cep-Kan-Neo in 4.14 % and 8.20% respectively. The phenotypes Amo-Cep-Kan-Neo, Amp-Amo-Cep-Kan-Neo-Cip, Amp-Amo-Cep-Kan-Neo-Van, Amp-Cep-Kan-Neo-Oxy-Chl-Cip, Amp-Cep-Kan-Neo-Oxy-Chl-Cip–Ery, Amp-Amo-Cep-Kan-Neo-Oxy-Chl-Van-Cip–Ery were other dominating phenotypes which occurred at high frequency (5.92%, 2.92%, 7.01%, 4.70%, 3.52%, 4.70%) among enteroccoci isolates. These prevalent MAR patterns from antibiotic resistant phenotypes were determined and are given in Table 4.4. In Appendix C (Table C.5 & Table C.6) a complete list of the phenotype antibiotic resistance profiles is provided. The results in Table 4.4 and 4.5 are thus summaries of phenotype antibiotic resistance profiles.

**Table 4.4:** Most prevalent antibiotic resistance patterns for individual faecal coliform isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source.

| Faecal coliform phenotypes | | | | | | | | No. of upstream Isolates | No. of Potch Isolates | No. of downstream Isolates | Total | Percentage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amp | Amo | Cep | Oxy | | | | | 0 | 3 | 1 | 4 | 2.40% |
| Amp | Amo | Cep | Cip | | | | | 0 | 4 | 0 | 4 | 2.40% |
| Amp | Kan | Neo | Oxy | | | | | 0 | 3 | 1 | 4 | 2.40% |
| Amp | Amo | Cep | Kan | Ery | | | | 0 | 3 | 1 | 4 | 2.40% |
| Amp | Amo | Cep | Oxy | Ery | | | | 2 | 1 | 1 | 4 | 2.40% |
| Amp | Amo | Cep | Neo | Oxy | Chl | | | 0 | 7 | 0 | 7 | 2.40% |
| Amp | Amo | Kan | Neo | Oxy | Chl | | | 0 | 5 | 3 | 8 | 4.14% |
| Amp | Amo | Cep | Kan | Oxy | Cip | Ery | | 0 | 4 | 0 | 4 | 2.40% |
| Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | 2 | 2 | 3 | 7 | 4.14% |
| Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Ery | 2 | 2 | 0 | 4 | 2.40% |

**Table 4.5:** Most prevalent antibiotic resistance patterns for individual enteroccoci isolates resistant to more than 4 antibiotics. Percentages were obtained from fraction of the number of isolates observed that were resistant to more than 4 antibiotics and total number of isolates from the sample source.

| Enteroccoci phenotypes | No. of upstream Isolates | No. of Potchefstroom Isolates | No. of downstream Isolates | Total | Percentage |
|---|---|---|---|---|---|
| Amo Cep Kan Neo | 1 | 1 | 8 | 10 | 5.92% |
| Amp Amo Cep Kan Neo | 1 | 9 | 4 | 14 | 8.20% |
| Amp Amo Cep Kan Neo Cip | 2 | 2 | 1 | 4 | 2.92% |
| Amp Amo Cep Kan Neo Van | 4 | 6 | 3 | 13 | 7.01% |
| Amp Cep Kan Neo Oxy Chl Cip | 2 | 6 | 0 | 8 | 4.70% |
| Amp Cep Kan Neo Oxy Chl Cip Ery | 2 | 3 | 1 | 6 | 3.52% |
| Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery | 0 | 8 | 0 | 8 | 4.70% |

The predominant seven faecal coliform and eight enteroccoci phenotypes originated from all three Potchefstroom urban sites. However, a greater proportion of the isolates that had these phenotypes were from either Potchefstroom sites or those downstream from Potchefstroom (Appendix C: Table C.5 & Table C.6).

### 4.3.3 Cluster analysis

Cluster analysis is used as a tool to determine the commonness and resolve differences between the bacteria isolated from different sample sources. Dendograms were constructed using the antibiotic inhibition zone diameter data (Appendix C: Table C.4 & Table C.5) obtained for all faecal coliform and enteroccoci isolates and are presented in Figure 4.8 and 4.9, respectively. Such dendograms may link samples with a common antibiotic exposure history.

(a) Faecal coliform cluster analysis

Analysis of the dendogram in Figure 4.8 below revealed patterns of association between Potchefstroom, downstream and upstream isolates.

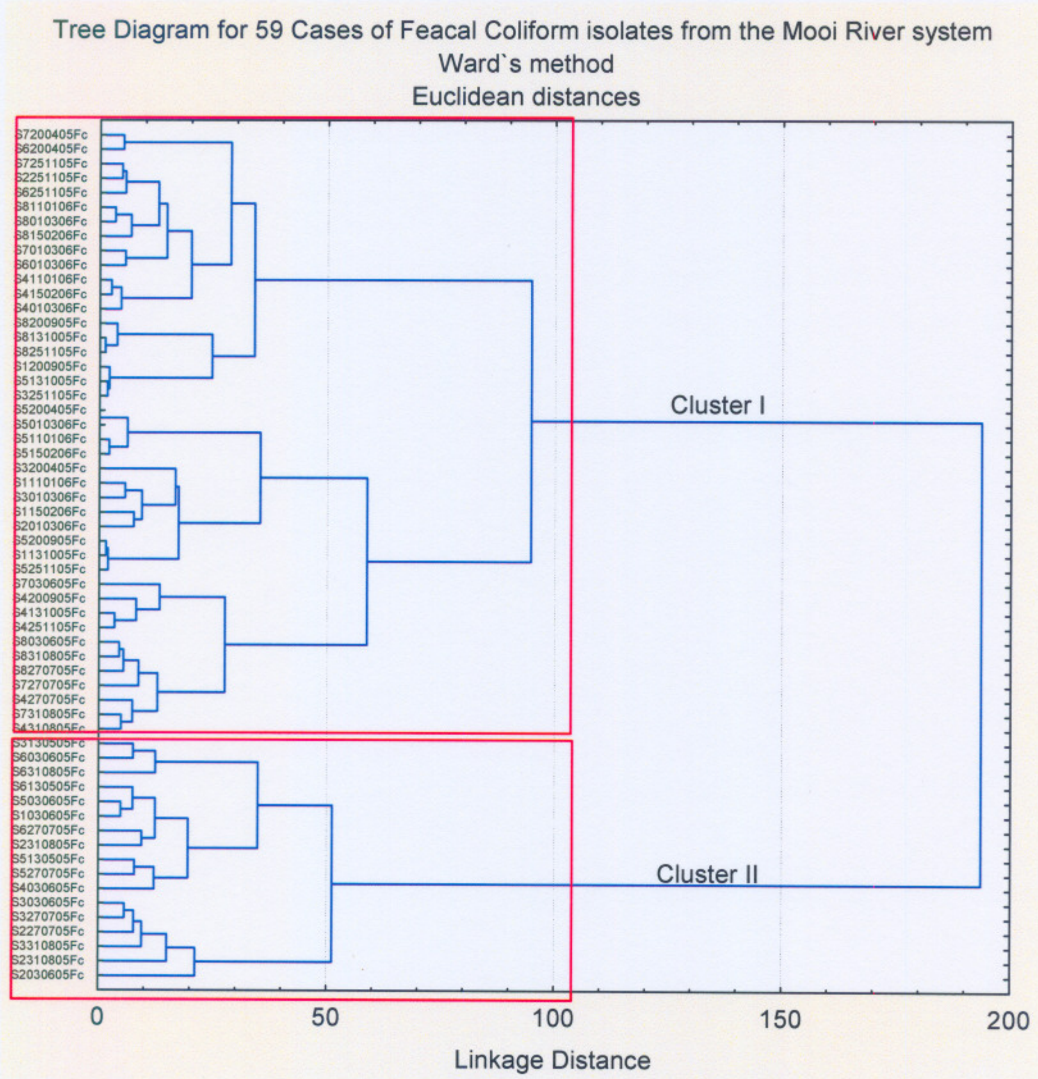



**Figure 4.8:** Dendograms showing relatedness of faecal coliforms isolated from the Mooi river system (upstream, Potchefstroom and downstream segments).

**Table 4.6:** Table indicating results of analysis of clusters from Figure 4.9. The number (N) and the percentage (%) of faecal coliform isolates from all the sites are indicated.

| **Microbial type** | **Sample type/ Site name** | **Cluster I , N= 42** | **Cluster II, N= 17** |
|---|---|---|---|
| **Faecal Coliform** | **Upstream** | 9(21.5%) | 8(47.0%) |
| | **Potchefstroom** | 18(42.9%) | 9(53.0%) |
| | **Downstream** | 15(35.7%) | 0(0%) |

Although clusters I (larger cluster) and II contained isolates from upstream, Potchefstroom and downstream, cluster I contained a large proportion of isolates from Potchefstroom and downstream from Pothefstroom water samples (Figure 4.8 and Table 4.6). From these results it is evident that the Potchefstroom urban area impacted on the downstream segment. In cluster II a large portion of samples from upstream clustered with Potchefstroom samples indicating that the contribution of faecal pollution from Potchefstroom and also from the upstream sources. There were no downsream isolates in cluster II.

(b) Enteroccoci cluster analysis

The dendogram for the enterococci isolates could not resolve differences between samples from different sources (Figure 4.9 and Table 4.7).

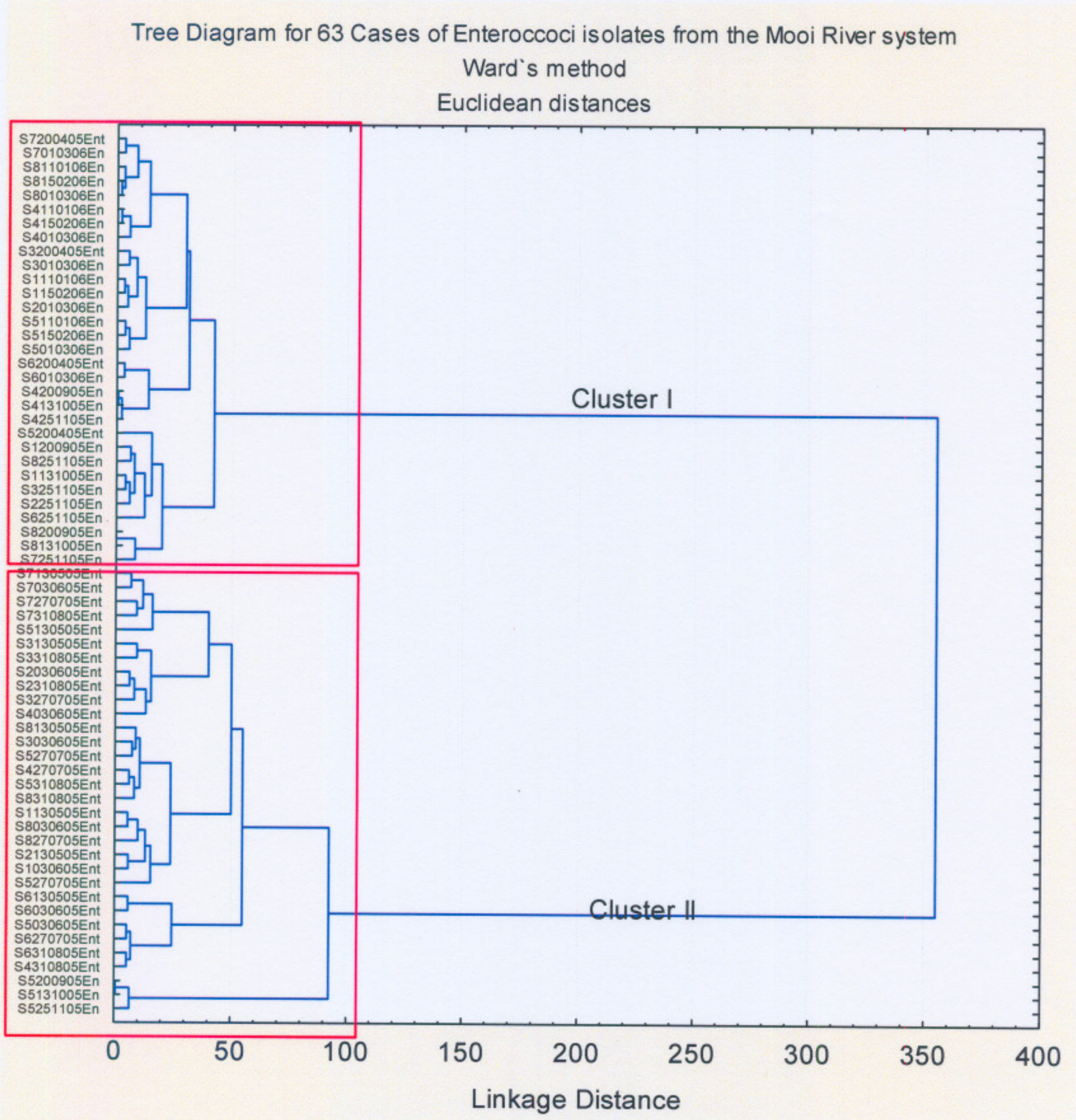



**Figure 4.9:** Dendograms showing relatedness of enteroccoci isolated from the Mooi river system (upstream, Potchefstroom and downstream segments).

**Table 4.7**: Table indicating results of cluster analysis from Figure 4.9. The number (N) and the Percentage (%) of enteroccoci isolates from all the sites are indicated.

| Microbial type | Sample type/ Site name | Cluster I , N = 31 | Cluster II, N= 32 |
|---|---|---|---|
| **Enteroccoci** | Upstream | 10(32.2%) | 10(31.3%) |
| | Potchefstroom | 13(41.9%) | 14(43.8%) |
| | Downstream | 8(25.8%) | 8(25.0%) |

Cluster I & II contained equal proportions of enteroccoci isolates from upstream, Potchefstroom and downstream segments of the river. Therefore mixed sources of faecal pollution were indicated by both clusters. Such results are indicative of similar antibiotic exposure histories along the river continuum.

### 4.3.4 Multiple antibiotic resistance (MAR) index

The MAR index for each site was calculated by using the formula presented in Materials and Methods (Section 3.6.2). Results are presented in Table 4.8.

**Table 4.8:** Multiple antibiotic resistance (MAR) indices for faecal coliform and enteroccoci isolates per river segment.

| Organism | Upstream | Potchefstroom | Downstream |
|---|---|---|---|
| **Faecal Coliform** | 0.18 | 0.32 | 0.23 |
| **Enteroccoci** | 0.18 | 0.28 | 0.15 |

Among the 6 group MAR indices, the highest indices were for the Potchefstroom urban area (0.32 for Faecal coliform and 0.28 for enteroccoci). The values for faecal coliform and enteroccoci upstream from Potchefstroom were both 0.18 and downstream 0.23 and 0.15 respectively. These results also demonstrate the existence of the faecal pollution gradient along the Mooi River system.

## 4.4 SUMMARY OF RESULTS

The physico-chemical parameters which were determined were temperature, pH, total dissolved solids (TDS), electro-conductivity, total dissolved oxygen (DO), chemical oxygen demand (COD), faecal indicators included faecal coliform, enteroccoci, heterotrophic bacteria and total coliforms. Results indicated seasonal and locational variation in most of the physico-chemical parameters and faecal indicators studied. Rainfall was an important factor which strongly influenced the characteristics of these parameters. Also temperature, pH and rainfall influenced the elevated levels of the microbiological indicators observed. High levels of faecal indicator bacteria were observed in the Potchefstroom urban area when compared to upstream and downstream segments, with exception of heterotrophic bacteria were there no marginal and log differences in heterotrophic plate count enumerated on media without and with ampicillin. The results of faecal coliform to enteroccoci ratio suggested that non-human sources contributed greater towards faecal pollution. The highest levels of antibiotic resistant bacteria were observed for the Potchefstroom urban area compared to upstream and downstream levels. Faecal coliform cluster analysis revealed patterns of association between Potchefstroom, downstream and upstream isolates. Enteroccoci cluster analysis could not resolve differences between samples from different sources. River water isolates from the Potchefstroom sites contained faecal coliform and enteroccoci that exhibited resistance to multiple antibiotics. Among the 6 group MAR indices, the highest indices were for the Potchefstroom urban area (0.32 for Faecal coliform and 0.28 for enteroccoci). Urban-Rural gradient were recognized in terms of faecal indicator bacteria such total coliform, faecal coliforms and enterococci and also in terms of MAR index.

# CHAPTER 5

# DISCUSSION AND CONCLUSIONS

## 5.1 INTRODUCTION

South Africa is a water scarce country where the demand for water exceeds its availability and most of its fresh water resources are heavily impacted by decades of urbanization followed by population and economic growth (Water Wheel, 2006). The problem that the country faces that needs greater priority is the faecal contamination of these fresh water resources. There are few studies conducted to tackle and solve this problem. Surface water contamination enhances the risk of human exposure to pathogenic enteric bacteria of intestinal origin and has been raising serious concerns in recent years (Shehane and Harwood, 2005). A motivation for this study was prompted by the fact that faecal pollution from human sources was implicated in the occurrence of pollution "hot spots" associated with urbanization that contributed to the deterioration of water quality (Webster *et al.*, 2004). This study would be of importance, since rapid urbanization is expected in Potchefstroom, and elsewhere in the North-West Province in future (Cilliers *et al.*, 2003). With recognition of the ecological, economic, social and cultural significance of rivers and their sensitivity to anthropogenic activities, it is essential that these river systems be managed in a sustainable manner (Newham *et al.*, 2004). To do so, base-line data obtained from the present study may provide solution oriented approach in future environmental management strategies.

Therefore, water quality monitoring and assessments using physical and microbiological variables were of paramount importance in the present study to identify the river confluence vulnerable to the pollution impacts of urbanization (Holland *et al.*, 2004). The Mooi River in North-West Province of South Africa presented the ideal setting due to increased development in the Potchefstroom urban area particularly in the vicinity of the river. The question posed by this study was to what extent urbanization has made an impact in the development of antibiotic resistant bacteria and if the antibiotic resistance data obtained could be used as a pollution biomonitoring tool.

## 5.2 LEVELS OF PHYSICO-CHEMICAL PARAMETERS

According to South African guidelines, physico-chemical parameters are regarded as good indicators of physical and chemical quality of river water (DWAF, 1998; DOH, 1998; WRC, 1998). The physico-chemical parameters of the Mooi River were compared with other results from a previous study of the Mooi River and other previous studies of the rivers in South Africa, Africa, worldwide, and showed distinct similar and dissimilar trends.

The South African rivers that were of interest and compared to the Mooi River were the Chunies River in Limpopo Province (Germs *et al.*, 2004), Mhlathuze River in KwaZulu-Natal (Bezuidenhout *et al.*, 2002), water sources in Venda (Obi *et al.*, 2002) and the African river was the Marimba River in Zimbabwe (Nhapi and Tirivarombo, 2004). The international water resources were Gaza Beach in Gaza Strip (Elmanama *et al.*, 2005), and Tidal creek in South Carolina (Holland *et al.*, 2004). All the sampling sites in the Mooi River were selected and considered to represent a range of water quality and the impact of some point and non-point sources. The middle urban segment of the Mooi

River was nominated as the focal point (reference) in this investigation of faecal pollution.

The results of physico-chemical variables for various sites which were selected and sampled in the Mooi River system at the same period and some at the same sites as the present study were indicated and grouped into river segments in Table 2.1 in Section 2.2 in the literature review Chapter 2 (De la Rey *et al.*, 2004). The physico-chemical parameters of this previous study of the Mooi River as explained with other previous studies of river systems, demonstrated similar and dissimilar trends when compared to those of the present study. This may be due to the fact that most of the sites which were selected and sampled in the previous study were not in precisely the same locations as in the current study. The differences may also be due to the changes in the riverine health within the short space of time. Faecal indicators, rainfall and downstream from Potchefstroom sites were not taken into consideration in the observations of the previous study and turbidity, phosphates were also not part of the present study.

In the present study the only physico-chemical parameters which were determined were temperature, pH, chemical parameters were total dissolved solids (TDS), eletroconductivity dissolved oxygen (DO) and chemical oxygen demand (COD). Almost all the sites which were monitored over a one year period included a rainy and a non-rainy season, clearly demonstrated locational trends with progressive changes in seasonal patterns. Similar trends were observed during interpretation of the results of the previous studies on river systems which were mentioned in the first paragraph of this section. In addition, rainfall was an important factor which strongly influenced the characteristics of the physico-chemical parameters in the present study and also in some of the previous studies. In contrast to the present study, seasonal variations were not taken into consideration in some of these previous studies (Obi *et al.*, 2002; Germs *et al.*, 2004).

In the present study, the monthly rainfall during the wet season ranged from 13.2 mm to 150.8 mm and during the dry season ranged from 0 mm to 3 mm (South African Weather Services, 2006). Average temperature of the river water during the entire monitoring period of this present study varied between 15 $^{0}$C and 25 $^{0}$C. The mean monthly pH values of the sites were within an acceptable range (7.6- 8.6) during the entire monitoring period (DWAF, 1996). Trends in water temperature and pH after each rain event were lower at the sites studied. The water temperatures of the Mhlathuze River in KwaZulu-Natal (Bezuidenhout *et al.*, 2002), Marimba River in Zimbabwe (Nhapi and Tirivarombo, 2004) and Tidal creek in South Carolina (Holland *et al.*, 2004) followed a typical summer and winter trends, similar to the trends observed in the present study. However, the average water temperatures of all these rivers during the summer period were higher than those observed in the Mooi River of the present study.

Electro-conductivity values of this present study were generally equivalent and directly proportional to the total dissolved solids and varied from138.25 to 319.87 µS/m and from 156.5 to 321.1 mg/L respectively. According to DWAF (1998) health effects from electro-conductivity in surface waters occur only at levels above 370µ µS.m$^{-1}$. In a study of Marimba River in Zimbabwe electro-conductivity levels were mostly higher (500

$\mu S.m^{-1}$) than those of the present study (319.87 $\mu S.m^{-1}$) and also much higher than guidelines of the Department of Water Affairs (1998) and Forestry and World Health Organization safety limits (2004).

Dissolved oxygen levels in water resources are influenced by several factors such as nutrients, turbidity, and faecal coliforms (Elmanama *et al.*, 2005). Schulze *et al.* (2001) showed that pristine unimpacted waters have the dissolved oxygen (DO) of 5 mg/L. In the present study relatively high dissolved oxygen levels (between 4 and 6 mg/l) were measured during the period May 2005 to September 2005 (non -rainy season). Dissolved oxygen decreased to 3 mg/l at almost all sites during the rainy season- October 2005 to April 2006 collection periods and the average was 3.2 mg/l which did not vary greatly. The highest levels of dissolved oxygen were detected in downstream sites, which is the location indicative of least faecal pollution and subsequent large amount of algal growth.

Low dissolved oxygen may be linked to the high levels of bacteria in the water. This observation is supported by evidence from a study by Coombes (2006). He proved that high dissolved oxygen and lower bacterial levels are indicative of better water quality favorable conditions for swimming (recreational) and agricultural use of water. Chemical oxygen demand (COD) is a measure of total utilizable organic matter. In the present lowest levels of COD were detected in the downstream sites. According to the data colleted during the dry and wet season in the present study, dissolved oxygen demand was inversely proportional to COD.

Most of the physical and chemical values obtained in this study were according to the South African and international guidelines in the ranges for recreational and agricultural use (DWAF, 1998; DOH, 1998; WHO, 2004). Therefore, during the entire monitoring period the river water was acceptable and fit for recreational and agricultural use, but not suitable for domestic purposes (DWAF, 1999; De la Rey *et al.*, 2004).

Unpolluted waters represent an important health-enhancing recreational resource. In terms of agriculture, there is no definitive possibility of contamination from vegetables and other crops eaten raw. Although the physicochemical parameters of the Potchefstroom urban segment of the river were higher than those obtained for the upstream and downstream sites. These results were also well within the DWAF guidelines for agricultural and recreational waters. There were no sudden fluctuations observed in the physico-chemical parameters, indicative of changing and adverse conditions in the Mooi River system of the present study for the entire monitoring period. The information collected on characteristics of these physico-chemical parameters in the Mooi River system presented in this study may be useful in future in setting standard guidelines for acceptable levels of human disturbances on river water quality.

## 5.3 MICROBIOLOGICAL OBSERVATIONS

Combinations of indicator organisms are more useful as a tool to identify the contaminant sources and predict the environmental impact of pollution (Whitlock *et al.*, 2002). In this study faecal coliform bacteria, enterococci bacteria, total coliform bacteria and heterotrophic bacterial population were used to determine the bacteriological quality of river water.

### 5.3.1 Faecal indicator bacterial levels

The faecal indicator bacterial levels bacterial levels presented in this study increased as the river developed from rural to urban area. Seasonal changes affected the water quality especially in Pothefstroom urban area. Local seasonal patterns, including rainfall facilitated the delivery of faecal indicator bacteria and urban runoff discharges into the Mooi River, leading to deterioration of water quality.

The rainfall events could negatively impact the rehabilitation and self-purification capacity of a river. Researchers showed that faecal coliforms, more specific *E. coli* counts in surface waters often peaked up after a rain event and thereafter, decreased or disappeared from the water column with time, through death and sedimentation processes (Webster *et al.*, 2004; Chigbu *et al.*, 2005; Elmanama *et al.*, 2005). In this study similar trends were observed during the rainy season, where elevated levels of faecal indicators were observed after rain events. Furthermore Crowther *et al.* (2002) demonstrated that faecal indicator levels could also be ascribed to runoff due to rainfall. In Crowther's demonstration elevated levels faecal coliform were detected during high flow rate conditions because of the microorganism's attachment to the surfaces of solids during runoff. Others studies also revealed the fact that the runoff from high rainfall figures influences the increased numbers of bacteria detected during the summer period (Nübel *et* *al.*, 1999; Lobitz *et al.*, 2000; Nishiguchi, 2000). It is possible that the rainfall events in the Potchefstroom urban area could also negatively impact on the Mooi River.

The Mooi River sampling sites 4, 5 and 6 (Potchefstroom sites) may be associated with urban runoff discharges from Potchefstroom to the river and had highest faecal indicator levels. These sites are downstream from the Wasgoed Spruit Tributary which receive polluted water from a wide variety of points and diffuse sources, including industrial effluents. The results also indicated that there were high levels of faecal coliform and enterococci bacteria isolated at Potchefstroom urban sites compared to upstream and downstream sites.

In the present study, there were no marginal and log differences in HPC enumerated on media with or without ampicillin. Contrary to the present study, the results of heterotrophic plate count bacteria detected in Mhlathuze River study during the summer season showed a peak which had a four log difference compared to the winter counts. The changes in heterotrophic bacterial counts in this study were similar to those of the indicator micro-organisms (Bezuidenhout *et al.*, 2002).

In a study of Mhlathuze River in KwaZulu-Natal (Bezuidenhout *et al.*, 2002) the total coliform population showed large fluctuations, whereas in the follow-up study done by Lin *et al.* (2004) elevated levels of total coliform counts were detected and there were no fluctuations observed. However in the present study, fluctuations in total coliform were not observed and higher levels were detected in the Potchefstroom urban area when compared to upstream and down stream segments.

The faecal indicator bacterial levels obtained in the present study were according to the South African and international guidelines in the ranges for recreational and agricultural use (DWAF, 1998; DOH, 1998; WHO, 2004).

### 5.3.2 Faecal coliform to faecal enteroccoci ratio

The faecal coliform- faecal streptococci ratio may be used to identify faecal pollution either as human or as nonhuman. In this study faecal enteroccoci instead of faecal streptococci were used. Identification of sources of faecal pollution using general faecal coliforms to faecal streptoccoci ratio was based on the premise that a ratio of $\geq$4.0 would indicate human pollution and a ratio of $\leq$0.6 would indicate non-human pollution (Gildreich and Kenner, 1969). The FC/FS ratios between 0.6 and 4.0 are difficult to interpret, and in the present study faecal coliform /faecal enteroccoci ratio ranged from 0.829 to 1.15. However, when the ratios of only ampicillin resistant faecal coliform to ampicillin enteroccoci were analyzed values were generally 0.6 or smaller. Both these sets of results suggest that non-human sources contributed greater towards faecal pollution. The findings of the present study were consistent with Jagals *et al.* (1995) in a study in South Africa, where the addition of human fecal material into an agriculturally impacted river showed a rise in the FC/FS ratios. However, further downstream the ratio fell to levels that would not indicate the presence of domestic sewage. Therefore based on this ratio alone, it is difficult to interpret the origin of faecal contamination due to the differential survival rates and other factors. This ratio is not reliable if the feacal contamination is not fresh, or if the concentrations of faecal streptococci are less than 100 cfu/100 ml (Sankaramakrishnan and Guo, 2005).

### 5.3.3 Antibiotic resistance and multiple resistance among faecal coliforms and enteroccoci isolates

In the present study faecal coliform and enteroccoci were indexed using antibiotics to determine sources of faecal pollution. It showed that Potchefstroom urban waters harbored higher percentages of antibiotic resistant bacteria than rural waters during entire monitoring period.

Previous studies have shown that low-level antibiotic resistance in bacteria can be found in pristine habitats suggesting that antibiotic resistance is of minimal importance under natural conditions. Further more, tolerance and resistance of bacteria increase proportionally along industrial contamination gradients (McArthur and Tuckfield, 1997). A study in Tillamook, Oregon has shown that when multiple antibiotics resistance (MAR) is indexed for specific sources, wild animals are generally low while human and livestock sources are much higher (Krumperman, 1983). As a result of the disposal of untreated sewage, industrial and agricultural waste into fresh waters resistance of naturally occurring bacteria to some antibiotics occurs.

Further studies have shown that faecal coliforms are the main carriers of resistance in faecal flora, associated with their source of pollution. In addition, largest numbers of antibiotic-resistant enterobacteriaceae were detected in biofilms from hospital wastewaters Furthermore, isolates from marsh sediments and urban runoff exhibited greater antibiotic resistance than isolates from other sources (Osterblad, 2002; Choi *et al.*, 2003; Schwartz, *et al.*, 2003).

In the present study there were large numbers (+80%) of resistant to ß-lactam antibiotics. More than 60% of enteroccoci were resistant to vancomycin, oxy-tetracycline, neomycin, chloramphenicol and between 40 and 50 % were resistant to erythromycin, and ciproflaxin. Between 60 and 80 % of the faecal coliform were resistant to kanamycin, cephalothin, neomycin, oxy-tetracycline, ciproflaxin, and 20 to 40 % resistant to chloramphenicol and erythromycin. Greater proportions of faecal coliform were resistant to multiple (more than 4) antibiotics than enteroccoci isolates. The highest resistance levels were observed for the Potchefstroom urban area compared to lower upstream and elevated downstream levels. The most predominant faecal coliform and enteroccoci resistance phenotypes Amp-Amo-Cep- Kan and Amp-Amo-Cep respectively formed the basis of all the phenotypes obtained. The most common multiple antibiotic resistance pattern for an individual faecal coliform and enteroccoci isolates was Amp-Amo-Cep-Kan-Neo-Oxy and Amp-Amo-Cep-Kan-Neo in 4.14 % and 8.20% respectively.

In the presents study cluster analysis was used as a tool to determine the commonness and resolve differences between the faecal coliform and enteroccoci isolated from different sample sources. The resulting dendograms for faecal coliform revealed patterns of association between Potchefstroom, upstream and downstream isolates. The dendogram for the enterococci isolates could not resolve differences between samples from different sources. The cluster patterns in the latter dendogram suggested the possibility of similar antibiotic exposure histories of all isolates. These trends observed in the present study are in accord with an earlier study which described the use of cluster analysis to categorize faecal coliform isolated from calves from several different farms (Berge *et al.*, 2003). A study of identification of sources of *Escherichia coli* in South Carolina estuaries using antibiotic resistance analysis supported the results of the present study (Webster *et al.*, 2004).

In this study among the 6 group MAR indices, the highest indices were for the Potchefstroom urban area (0.32 for faecal coliform and 0.28 for enteroccoci). The values for faecal coliform and enteroccoci upstream from Potchefstroom were both 0.18 and downstream 0.23 and 0.15 respectively. These results also demonstrate the existence of the faecal pollution gradient along the Mooi River system.

### 5.3.4 River health categorization

Ecological categorization of the state of the Mooi River system was described in terms of a health category ranging between good and poor water quality, as described in Section 2.1 and Table 2.1 in the literature review (DWAF, 1999). From this ecological classification, microbial categorization of the state of the Mooi River based on the faecal coliform and enteroccoci results can also be described in terms of health category ranging between good and poor water quality Appendix D (Table D.1).

The scenario observed during the interpretation of microbial data of the faecal coliform and enteroccoci bacteria in the current study in terms of water quality is that, the upper and lower reaches of the Mooi river water are the least polluted and the middle portion entirely urban segment is the water pollution "hot spot" as shown in Appendix D (Figure D.1). Urban-rural gradients were recognized in terms of faecal indicator bacteria such total coliform, faecal coliforms and enterococci and also in terms of MAR index.

The occurrence of antibiotic resistant faecal coliform and enteroccoci at all sites was of particular importance as antibiotic resistance is a key evaluation tool in determining faecal pollution sources along the urban and rural segments of the Mooi River system. Currently, limited data has been accumulated on the faecal coliforms and enteroccoci levels and their antibiotics resistance throughout the Mooi River system. Therefore, further work is needed to properly evaluate the effects of the tributaries entering the Mooi River system.

## 5.4 CONCLUSION

Physico-chemical parameters and faecal indicator bacteria were useful as tools in evaluation of physical, chemical and bacteriological quality of river water in the present study. Results indicated seasonal and locational variation in most of the physico-chemical parameters and faecal indicators studied. Rainfall was an important factor which strongly influenced the characteristics of these parameters. Also temperature, pH and rainfall influenced the elevated levels of the microbiological indicators observed. High levels of faecal indicator bacteria were observed in the Potchefstroom urban area when compared to upstream and downstream segments of the Mooi River system. The highest peaks of faecal indicator bacterial were during the rainy-season. The results of faecal coliform to enteroccoci ratio suggested that non-human sources contributed greater towards faecal pollution. This ratio is not reliable if the fecal contamination is not fresh, or if the concentrations of faecal streptococci are less than 100 cfu/100 ml.

Most of the physico-chemical parameters and faecal indicators obtained from the selected sampling sites of the Mooi River system during the entire monitoring period were according to the South African and international guidelines in the ranges for recreational and agricultural use. Therefore, the river water quality during the entire monitoring period was satisfactory for recreational and agricultural use, but not suitable for domestic purposes. It can be concluded that faecal pollution due to urbanization is a major contributor of antibiotic resistance bacteria in river system as observed in this study. Higher levels of antibiotic resistant bacteria were detected in the Potchefstroom urban sites were compared to lower upstream and elevated downstream levels. These observations are supported by various studies concerning antibiotic resistance in river systems. It can, be concluded that urbanization has had a definite impact on the levels of antibiotic resistant bacteria in the aquatic environment. Urban-rural gradients were recognized in terms of faecal indicator species (faecal coliforms, total coliforms and enterococci), but were not observed in terms of physico-chemical parameters and heterotrophic bacterial indicators. Therefore, it is evident that the river water quality in the Potchefstroom region is impacted by human contamination due to urbanization.

This study has shown the presence of antimicrobial resistant bacteria both in the urban and rural settings of the river. The fact that large numbers of resistant bacteria were isolated in the Potchefstroom urban area can not be ignored. Further research is urgently needed to assess the effects of pollution in all major tributaries of the Mooi River both environmental and urban. The use of antibiotic resistant bacteria as tools in faecal pollution source identification along urban and rural gradients in a river system may prove a valuable biomonitoring tool that needs to be refined and could be implemented in other rivers in the region, South Africa and Worldwide. Such base-line data may provide solution oriented approach in future environmental impact studies in setting standards and guidelines for the effects of human disturbances on river water quality to be reduced to acceptable levels.

## 5.5 RECOMMENDATIONS FOR FURTHER STUDY

The antibiotic resistance proved to be an invaluable tool in the investigation of faecal pollution along the urban rural gradients of the Mooi River system in this study. Given the lack of information regarding the role of antibiotic resistant bacteria as tools to assess the river water quality along urban rural gradients of a river system, there is a definitive need for further research. In addition it is thus also recommended that the following be in included in determining the river water quality.

(i) Extended evaluation of the effects of the tributaries entering the Mooi River system.

(ii) Sampling sites along the river system also have to be selected adjacent to disturbed environment in order to determine the effects of these areas.

(iii) Additional soil data has to be measured to determine the effects of metal on faecal indicators.

(iv) Identification and classification of fecal indicator bacteria to species level would be necessary

(v) The study period has to be extended over at least a two period in order to gain sufficient data.

Various currently used molecular techniques can also be implemented to identify and characterize bacteria.

## REFERENCES

BAUER, A.W., KIRBY, M.M., SHERRIS, J.L. & TURCK, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology.* 45: 493-496.

BERGE, A.C.B., ATWILL, E.R. & SISCHO, W.M. 2003. Assessing antibiotic resistance in faecal *Escherichia coli* in young calves using cluster analysis techniques. *Preventative Veterinary Medicine*, 61: 91 – 102.

BERNHARD, A.E., GOYARD, T., SIMONICH, M.T. & FIELD, K.G. 2003. Application of a rapid method for identifying faecal pollution sources in a multi-use estuary. *Water Research*, 37: (2003) 909–913.

BEZUIDENHOUT, CC., MTHEMBU, N., PUCKREE, T. & LIN, J. 2002. Microbiological evaluation of the Mhlathuze River, Kwazulu Natal (RSA). *Water SA*, 3(28): 280-286.

BLAIR, R.B. 1996. Land use and avian species diversity along an urban gradient, *Ecological Applications*, 6: (1996) 506–519.

CHEUNG, K. C., POON, B. H. T., LAN, C. Y. & WONG, M. H. 2003. Assessment of metal and nutrient concentrations in river water and sediment collected from the cities in the Pearl River Delta, South China. *Environmental and Public Health Management*, 52(9): 1431-1440.

CHIGBU, P., GORDON, S. & STRANGE, T.R. 2005. Feacal coliform bacteria disappearance rates in a north-central Gulf of Mexico estuary. *Estuarine, Coastal and Shelf Science*, 65(2005): 309 -318.

CHOI, S., WEIPING, C., BROWN, J., BECKER, S.J., HARWOOD, V.J. & JIANG, S.C. 2003. Application of enterococci antibiotic resistance patterns for contamination source identification at Huntington Beach, California. *Marine Pollution Bulletin*. 46: 48–755.

CILLIERS, S.S., BOUMAN H. & DREWES, J.E. 2003. Comparative Urban Ecological research in developing countries: settled areas in the North-West Province, South Africa. *Urban Ecology* (In press).

CLOETE, T.E. 2003. Resistance mechanisms of bacteria to antimicrobial compounds. *International Biodeterioration and Biodegradation*, 51: 277-282.

COOMBES P.J. ARGUE J.R & KUCZERA, G. Figtree Place: A Case Study in Water Sensitive Urban Development (WSUD). Web:www.w.sud.org/downloads/info%20Exchange%20&%20Lit/FIGTREE%20LAST21pd (Accessed 23 April 2006).

CROWTHER, J., KAY, D. & WYER, M.D. 2002. Feacal- indicator concentrations in waters draining lowland pastoral catchments in UK: relationships with land use and farming practices. *Water Research*, 36(2002):1725-1734.

DE LA REY P.A., TAYLOR, J.C. LAAS, A., VAN RENSBURG, L. & VOSLOO, A. 2004. Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5. *Water SA*, 30(3): 325 – 332.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). 1996. South African Water Quality Guidelines. 2nd ed. 1: Domestic Use 2: Agricultural Use.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF), DEPARTMENT OF HEALTH (DOH) AND WATER RESEARCH COMMISSION (WRC). 1998. *Quality of Domestic Water Supplies: Assessment Guide*, 1.

DEPARTMENT OF HEALTH. 1998. *Quality of Domestic Water Supplies: Assessment Guide*, 1.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). 1999. South African Water Quality Guidelines. *Aquatic Ecosystems*, 7.

DESMARAIS, T.R., SOLO-GABRIELE, H.M. & PALMER, C.J. 2003. Influence of soil on feacal indicator organisms in a tidally influenced subtropical environment. *Applied and environmental microbiology*, 68(3): 1165-1172.

DURAN, M., HAZNEDAROĞLU, B.Z. & ZITOMER, D.H. 2006. Microbial source tracking using host specific FAME profiles of feacal coliforms. *Water Research*, 40(1): 67-74.

ELMANAMA, A.A., AFIFI, S., BAHR, S. 2005. Seasonal and spatial variation in the monitoring parameters of Gaza beach during 2002-2003. *Environmental Research*, 1-9.

EVANSON, M. & AMBROSE, R.F. 2006. Sources and growth dynamics of feacal indicator bacteria in a coastal wetland system and potential impacts to adjacent waters. *Water Research*, 40(3): 475-486.

FUHRMAN, J. & NOBLE, R. T. 2000. Enteroviruses detected in the coastal waters of Santa Monica Bay, CA: low correlation to bacterial indicators. *Hydrobiologia,* 460:175-184.

GANNON, V.P.J., DUKE, G.D., THOMAS, T. J.E., VANLEEUWEN, J., BYRNE, J., JOHNSON, D., KIENZLE, S.W., LITTLE, J., GRAHAM, T. & SELINGER, B. 2005. Use of in-stream reservoirs to reduce bacterial contamination of rural watersheds. *Science of the Total Environment*, 348(2005): 19– 31.

GERMS, W., COETZEE, M.S., VAN RENSBURG, L. & MABOETA, M.S. 2004. A preliminary assessment of the chemical and microbial water quality of the Chunies River - Limpopo. *Water SA*, 30(2).

GILDREICH, E. E. & KENNER, B. A. 1969. Concepts of feacal streptococci in stream pollution. *Journal of Water Pollution: Control Fedding*, 41:R336-R352.

HANS, A. K. & MCDONNELL, M. J. 2006. Selecting independent measures to quantify Melbourne's urban–rural gradient. *Landscape and Urban Planning,* 1-13.

HARWOOD, V.J., WHITLOCK, J. & WITHINGTON, V. 2000. Classification of Antibiotic Resistance of Indicator Bacteria by Discriminant Analysis: Use in Predicting the Source of Feacal Contamination in Subtropical Waters. *Journal of Antibiotic Resistance as Indicator Source Identifier*, 66(9): 3698-3704.

HOLLAND, A.F., SANGERA, D.M., GAWLEA, C.P., LERBERG, S.B., SANTIAGO, S.M., RIEKERK, G, H.M., ZIMMERM, L.E. & SCOTT, G. 2004. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *Journal of Experimental Marine Biology and Ecology*, 298: 151– 178.

INSTITUTE FOR WATER QUALITY STUDIES (IWQS) & KEMPSTER, 1999. Report of radioactivity monitoring programme. The Mooi River (wonderfonteinspruit) catchment North-West Province SA.

JAGALS, P., GRABOW, W. O. K. & DE VILLIERS, J. C. 1995. Evaluation of indicators for assessment of human and animal faecal pollution of surface run-off. *Water Science Technology,* 31:235-241.

JEENA, M.I., DEEPA, P., K.M. MUJEEB RAHIMAN., SHANTHI, R.T. & HATHA, A.A.M. 2006. Risk assessment of heterotrophic bacteria from bottled drinking water sold in Indian markets. *International Journal of Hygiene and Environmental Health,* 209(2): 191-196.

JENG, H.A.C., ENGLAND, A.J., BAKEER, R.M. & BRADFORD, H.B. 2005. Impact of urban stormwater runoff on estuarine environmental quality. *Estuarine, Coastal and Shelf Science, 63(4):* 513-526.

KAYE, J. P. GROFFMAN, P. M. GRIMM, N. B. BAKER, L.A. & POUYAT, R.V. 2006. A distinct urban biogeochemistry? *Trends in Ecology & Evolution* ,21(4):192-199.

KAYSER, F.H. 2003. Safety aspects of enteroccoci from the medical point of view. *International Journal of Food Microbiology*, 88(2-3): 255-262.

KEMPSTER, 1999. Report of radioactivity monitoring programme. The Mooi River (wonderfonteinspruit) catchment North-West Province SA.

KIM, G., CHOI, E.T., LEE, D. 2005. Diffuse and point pollution impacts on the pathogen indicator organism level in the Geum River, Korea. *Science of the Total Environment*, 1-12.

KRUMPERMAN, P.H. 1983. Multiple antibiotic resistance indexing of Escherichia coli to identify high risk sources of faecal contamination of foods. *Applied and Environmental Microbiology*, 46: 165-170.

LIN, J., BIYELA, P.T. & PUKREE, T. 2004. Antibiotic Resistant profiles of environmental isolates from Mhlathuze River, KwaZulu Natal (RSA). *Water SA*, 1(30): 23-28.

LOBITZ, B., BECK, L., HUQ, A., WOOD, B., FUCH, G., FARUQUE, A.S.G., COLWELL, R. 2000. Climate and infectious disease: Use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. *Procedures of National Academy of Scieince*, 97, (4): 1438-1443.

MALLON, L. & CORKILL, K. 2002. Excretion of vancomycin resistant enterococci by wild mammals. *Journal of Emerging Infectious Diseases*, 8(6): 637-638.

MARZLUFF, J.M., BOWMAN, R. & DONNELLY, R. 2001. Quantifying the urban gradient: Linking urban planning and ecology. *Avian Ecology and Conservation in an Urbanizing*, 89–115.

McARTHUR, J.V. AND TUCKFIELD, R.C. 1997. Information length: spartial and temporal parameters among stream bacterial assemblages. *Journal of North American Benthological Society*, 16: 347-357.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS. 1999. Performance standard for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. *National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania,* Approved Standard M 31-A19 (11).

MAPLANDIA. POTCHEFSTROOM SATELLITE IMAGES. Web: http://www.maplandia.com/South-africa/north-west/potchefstroom/Potchefstroom/ (Accessed 20 May 2006).

NEWHAM, L. T. H. LETCHER, R. A. JAKEMAN, A. J. & KOBAYASHI, T. 2004. A framework for integrated hydrologic, sediment and nutrient export modelling for catchment-scale management. *Environmental Modelling & Software : Integrated Catchment Modelling and Decision Support*, 19(11): 1029-1038.

NHAPI, I. & TIRIVAROMBO, S. 2004. Sewage discharges and nutrients levels in Marimba River, Zimbabwe. *Water SA*. 30: 107-113.

NISHIGUCHI, M.K. 2000. Temperature affect species distribution in symbiotic populations of *Vibrio* spp. *Applied Environmental Microbiology*, 66 (8): 3550-3555.

NOBLE, R. T. & FUHRMAN, J. A. 2001. Enteroviruses detected in the coastal waters of Santa Monica Bay, CA: low correlation to bacterial indicators. *Hydrobiologia*, 460:175-184.

NÜBEL U, GARCIA-PICHEL F, KUHL M & MUYZER G. 1999. Quantify microbial diversity: Morphotypes,16S rRNA genes and caretenoids of organic phototropesin microbial mats. *Applied Environmental Microbiology*, 65 (2): 422-430.

N.W. North West Province-SOTE. 2002. North-West Province State of the environment Report. Web: http:www.environment.gov.za/soer/reports/northwest/01%20Contents.pdf (Accessed 20 July 2006).

OBI, C.L., POTGIETER, N., BESSONG, P.O., MATSAUNG, G. 2002. Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA*, 28(3): 287-292.

OSTERBLAD, M. AND HAKANEN, A. 2002. A between-Species Comparison of antimcrobial Resistance in Enterobacteria in fecal Flora. *American society for Microbiology,* 1479-1484.

PARVEEN, S., MORPHEME, R.L., EDISTO. L., KASPAR, C.W., PORTIERE, K.M. & TRAMPLING, M.L. 1997. Association of multiple antibiotic- resistance profiles with point and nonpoint sources of E. coli in Apalachicola Bay. *Applied Environmental Microbiology*, 63:2607–12.

PICKECTT, S., BUNRCH, W.R. AND DALTON, S.E. 1997. Integrated urban ecosystem research. *Urban ecosystem*, 1:183-184.

PRESCOTT, J.P. H & KLEIN, D.A. 2002. Laboratory exercise in Microbiology, New York, (fifth edition), 133.

(RED CROSS INTERNATIONAL), Red Cross International federation of and Red Crescent Societies. Health and community care. Web: http://www.ifrc.org/what/health/water/ (Accessed 25 August 2006).

SANKARAMAKRISHNAN, N. & GUO, Q. 2005. Chemical tracers as indicator of human feacal coliforms at storm water outfalls. *Journal of Environmental International*, 31:1133 – 1140.

SCHWARTZ, T., KOHNEN, W., JANSEN. & OBST, U. 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilm. *Journal of FEMS: Microbiology Ecology*, 43: 325-335.

SCHULZE, D.L., EGGERS, J.S., ESSER, L.R., 2001. Water quality studies red rock and Saylorville Reservoirs Des Moines River, Iowa. Annual Report. Department of the Army, Rock Island District, Corps of Engineers, Rock Island, IL.

SCOTT, T.M., ROSE, J.B., JENKINS, T.M., FARRAH, R.S. & LUKASIK, J. 2003. Microbial Source Tracking: Current Methodology and Future Directions. *Applied and Environmental Microbiology*, 68 (12): 5796-5803.

SHEHANE, S.D., HARWOOD, V.J., WHITLOCK, J.E. & ROSE, J.B. 2005. The influence of rainfall on the incidence of microbial feacal indicators and the dominant sources of feacal pollution in a Florida river. *Journal of Applied Microbiology*, 98(5): 1127-1136.

SOUTH AFRICAN WEATHER SERVICES. North-West Monthly Rainfall. Web: www.weathersa.co.za (Accessed 26 April 2006).

STATISTICS SOUTH AFRICA CENSUS 2001. Census 2001 at a glance, Web: http://www.statssa.gov.za/census01/html/default.asp (Accessed 7 December 2006).

TANG, Z., ENGEL., B.A., PIJANOWSKI, B.C. 2005. Forecasting land use change and its environmental impact at the watershed scale. *Journal of Environmental Management*, 76(1):35-45.

THEOBALD, D.M. 2005. Placing exurban land-use change in a human modification framework. *Frontal Ecological Environment*, 2(2004):139–144.

TICEHURST., J.L., LACHLAN T.H., NEWHAM, T.H., RISSIK, L.D., REBECCA A. LETCHER, A.R. & JAKEMAN, A.J. 2006. A Bayesian network approach for assessing the sustainability of coastal lakes in New South Wales, Australia. *Environmental Modelling & Software*, 2-353.

USGS SCIENCE FOR A CHANGING WORLD. Water science for schools Web: http://ga.water.usgs.gov/edu/urbanpop.html (Accessed 18 March 2006).

WATER WHEEL. 2006. Evaporation in the spotlight. *Freshwater ecosystem*, 5(4):20-23.

WATER RESEARCH COUNCIL. 1998. *Quality of Domestic Water Supplies: Assessment Guide*, 1.

WEBSTER, L.F., THOMPSONA, B.C., FULTONA, M.H., CHESTNUT, D.E., VAN DOLAH, R.F., LEIGHT, A.K. & SCOTT, G.I. 2004. Identification of sources of

Escherichia coli in South Carolina estuaries using antibiotic resistance analysis. *Journal of Experimental Marine Biology and Ecology*, 298 (2004) 179– 195.

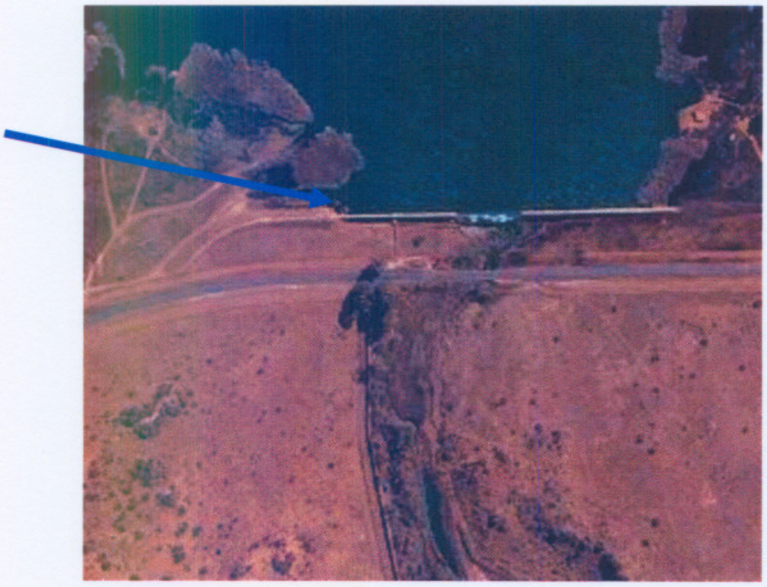
WHITLOCK, J. E., JONES, D.T. AND HARWOOD, V. J. 2002. Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research*, 36(17): 4273-4282.

WIGGINS, B.A. 1996. Discriminant analysis of antibiotic resistance patterns in feacal streptococci, a method to differentiate human and animal sources of feacal pollution in natural waters. *Applied Environmental Microbiology*, 62: 3997– 4002.

WORLD HEALTH ORGANIZATION. 2004. Guidelines for drinking-water quality, recommendations. Switzerland, Geneva, 1.

# APPENDIX A

**Figure A.1**: Enlarged satellite pictures of the exact location of the sampling sites in the Mooi River system.



**Klerkskraal dam – Site 1**

**Muiskraal bridge- Site 2**

**Potchefstroom Dam weir – Site 3**

**Wasgoed Spruit tributary- Site 4**

**Opposite police rugby field – Site 5**

**Opposite River Walk mall -   Site 6**

**Upstream from the Sewage Treatment Plant on the bridge opposite Potchefstroom prison – Site 7**

**Mooi River Mouth (on the Scandinavia river drift bridge) – Site 8**

**Table A.1:** Average temperature data ($^0$C) collected from collected from April 2005 to March 2006.

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Average temperature | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| **Apr-05** | 19 | 18.5 | 18.8 | 18.6 | 18.3 | 18.7 | 19.2 | 18.4 | 18.6875 | 48.2 |
| **May** | 18 | 17.5 | 17.1 | 17 | 17.3 | 18 | 17.6 | 17.6 | 17.5125 | 3 |
| **June** | 16 | 15.9 | 16.2 | 17.6 | 16.5 | 16.3 | 17.9 | 16.4 | 16.6 | 0 |
| **July** | 18 | 12 | 14.3 | 15 | 14.9 | 14.5 | 15.9 | 13.2 | 14.725 | 0 |
| **August** | 20 | 16.4 | 16.5 | 16.2 | 16.01 | 16 | 16.4 | 16.5 | 16.75125 | 0 |
| **September** | 21 | 18.2 | 17.2 | 17.4 | 17.2 | 17.3 | 16.5 | 17 | 17.725 | 0 |
| **October** | 23.9 | 23.7 | 20.5 | 23.5 | 22.4 | 21.7 | 23.2 | 23.2 | 22.7625 | 13.2 |
| **November** | 23.8 | 23.4 | 21.2 | 23.4 | 24.6 | 23.4 | 25.9 | 23.3 | 23.625 | 41.2 |
| **Jan-06** | 26.1 | 24.4 | 27.6 | 25.2 | 25 | 25.5 | 26.7 | 24.6 | 25.6375 | 82.4 |
| **Feb-06** | 25.7 | 24.2 | 26.7 | 24.9 | 24.7 | 25.3 | 25.8 | 25 | 25.2875 | 48.8 |
| **Mar-06** | 26 | 25.3 | 26.3 | 26 | 26 | 26 | 26 | 25.9 | 25.9375 | 150.8 |

**Table A.2:** Average pH data collected from collected from April 2005 to March 2006.

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Average pH | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| Apr-05 | 7.6 | 7.2 | 8 | 7.5 | 7.8 | 7.4 | 7.7 | 7.5 | 7.5875 | 48.2 |
| May | 7.8 | 7.3 | 7.7 | 7.9 | 7.4 | 7.6 | 7.8 | 7.1 | 7.575 | 3 |
| June | 8.7 | 8.4 | 8.7 | 8.6 | 8.2 | 8.8 | 8.9 | 8.5 | 8.6 | 0 |
| July | 8.5 | 8.5 | 8.6 | 8.4 | 8.5 | 8.6 | 8.5 | 8.6 | 8.525 | 0 |
| August | 8.4 | 8.3 | 8.5 | 8.4 | 8.3 | 8.2 | 8.2 | 8.4 | 8.3375 | 0 |
| September | 8.8 | 8.4 | 8.6 | 8.2 | 8.2 | 8.3 | 8.3 | 8.1 | 8.3625 | 0 |
| October | 8.7 | 8.3 | 8.5 | 8.6 | 8.3 | 8.6 | 8.5 | 8.4 | 8.4875 | 13.2 |
| November | 8.6 | 8.5 | 8.6 | 8.5 | 8.5 | 8.2 | 8.3 | 8.6 | 8.475 | 41.2 |
| Jan-06 | 8.43 | 7.98 | 8.594 | 7.978 | 7.917 | 7.55 | 7.5 | 7.67 | 7.952375 | 82.4 |
| Feb-06 | 8.52 | 8.1 | 7.98 | 7.86 | 8.1 | 7.64 | 7.6 | 7.56 | 7.92 | 48.8 |
| Mar-06 | 8.3 | 7.9 | 8.5 | 8.4 | 8.3 | 8.6 | 7.5 | 7.5 | 8.125 | 150.8 |

**Table A.3:** Total dissolved solids data collected from collected from April 2005 to March 2006.

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | AverageTDS | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| Apr-05 | 310 | 667 | 315 | 200 | 274 | 268.9 | 315 | 219 | 321.1125 | 48.2 |
| May | 311 | 538 | 413 | 199 | 236 | 360 | 278 | 209 | 318 | 3 |
| June | 200 | 350 | 488 | 178.5 | 164 | 350 | 100 | 148 | 247.3125 | 0 |
| July | 125 | 133 | 422 | 150 | 199 | 345 | 167 | 229 | 221.25 | 0 |
| August | 160 | 630 | 332 | 155 | 175 | 275 | 179 | 0 | 238.25 | 0 |
| September | 315 | 415 | 270 | 167.5 | 149 | 169 | 245 | 0 | 216.3125 | 0 |
| October | 209 | 540 | 143 | 163.8 | 171 | 266 | 85 | 0 | 197.225 | 13.2 |
| November | 142 | 450 | 166 | 164.8 | 150 | 270 | 300 | 142 | 223.1 | 41.2 |
| Jan-06 | 139 | 460 | 0 | 123 | 0.1 | 244 | 254 | 136 | 169.5125 | 82.4 |
| Feb-06 | 120 | 420 | 0 | 110 | 0 | 236 | 240 | 126 | 156.5 | 48.8 |
| Mar-06 | 166 | 500 | 0 | 148 | 0 | 266 | 268 | 149 | 187.125 | 150.8 |

**Table A.4:** Average electro-conductivity data collected from collected from April 2005 to March 2006.

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Average Conductivity | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| Apr-05 | 220 | 660 | 300 | 331 | 270 | 160.8 | 310 | 215 | 308.35 | 48.2 |
| May | 210 | 530 | 410 | 340 | 233 | 358 | 278 | 200 | 319.875 | 3 |
| June | 185 | 348 | 480 | 320.8 | 165 | 357 | 100 | 134 | 261.225 | 0 |
| July | 150 | 130 | 420 | 318 | 174 | 344 | 167 | 220 | 240.375 | 0 |
| August | 121.8 | 620 | 330 | 315 | 148 | 272 | 179 | 140.6 | 265.8 | 0 |
| September | 230 | 400.5 | 268 | 300 | 170 | 269 | 244.9 | 220 | 262.8 | 0 |
| October | 116 | 538 | 143 | 200 | 149 | 242 | 84.8 | 0 | 184.1 | 13.2 |
| November | 110 | 132 | 165 | 163 | 0 | 237 | 299 | 0 | 138.25 | 41.2 |
| Jan-06 | 144 | 190 | 120 | 260 | 139 | 244 | 256 | 200 | 194.125 | 82.4 |
| Feb-06 | 135 | 170 | 110 | 250 | 120 | 200 | 240 | 190 | 176.875 | 48.8 |
| Mar-06 | 188 | 210 | 140 | 280 | 160 | 290 | 280 | 220 | 221 | 150.8 |

**Table A.5:** Average dissolved oxygen data collected from collected from April 2005 to March 2006

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Average DO | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| Apr-05 | 8.54 | 5.99 | 8.6 | 7.9 | 4.4 | 5.65 | 6.2 | 3.8 | 6.385 | 48.2 |
| May | 10.6 | 5.89 | 8.34 | 7.88 | 3.4 | 5 | 6 | 3.8 | 6.36375 | 3 |
| June | 12.8 | 5.66 | 8 | 7.9 | 3.43 | 4.23 | 5.54 | 3.6 | 6.395 | 0 |
| July | 9.5 | 5.78 | 7.45 | 7.6 | 3.9 | 4.22 | 5.3 | 3.6 | 5.91875 | 0 |
| August | 6 | 6 | 7.77 | 7.66 | 3.7 | 3.15 | 5 | 3.5 | 5.3475 | 0 |
| September | 8 | 6.7 | 7.44 | 7.45 | 3.5 | 3.45 | 5 | 3.42 | 5.62 | 0 |
| October | 5.8 | 5.12 | 7.8 | 5.36 | 2.59 | 2.9 | 4.7 | 3.4 | 4.70875 | 13.2 |
| November | 7 | 4.5 | 6.7 | 5.3 | 2 | 4.11 | 3.8 | 3.3 | 4.58875 | 41.2 |
| Jan-06 | 4.85 | 4.12 | 5.11 | 3.6 | 3.42 | 3.74 | 3.15 | 3.3 | 3.91125 | 82.4 |
| Feb-06 | 4.61 | 3.44 | 4.2 | 3.5 | 4.6 | 3.66 | 2.71 | 2.9 | 3.7025 | 48.8 |
| Mar-06 | 4.4 | 3.18 | 4.96 | 3.7 | 4 | 3.74 | 2.1 | 2.1 | 3.5225 | 150.8 |

**Table A.6**: Average chemical oxygen demand data collected from collected from April 2005 to March 2006

| DATE | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Average COD | Rainfall |
|---|---|---|---|---|---|---|---|---|---|---|
| Apr-05 | 140 | 406 | 643 | 6 | 20 | 60 | 120 | 660 | 256.875 | 48.2 |
| May | 176 | 389 | 658 | 20 | 40 | 84 | 169 | 786 | 290.25 | 3 |
| June | 228 | 616 | 620 | 15 | 30 | 66 | 130 | 760 | 308.125 | 0 |
| July | 270 | 578 | 600 | 27 | 50 | 100 | 200 | 772 | 324.625 | 0 |
| August | 220 | 534 | 544 | 30 | 62 | 124 | 244 | 658 | 302 | 0 |
| September | 300 | 520 | 629 | 46 | 88 | 164 | 328 | 700 | 346.875 | 0 |
| October | 349.6 | 578.9 | 600 | 109 | 150 | 303 | 364 | 650 | 388.0625 | 13.2 |
| November | 477 | 567 | 580 | 200 | 400 | 460 | 466 | 649 | 474.875 | 41.2 |
| Jan-06 | 209 | 406 | 623 | 338 | 457 | 412 | 442 | 786 | 459.125 | 82.4 |
| Feb-06 | 228 | 389 | 590 | 320 | 430 | 400 | 434 | 760 | 443.875 | 48.8 |
| Mar-06 | 270 | 415 | 630 | 357 | 475 | 440 | 455 | 770 | 476.5 | 150.8 |

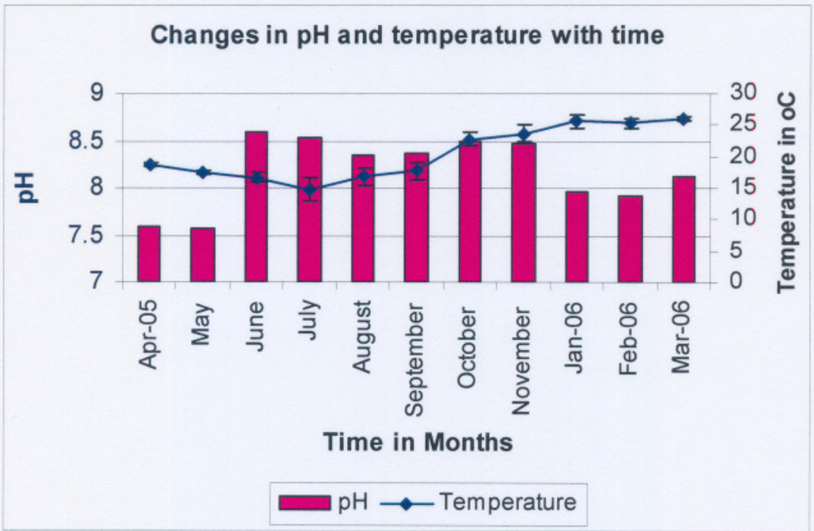
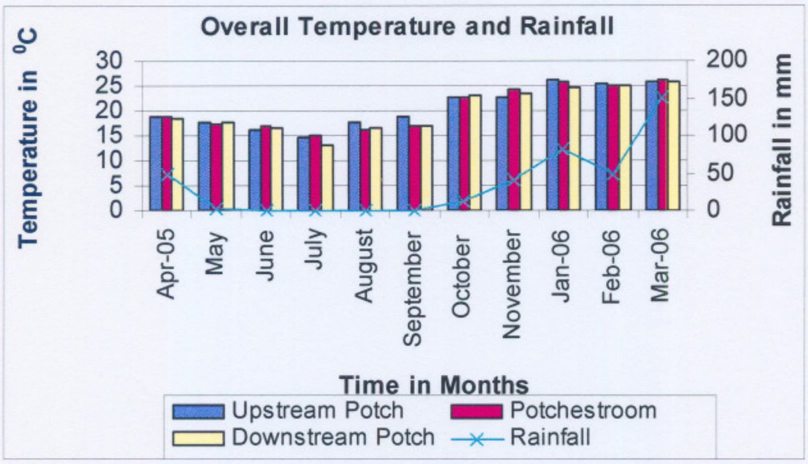



**Figure: A.2** Relationship between average temperature, pH and rainfall amount using data collected from April 2005 to March 2006.



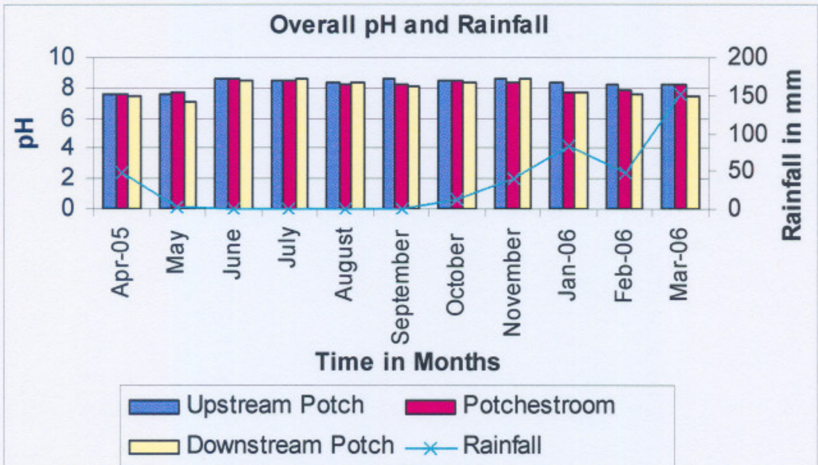




**Figure A.3:** Relationship of the overall temperature, pH and rainfall data for the non-rainy and the rainy season.

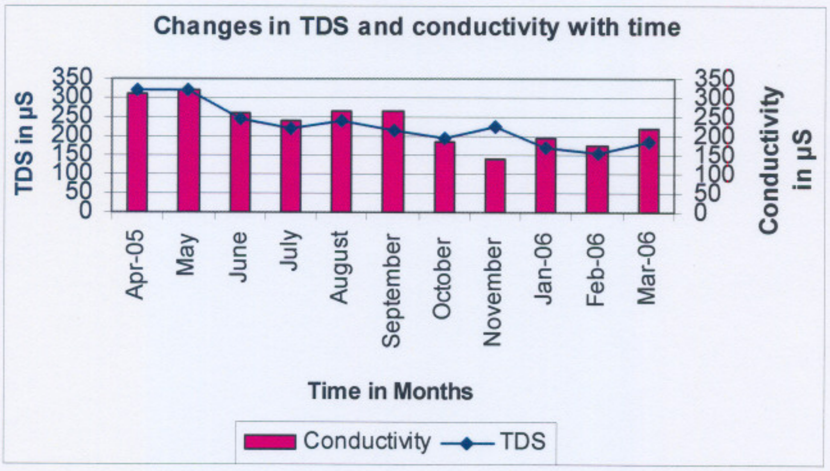
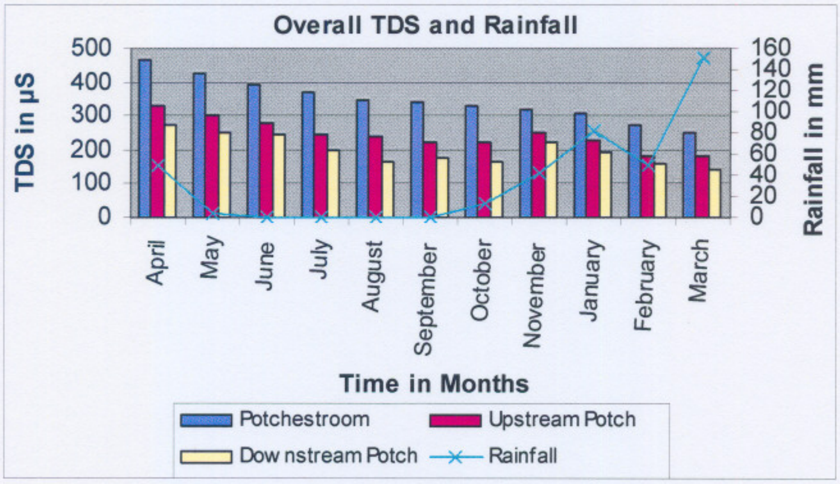



**Figure: A.4** Relationship between total dissolved solids, electrocoductivity and rainfall for wet and dry season.



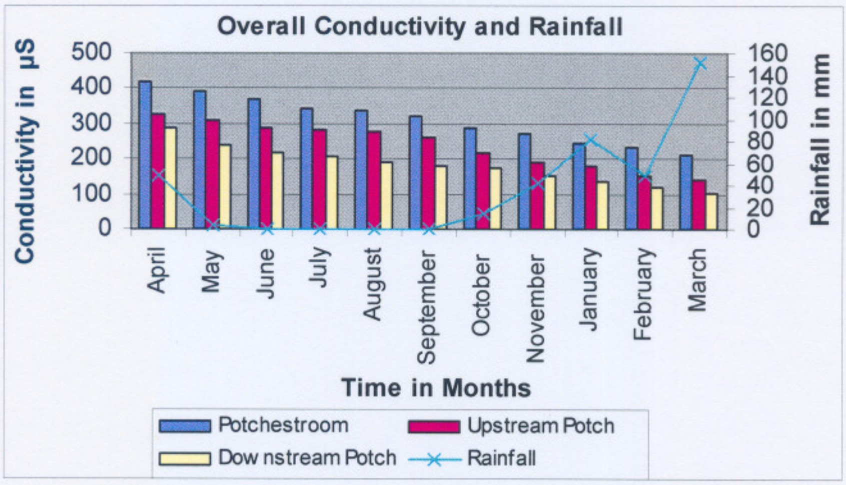




**Figure: A.5** Relationship between mean monthly total dissolved solids values and electrocoductivity for wet and dry season.

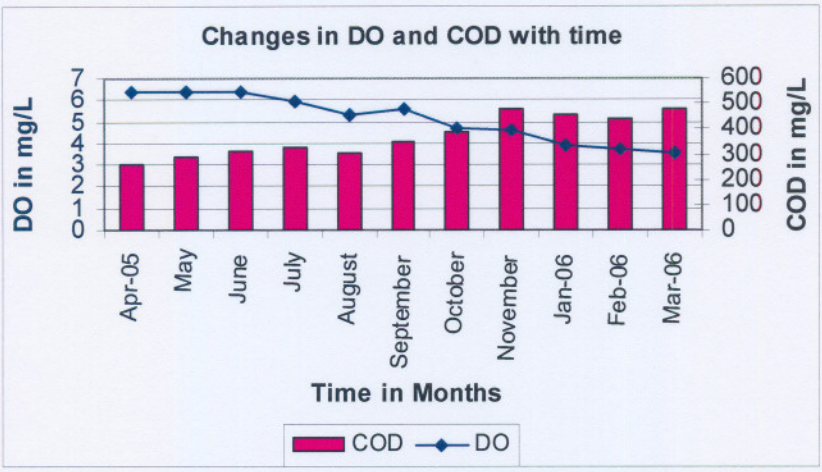
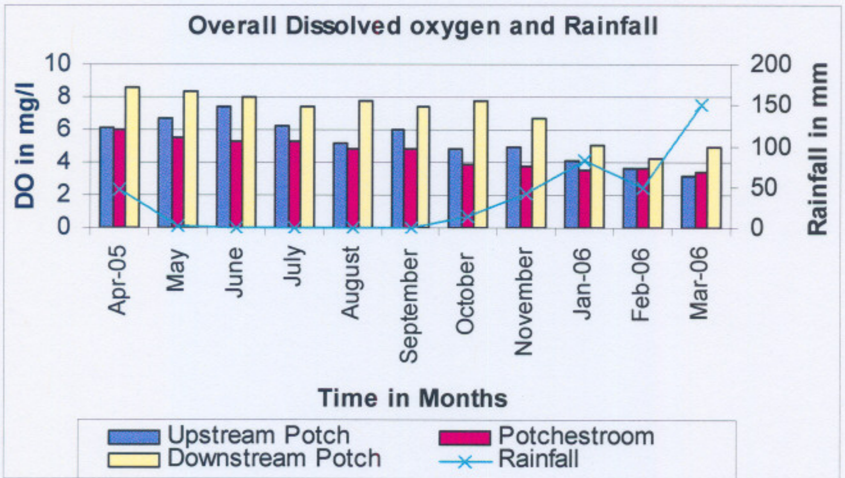



**Figure A.6:** Relationship of the average dissolved oxygen, chemical oxygen demand and rainfall.



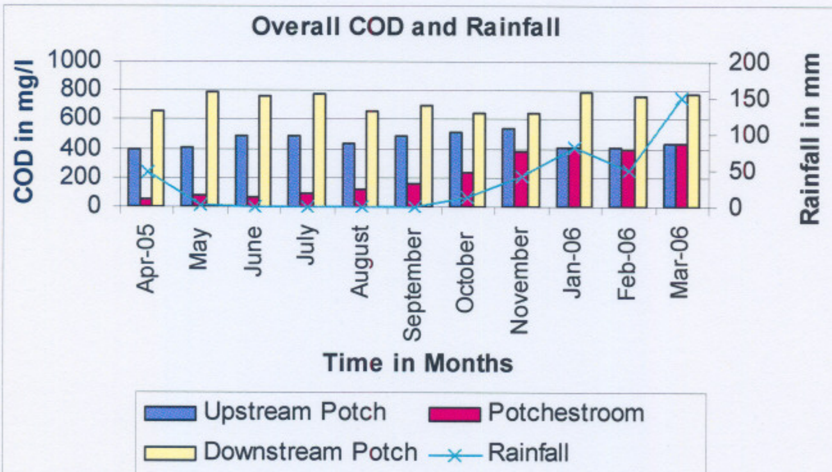




**Figure A.7:** Seasonal variations in DO, COD and rainfall at all the sites during the period of study.

# APPENDIX B

**Table B.1:** Faecal colifom counts (cfu/100ml) without Ampicillin over a one period

| DATE | Upstream | Stdev | Potchefstroom | Stdev | Downstream | Stdev |
|---|---|---|---|---|---|---|
| Apr-05 | 61.33333 | 15.56706 | 153.5 | 34.12843 | 100 | 9.899495 |
| May | 62 | 20.29778 | 153 | 39.5664 | 100 | 6.363961 |
| June | 69.66667 | 11.93035 | 156.25 | 34.80212 | 104 | 7.071068 |
| July | 65.33333 | 13.61372 | 153.5 | 31.69779 | 104 | 8.485281 |
| August | 64.66667 | 20.55075 | 155.75 | 36.58125 | 98 | 8.485281 |
| September | 71.66667 | 8.504901 | 153 | 33.54102 | 100 | 9.899495 |
| October | 61.33333 | 19.42507 | 153.25 | 31.236 | 98 | 12.72792 |
| November | 59.33333 | 22.72297 | 149.5 | 37.42659 | 90 | 5.656854 |
| Jan-06 | 102.6667 | 16.16581 | 198.25 | 39.51819 | 141 | 2.12132 |
| Feb-06 | 46 | 13.52775 | 124.25 | 33.48414 | 73 | 7.071068 |
| Mar-06 | 54 | 15.09967 | 120.5 | 27.29011 | 76 | 8.485281 |

**Table B.2:** Faecal coliform counts (cfu/100ml) on ampicillin containing plates over a one period

| DATE | Upstream | Stdev | Potchefstroom | Stdev | Downstream | Stdev |
|---|---|---|---|---|---|---|
| **Apr-05** | 29 | 7 | 77.575 | 22.21807 | 45.7 | 4.454773 |
| **May** | 30.96667 | 5.95007 | 81.825 | 19.94298 | 72 | 8.980256 |
| **June** | 37.66667 | 8.504901 | 81.5 | 20.87263 | 52 | 5.656854 |
| **July** | 31.63333 | 8.504313 | 78.65 | 23.03743 | 53 | 0.353553 |
| **August** | 30.73333 | 6.634255 | 79.75 | 20.87063 | 54 | 1.414214 |
| **September** | 29.03333 | 8.951164 | 82.025 | 21.12035 | 52.5 | 3.889087 |
| **October** | 34.66667 | 11.50362 | 81.575 | 21.15788 | 64 | 4.030509 |
| **November** | 30.66667 | 11.01514 | 84 | 18.45716 | 57 | 4.242641 |
| **Jan-06** | 43.76667 | 8.292366 | 100.4 | 26.30247 | 71 | 2.05061 |
| **Feb-06** | 24.6 | 7.1631 | 63.75 | 16.54297 | 36.9 | 5.515433 |
| **Apr-05** | 26.66667 | 7.767453 | 64.975 | 16.61734 | 45.7 | 0.919239 |

**Table B.3:** Seasonal enteroccocal counts (cfu/100ml) without Ampicillin

| DATE | Upstream | Stdev | Potchefstroom | Stdev | Downstream | Stdev |
|---|---|---|---|---|---|---|
| **Apr-05** | 63.66667 | 8.144528 | 152.25 | 48.38733 | 87 | 7.778175 |
| **May** | 67.66667 | 11.23981 | 154.75 | 43.40891 | 90 | 9.192388 |
| **June** | 71.33333 | 12.05543 | 154.5 | 45.36886 | 93 | 8.485281 |
| **July** | 69.33333 | 12.05543 | 152.25 | 46.19885 | 93 | 3.535534 |
| **August** | 67.33333 | 11.50362 | 154.25 | 43.48563 | 89 | 10.6066 |
| **September** | 70.33333 | 8.504901 | 153 | 48.38733 | 95 | 3.535534 |
| **October** | 68.33333 | 7.094599 | 155 | 47.1593 | 93 | 3.535534 |
| **November** | 71 | 10.14889 | 153.5 | 45.17743 | 90 | 7.778175 |
| **Jan-06** | 89 | 9.539392 | 191 | 55.42863 | 115 | 10.6066 |
| **Feb-06** | 51.66667 | 7.637626 | 124.75 | 35.57152 | 76 | 2.828427 |
| **Apr-05** | 53.33333 | 7.571878 | 126.75 | 43.71499 | 73 | 4.242641 |

**Table B.4:** Seasonal enteroccocal counts (cfu/100ml) on ampicillin containing plates.

| DATE | Upstream | Stdev | Potchefstroom | Stdev | Downstream | Stdev |
|---|---|---|---|---|---|---|
| Apr-05 | 47.66667 | 7.071068 | 116 | 38.84156 | 66 | 7.071068 |
| May | 50.33333 | 9.192388 | 115.25 | 35.0464 | 68 | 7.071068 |
| June | 51.33333 | 9.192388 | 113.25 | 40.19432 | 76 | 5.656854 |
| July | 66.66667 | 6.363961 | 127.25 | 25.10478 | 90 | 7.071068 |
| August | 53.33333 | 16.97056 | 115.75 | 34.34506 | 73 | 4.949747 |
| September | 65.66667 | 16.26346 | 127 | 24.79247 | 88 | 8.485281 |
| October | 67.66667 | 12.02082 | 118.75 | 31.48942 | 90 | 5.656854 |
| November | 67.66667 | 12.02082 | 127 | 24.79247 | 93 | 4.949747 |
| Jan-06 | 85 | 13.43503 | 171 | 46.02898 | 112 | 5.656854 |
| Feb-06 | 43.66667 | 6.363961 | 98.75 | 36.28935 | 70 | 7.071068 |
| Apr-05 | 35.66667 | 6.363961 | 97.5 | 30.69745 | 70 | 4.242641 |

**Table B.5**: Paired t test and the Pearson correlation results for temperature, pH, conductivity, DO, COD and rainfall in Mooi River water samples.

| Physico-chemical parameters & Rainfall | t-test | Pearson correlation |
|---|---|---|
| Temperature | 0.294043 | 0.777991 |
| pH | 0.088167 | -0.300232 |
| TDS | 1.942200E-05 | -0.390260 |
| Conductivity | 1.543610E-05 | -0.325464 |
| pH & eletro-conductivity | 1.226551E-07 | -0.432791 |
| TDS& eletro-conductivity | 0.528178 | 0.793398 |
| DO | 0.0650952 | -0.721375 |
| COD | 5.259592E-09 | 0.669660 |
| DO&COD | 4.292341E-08 | -0.923881 |

**Table B.6:** Paired t test and the Pearson correlation results for faecal coliform and enteroccci bacteria over a one year period

| Paired river segments | faecal coliforms | | faecal coliforms on ampicillin containing plates | | enteroccoci | | enteroccoci on ampicillin containing plates | |
|---|---|---|---|---|---|---|---|---|
| | t-test | Pearson correlation | t-test | Pearson correlation | t-test | Pearson correlation | t-test | Pearson correlation |
| Up & Potch | 1.011E-11 | 0.933 | 8.78E-11 | 0.867 | 6.470E-12 | 0.978 | 4.431E-10 | 0.910 |
| Potch &Down | 1.310E-12 | 0.982 | 2.03E-07 | 0.797 | 5.322E-11 | 0.974 | 1.210E-07 | 0.874 |

**Table B.7:** Paired t test and the Pearson correlation results for seasonal FC/FE ratio and total coliforms

| **Paired river segments** | **faecal/enteroccoci ratio** | | **faecal/enteroccoci on ampicillin contaning plates** | | **Total coliforms without ampicillin & Total coliform on ampicillin containing plates** | |
|---|---|---|---|---|---|---|
| | t-test | Pearson correlation | t-test | Pearson correlation | t-test | Pearson correlation |
| Up & Potch | 0.138 | 0.457 | 0.018 | -0.055 | 0.000 | 0.000 |
| Potch &Down | 0.000576 | 0.742 | 0.942 | 0.148 | 0.000 | 0.000 |
| Up&Up | 0.000 | 0.000 | 0.000 | 0.000 | 3.630E-09 | 0.882 |
| Potch&Potch | 0.000 | 0.000 | 0.000 | 0.000 | 7.423E-12 | 0.946 |
| Down&Down | 0.000 | 0.000 | 0.000 | 0.000 | 9.580E-09 | 0.704 |

# APPENDIX C

**Table C.1:** Inhibtion zone diameter measured for all enteroccoci isolates during the determination of antibiotic resistance

| CODE | VAN | CIP | KF | OXY | AMO | NE | AMP | KAN | CHL | ERY |
|---|---|---|---|---|---|---|---|---|---|---|
| **1**12004Ent | 18 | 20 | 12 | 25 | 14 | 6 | 15 | 6 | 20 | 12 |
| **2**12004Ent | 16 | 18 | 10 | 20 | 12 | 14 | 16 | 15 | 17 | 15 |
| **3**12004Ent | 15 | 17 | 11 | 22 | 13 | 10 | 10 | 6 | 21 | 20 |
| **4**22004Ent | 15 | 20 | 12 | 26 | 10 | 6 | 9 | 10 | 22 | 22 |
| **5**22004Ent | 11 | 19 | 17 | 21 | 16 | 7 | 13 | 14 | 24 | 21 |
| **6**22004Ent | 15 | 20 | 11 | 22 | 17 | 10 | 11 | 6 | 22 | 20 |
| **7**32004Ent | 14 | 18 | 20 | 18 | 10 | 20 | 14 | 6 | 20 | 21 |
| **8**32004Ent | 13 | 16 | 17 | 24 | 12 | 14 | 11 | 10 | 21 | 19 |
| **9**32004Ent | 12 | 17 | 22 | 25 | 12 | 22 | 10 | 6 | 20 | 18 |
| **10**42004Ent | 15 | 18 | 9 | 18 | 12 | 15 | 15 | 6 | 19 | 25 |
| **11**42004Ent | 14 | 14 | 10 | 18 | 14 | 6 | 16 | 9 | 18 | 23 |
| **12**42004Ent | 15 | 17 | 12 | 17 | 16 | 11 | 9 | 11 | 16 | 21 |
| **13**11305Ent | 14 | 6 | 30 | 12 | 16 | 14 | 8 | 6 | 6 | 24 |
| **14** 11305Ent | 14 | 8 | 8 | 14 | 40 | 18 | 7 | 30 | 36 | 24 |
| **15**11305Ent | 20 | 20 | 14 | 6 | 28 | 14 | 15 | 6 | 6 | 6 |
| **16**21305Ent | 22 | 12 | 6 | 8 | 12 | 8 | 10 | 6 | 14 | 20 |
| **17**21305Ent | 32 | 6 | 6 | 18 | 26 | 10 | 16 | 20 | 8 | 16 |
| **18**21305Ent | 36 | 10 | 9 | 14 | 22 | 12 | 16 | 8 | 14 | 20 |
| **19**31305Ent | 20 | 6 | 21 | 6 | 14 | 25 | 16 | 8 | 24 | 14 |
| **20**31305Ent | 24 | 8 | 19 | 6 | 32 | 15 | 11 | 21 | 10 | 16 |
| **21**31305Ent | 20 | 8 | 18 | 11 | 18 | 19 | 14 | 12 | 0 | 14 |
| **22**611305Ent | 16 | 6 | 14 | 11 | 22 | 14 | 6 | 8 | 10 | 12 |
| **23**611305Ent | 16 | 6 | 12 | 8 | 20 | 16 | 10 | 6 | 10 | 12 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **24**611305Ent | 14 | 6 | 8 | 6 | 34 | 14 | 15 | 7 | 12 | 10 |
| **25**41305Ent | 22 | 8 | 20 | 6 | 24 | 10 | 16 | 6 | 18 | 10 |
| **26**41305Ent | 16 | 8 | 12 | 6 | 26 | 6 | 14 | 7 | 18 | 6 |
| **27**41305Ent | 36 | 6 | 12 | 6 | 26 | 8 | 11 | 9 | 8 | 8 |
| **28**711305Ent | 10 | 6 | 6 | 6 | 30 | 22 | 10 | 17 | 6 | 6 |
| **29**711305Ent | 20 | 10 | 8 | 10 | 24 | 6 | 12 | 8 | 22 | 14 |
| **30**711305Ent | 20 | 22 | 8 | 8 | 22 | 18 | 13 | 6 | 8 | 14 |
| **31**521305Ent | 10 | 8 | 6 | 6 | 20 | 8 | 10 | 6 | 10 | 8 |
| **32**521305Ent | 16 | 12 | 8 | 8 | 22 | 8 | 20 | 10 | 6 | 16 |
| **33**521305Ent | 22 | 22 | 14 | 8 | 32 | 14 | 6 | 6 | 11 | 10 |
| **34**10306Ent | 12 | 6 | 28 | 14 | 30 | 16 | 11 | 10 | 6 | 26 |
| **35**10306Ent | 24 | 10 | 8 | 16 | 34 | 20 | 15 | 30 | 36 | 24 |
| **36**10306Ent | 26 | 20 | 18 | 6 | 30 | 14 | 10 | 6 | 6 | 6 |
| **37**20306Ent | 24 | 12 | 10 | 6 | 6 | 8 | 6 | 8 | 14 | 22 |
| **38**20306Ent | 34 | 6 | 6 | 18 | 26 | 10 | 14 | 6 | 0 | 16 |
| **39**20306Ent | 36 | 10 | 6 | 14 | 22 | 12 | 16 | 8 | 14 | 20 |
| **40**30306Ent | 20 | 8 | 6 | 6 | 14 | 12 | 16 | 6 | 24 | 14 |
| **41**30306Ent | 24 | 6 | 10 | 6 | 30 | 8 | 12 | 6 | 14 | 18 |
| **42**30306Ent | 26 | 8 | 8 | 6 | 18 | 6 | 14 | 14 | 8 | 16 |
| 4340306Ent | 16 | 6 | 14 | 10 | 24 | 8 | 6 | 16 | 10 | 14 |
| **44**40306Ent | 18 | 6 | 16 | 6 | 22 | 10 | 14 | 6 | 12 | 16 |
| **45**40306Ent | 16 | 14 | 12 | 8 | 32 | 6 | 11 | 6 | 16 | 12 |
| **46**50306Ent | 14 | 6 | 22 | 6 | 26 | 14 | 16 | 8 | 12 | 10 |
| **47**50306Ent | 18 | 6 | 14 | 16 | 28 | 6 | 18 | 22 | 20 | 8 |
| **48**50306Ent | 34 | 8 | 14 | 6 | 26 | 10 | 18 | 6 | 10 | 12 |
| **49**60306Ent | 4 | 6 | 6 | 20 | 32 | 24 | 9 | 18 | 6 | 6 |
| **50**60306Ent | 26 | 10 | 8 | 10 | 24 | 6 | 16 | 6 | 22 | 16 |
| **51**60306Ent | 24 | 26 | 8 | 8 | 22 | 18 | 15 | 8 | 8 | 16 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **52**70306Ent | 10 | 6 | 11 | 6 | 22 | 10 | 10 | 14 | 6 | 6 |
| **53**70306Ent | 16 | 14 | 10 | 10 | 24 | 6 | 12 | 8 | 8 | 18 |
| **54**70306Ent | 28 | 26 | 16 | 10 | 34 | 16 | 8 | 8 | 10 | 12 |
| **55**90306Ent | 24 | 18 | 22 | 8 | 26 | 12 | 16 | 10 | 20 | 12 |
| **56**90306Ent | 20 | 10 | 16 | 8 | 28 | 6 | 22 | 6 | 20 | 10 |
| **57**90306Ent | 40 | 10 | 14 | 6 | 24 | 8 | 28 | 10 | 10 | 10 |
| **58**12707Ent | 12 | 6 | 20 | 10 | 22 | 14 | 14 | 6 | 10 | 20 |
| **59**12707Ent | 24 | 10 | 6 | 14 | 20 | 18 | 14 | 24 | 34 | 22 |
| **60**12707Ent | 18 | 18 | 16 | 6 | 24 | 14 | 12 | 6 | 6 | 6 |
| **61**22707Ent | 22 | 6 | 6 | 6 | 14 | 12 | 16 | 6 | 22 | 20 |
| **62**22707Ent | 22 | 6 | 6 | 16 | 30 | 8 | 10 | 6 | 12 | 16 |
| **63**22707Ent | 20 | 10 | 8 | 6 | 18 | 6 | 14 | 10 | 6 | 18 |
| **64**32707Ent | 16 | 6 | 12 | 6 | 24 | 6 | 6 | 6 | 8 | 12 |
| **65**32707Ent | 14 | 6 | 10 | 8 | 18 | 10 | 24 | 6 | 8 | 14 |
| **66**32707Ent | 16 | 10 | 8 | 11 | 30 | 11 | 12 | 15 | 10 | 16 |
| **67**42707Ent | 20 | 6 | 22 | 12 | 26 | 12 | 18 | 6 | 16 | 12 |
| **68**42707Ent | 18 | 10 | 14 | 8 | 24 | 6 | 20 | 8 | 16 | 14 |
| **69**42707Ent | 30 | 12 | 12 | 10 | 24 | 18 | 24 | 14 | 10 | 10 |
| **70**52707Ent | 6 | 8 | 6 | 6 | 28 | 22 | 18 | 6 | 6 | 12 |
| **71**52707Ent | 18 | 10 | 10 | 12 | 20 | 6 | 22 | 8 | 10 | 6 |
| **72**52707Ent | 22 | 14 | 8 | 6 | 22 | 16 | 24 | 6 | 10 | 10 |
| **73**62707Ent | 6 | 10 | 6 | 11 | 18 | 17 | 10 | 10 | 6 | 6 |
| **74**62707Ent | 18 | 10 | 10 | 8 | 20 | 19 | 12 | 14 | 4 | 14 |
| **75**62707Ent | 20 | 20 | 14 | 6 | 28 | 21 | 8 | 6 | 12 | 16 |
| **76**92707Ent | 16 | 6 | 12 | 12 | 20 | 8 | 8 | 6 | 12 | 6 |
| **77**92707Ent | 14 | 6 | 14 | 6 | 18 | 8 | 24 | 6 | 10 | 14 |
| **78**92707Ent | 18 | 12 | 10 | 8 | 30 | 10 | 9 | 6 | 10 | 8 |
| **79**13108Ent | 12 | 6 | 22 | 10 | 20 | 12 | 16 | 8 | 8 | 22 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **80**13108Ent | 11 | 10 | 6 | 12 | 38 | 16 | 10 | 26 | 30 | 22 |
| **81**13108Ent | 13 | 22 | 12 | 8 | 24 | 12 | 14 | 10 | 6 | 6 |
| **82**23108Ent | 18 | 10 | 8 | 6 | 12 | 10 | 14 | 8 | 22 | 12 |
| **83**23108Ent | 20 | 6 | 9 | 8 | 28 | 10 | 10 | 6 | 12 | 14 |
| **84**23108Ent | 22 | 10 | 8 | 9 | 16 | 10 | 12 | 10 | 16 | 12 |
| **85**33108Ent | 14 | 6 | 12 | 6 | 10 | 8 | 6 | 8 | 12 | 10 |
| **86**33108Ent | 16 | 6 | 10 | 8 | 18 | 10 | 11 | 6 | 8 | 10 |
| **87**33108Ent | 12 | 10 | 14 | 10 | 30 | 6 | 13 | 10 | 10 | 12 |
| **88**43108Ent | 16 | 8 | 14 | 6 | 10 | 8 | 24 | 6 | 12 | 14 |
| **89**43108Ent | 14 | 12 | 10 | 10 | 30 | 6 | 20 | 8 | 14 | 10 |
| **90**43108Ent | 12 | 6 | 20 | 6 | 24 | 12 | 14 | 6 | 20 | 12 |
| **91**53108Ent | 14 | 6 | 22 | 10 | 24 | 14 | 12 | 10 | 10 | 8 |
| **92**53108Ent | 16 | 10 | 12 | 10 | 22 | 10 | 16 | 20 | 18 | 6 |
| **93**53108Ent | 32 | 6 | 12 | 6 | 20 | 6 | 16 | 6 | 12 | 10 |
| **94**63108Ent | 8 | 11 | 6 | 6 | 16 | 10 | 8 | 12 | 6 | 8 |
| **95**63108Ent | 20 | 6 | 8 | 10 | 18 | 6 | 16 | 10 | 6 | 12 |
| **96**63108Ent | 18 | 16 | 12 | 6 | 24 | 10 | 6 | 6 | 10 | 18 |
| **97**93108Ent | 18 | 6 | 10 | 6 | 12 | 14 | 14 | 6 | 22 | 16 |
| **98**93108Ent | 22 | 10 | 6 | 10 | 24 | 10 | 10 | 6 | 12 | 14 |
| **99**93108Ent | 24 | 6 | 8 | 6 | 16 | 10 | 16 | 10 | 8 | 18 |
| **100**32009Ent | 6 | 7 | 7 | 6 | 8 | 8 | 6 | 6 | 6 | 8 |
| **101**32009Ent | 8 | 7 | 8 | 6 | 6 | 6 | 7 | 17 | 7 | 7 |
| **102**32009Ent | 7 | 6 | 6 | 7 | 7 | 7 | 6 | 6 | 6 | 6 |
| **103**62009Ent | 15 | 19 | 12 | 20 | 10 | 10 | 20 | 8 | 14 | 17 |
| **104**62009Ent | 16 | 20 | 14 | 21 | 9 | 9 | 21 | 7 | 19 | 18 |
| **105**62009Ent | 15 | 18 | 13 | 19 | 11 | 10 | 22 | 6 | 17 | 17 |
| **106**72009Ent | 19 | 18 | 14 | 21 | 12 | 20 | 16 | 7 | 19 | 8 |
| **107**72009Ent | 13 | 19 | 15 | 19 | 9 | 12 | 14 | 8 | 17 | 19 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **108**72009Ent | 14 | 19 | 12 | 20 | 10 | 11 | 10 | 6 | 18 | 18 |
| **107**92009Ent | 17 | 24 | 11 | 21 | 12 | 7 | 15 | 8 | 20 | 20 |
| **108**92009Ent | 18 | 22 | 12 | 23 | 12 | 8 | 10 | 6 | 19 | 19 |
| **109**92009Ent | 17 | 20 | 10 | 20 | 14 | 7 | 16 | 6 | 21 | 20 |
| **110**31310Ent | 6 | 7 | 7 | 6 | 8 | 8 | 6 | 15 | 6 | 8 |
| **111**31310Ent | 8 | 7 | 8 | 6 | 6 | 6 | 7 | 6 | 7 | 7 |
| **112**31310Ent | 7 | 6 | 6 | 7 | 7 | 7 | 6 | 6 | 6 | 6 |
| **113**61310Ent | 15 | 19 | 12 | 20 | 10 | 10 | 20 | 8 | 14 | 17 |
| **114**61310Ent | 16 | 20 | 14 | 21 | 9 | 9 | 21 | 7 | 19 | 18 |
| **115**61310Ent | 15 | 18 | 13 | 19 | 11 | 10 | 22 | 6 | 17 | 17 |
| **116**71310Ent | 19 | 18 | 14 | 21 | 12 | 20 | 19 | 7 | 19 | 8 |
| **117**71310Ent | 13 | 19 | 15 | 19 | 9 | 12 | 17 | 8 | 17 | 19 |
| **118**71310Ent | 14 | 19 | 12 | 20 | 10 | 11 | 18 | 6 | 18 | 18 |
| **119**91310Ent | 17 | 24 | 11 | 21 | 12 | 7 | 11 | 8 | 20 | 20 |
| **120**91310Ent | 18 | 22 | 12 | 23 | 12 | 8 | 13 | 6 | 19 | 19 |
| **121**91310Ent | 17 | 20 | 10 | 20 | 14 | 7 | 15 | 6 | 21 | 20 |
| **122**12511Ent | 15 | 18 | 8 | 18 | 9 | 7 | 22 | 6 | 17 | 21 |
| **123**12511Ent | 16 | 18 | 9 | 20 | 11 | 8 | 12 | 6 | 19 | 20 |
| **124**12511Ent | 15 | 19 | 10 | 17 | 10 | 9 | 22 | 6 | 18 | 19 |
| **125**22511Ent | 17 | 21 | 10 | 21 | 19 | 17 | 20 | 7 | 18 | 23 |
| **126**22511Ent | 15 | 20 | 9 | 20 | 11 | 15 | 9 | 6 | 17 | 19 |
| **127**22511Ent | 16 | 18 | 8 | 19 | 10 | 21 | 22 | 6 | 19 | 20 |
| **128**32511Ent | 12 | 10 | 7 | 6 | 8 | 8 | 6 | 8 | 6 | 8 |
| **129**32511Ent | 8 | 7 | 8 | 6 | 10 | 6 | 7 | 17 | 10 | 7 |
| **130**32511Ent | 11 | 6 | 10 | 7 | 7 | 7 | 11 | 8 | 6 | 11 |
| **131**42511Ent | 19 | 19 | 15 | 24 | 12 | 22 | 20 | 6 | 20 | 6 |
| **132**42511Ent | 15 | 18 | 16 | 22 | 11 | 13 | 18 | 8 | 19 | 21 |
| **133**42511Ent | 13 | 21 | 15 | 23 | 10 | 10 | 17 | 6 | 18 | 20 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **134**52511Ent | 19 | 18 | 14 | 21 | 12 | 20 | 19 | 7 | 19 | 8 |
| **135**52511Ent | 13 | 19 | 15 | 19 | 9 | 12 | 17 | 17 | 17 | 19 |
| **136**52511Ent | 14 | 19 | 12 | 20 | 10 | 11 | 18 | 10 | 18 | 18 |
| **137**62511Ent | 15 | 19 | 12 | 20 | 10 | 10 | 16 | 8 | 14 | 17 |
| **138**62511Ent | 16 | 20 | 14 | 21 | 9 | 9 | 10 | 7 | 19 | 18 |
| **139**62511Ent | 15 | 18 | 13 | 19 | 11 | 10 | 22 | 6 | 17 | 17 |
| **140**92511Ent | 17 | 24 | 11 | 21 | 12 | 7 | 11 | 8 | 20 | 20 |
| **141**92511Ent | 18 | 22 | 12 | 23 | 12 | 8 | 14 | 10 | 19 | 19 |
| **142**92511Ent | 17 | 20 | 10 | 20 | 14 | 7 | 12 | 6 | 21 | 20 |
| **143**31101Ent | 14 | 18 | 10 | 18 | 10 | 6 | 13 | 6 | 20 | 21 |
| **144**31101Ent | 13 | 16 | 13 | 14 | 12 | 6 | 15 | 10 | 21 | 19 |
| **145**31101Ent | 12 | 15 | 10 | 25 | 12 | 8 | 14 | 6 | 13 | 18 |
| **146**61101Ent | 16 | 14 | 14 | 21 | 10 | 10 | 11 | 6 | 22 | 17 |
| **147**61101Ent | 14 | 18 | 13 | 20 | 16 | 6 | 16 | 8 | 20 | 18 |
| **148**61101Ent | 15 | 15 | 11 | 21 | 17 | 14 | 16 | 6 | 21 | 12 |
| **149**71101Ent | 16 | 13 | 9 | 17 | 11 | 6 | 20 | 6 | 18 | 20 |
| **150**71101Ent | 12 | 16 | 8 | 16 | 13 | 9 | 14 | 17 | 17 | 22 |
| **151**71101Ent | 13 | 18 | 15 | 15 | 9 | 8 | 10 | 6 | 15 | 19 |
| **152**91101Ent | 13 | 16 | 12 | 19 | 14 | 14 | 13 | 17 | 18 | 16 |
| **153**91101Ent | 12 | 15 | 13 | 18 | 16 | 9 | 11 | 6 | 19 | 8 |
| **154**91101Ent | 14 | 18 | 11 | 17 | 13 | 6 | 16 | 11 | 21 | 15 |
| **155**31502Ent | 14 | 18 | 10 | 18 | 10 | 6 | 14 | 6 | 20 | 13 |
| **156**31502Ent | 15 | 16 | 13 | 24 | 17 | 6 | 9 | 10 | 21 | 19 |
| **157**31502Ent | 12 | 17 | 10 | 14 | 12 | 8 | 16 | 6 | 12 | 18 |
| **158**61502Ent | 16 | 14 | 14 | 21 | 10 | 14 | 12 | 6 | 22 | 17 |
| **159**61502Ent | 14 | 18 | 13 | 20 | 11 | 6 | 16 | 8 | 20 | 18 |
| **160**61502Ent | 15 | 15 | 11 | 21 | 17 | 7 | 16 | 10 | 21 | 12 |
| **161**71502Ent | 16 | 13 | 9 | 17 | 11 | 10 | 15 | 6 | 18 | 20 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **162**71502Ent | 12 | 16 | 8 | 16 | 13 | 9 | 12 | 7 | 17 | 22 |
| **163**71502Ent | 13 | 18 | 15 | 15 | 9 | 8 | 10 | 11 | 15 | 19 |
| **164**91502Ent | 13 | 16 | 12 | 19 | 14 | 10 | 16 | 15 | 18 | 16 |
| **163**91502Ent | 12 | 15 | 13 | 18 | 12 | 9 | 15 | 6 | 19 | 8 |
| **164**91502Ent | 14 | 18 | 11 | 17 | 17 | 6 | 12 | 11 | 21 | 15 |
| **165**10103Ent | 13 | 20 | 12 | 25 | 14 | 6 | 14 | 6 | 20 | 12 |
| **166**10103Ent | 16 | 18 | 10 | 20 | 12 | 14 | 16 | 15 | 17 | 15 |
| **167**10103Ent | 15 | 15 | 11 | 22 | 13 | 10 | 10 | 6 | 13 | 20 |
| **168**20103Ent | 15 | 20 | 12 | 26 | 10 | 6 | 8 | 10 | 22 | 22 |
| **169**20103Ent | 17 | 19 | 17 | 21 | 16 | 7 | 6 | 14 | 24 | 21 |
| **170**20103Ent | 15 | 20 | 11 | 22 | 17 | 10 | 16 | 6 | 22 | 13 |
| **171**30103Ent | 14 | 18 | 10 | 18 | 10 | 6 | 15 | 6 | 20 | 21 |
| **172**30103Ent | 13 | 16 | 13 | 24 | 12 | 6 | 14 | 10 | 21 | 19 |
| **173**30103Ent | 12 | 17 | 10 | 25 | 13 | 8 | 10 | 6 | 20 | 18 |
| **174**40103Ent | 15 | 18 | 9 | 18 | 12 | 15 | 11 | 6 | 19 | 25 |
| **175**40103Ent | 14 | 14 | 10 | 18 | 14 | 6 | 13 | 9 | 18 | 23 |
| **176**40103Ent | 15 | 17 | 16 | 17 | 16 | 11 | 10 | 11 | 16 | 21 |
| **177**50103Ent | 16 | 13 | 9 | 17 | 17 | 6 | 8 | 6 | 18 | 20 |
| **178**50103Ent | 12 | 16 | 10 | 16 | 13 | 9 | 19 | 7 | 17 | 22 |
| **179**50103Ent | 13 | 18 | 15 | 15 | 9 | 8 | 20 | 6 | 15 | 19 |
| **180**60103Ent | 16 | 14 | 14 | 21 | 10 | 10 | 16 | 14 | 22 | 17 |
| **181**60103Ent | 14 | 18 | 13 | 20 | 11 | 6 | 12 | 8 | 20 | 18 |
| **182**60103Ent | 15 | 15 | 11 | 21 | 17 | 14 | 16 | 6 | 21 | 12 |
| **183**90103Ent | 13 | 16 | 12 | 19 | 14 | 6 | 16 | 10 | 18 | 16 |
| **184**90103Ent | 12 | 15 | 13 | 18 | 12 | 9 | 14 | 6 | 19 | 8 |
| **185**90103Ent | 14 | 18 | 11 | 17 | 16 | 6 | 22 | 11 | 21 | 15 |

**Table C.2:** Inhibtion zone diameter measured for all faecal coliform isolates during the determination of antibiotic resistance.

| CODE | ERY | KF | AMP | AMO | CHL | OXY | NEO | CIP5 | KAN |
|---|---|---|---|---|---|---|---|---|---|
| 112004Fc | 15 | 13 | 15 | 14 | 18 | 16 | 16 | 30 | 18 |
| 212004Fc | 20 | 12 | 12 | 13 | 17 | 15 | 17 | 25 | 19 |
| 312004Fc | 17 | 13 | 16 | 14 | 16 | 17 | 15 | 23 | 19 |
| 422004Fc | 16 | 10 | 14 | 12 | 17 | 15 | 19 | 22 | 20 |
| 522004Fc | 17 | 12 | 15 | 13 | 16 | 14 | 18 | 24 | 21 |
| 622004Fc | 15 | 11 | 14 | 12 | 17 | 13 | 15 | 21 | 15 |
| 732004Fc | 14 | 8 | 12 | 11 | 16 | 16 | 18 | 7 | 15 |
| 832004Fc | 11 | 7 | 6 | 10 | 15 | 14 | 16 | 6 | 7 |
| 932004Fc | 10 | 12 | 13 | 11 | 14 | 15 | 17 | 30 | 8 |
| 1042004Fc | 7 | 7 | 6 | 6 | 6 | 6 | 7 | 19 | 10 |
| 1142004Fc | 8 | 6 | 8 | 7 | 7 | 7 | 6 | 22 | 15 |
| 1242004Fc | 15 | 15 | 10 | 14 | 17 | 15 | 14 | 21 | 17 |
| 1311305Fc | 20 | 12 | 11 | 20 | 8 | 6 | 6 | 10 | 6 |
| 1411305Fc | 6 | 12 | 14 | 6 | 28 | 8 | 6 | 12 | 6 |
| 1511305Fc | 6 | 17 | 11 | 24 | 10 | 6 | 12 | 32 | 6 |
| 16121305Fc | 24 | 6 | 14 | 18 | 28 | 10 | 10 | 40 | 6 |
| 17121305Fc | 22 | 14 | 12 | 16 | 26 | 6 | 12 | 40 | 6 |
| 18121305Fc | 36 | 18 | 20 | 22 | 12 | 6 | 12 | 10 | 6 |
| 19211305Fc | 32 | 30 | 10 | 24 | 22 | 8 | 12 | 32 | 15 |
| 20211305Fc | 10 | 12 | 6 | 6 | 14 | 6 | 12 | 10 | 6 |
| 21211305Fc | 20 | 12 | 6 | 8 | 8 | 10 | 14 | 40 | 14 |
| 22311305Fc | 22 | 18 | 6 | 6 | 6 | 12 | 10 | 44 | 6 |
| 23311305Fc | 13 | 16 | 6 | 12 | 6 | 8 | 12 | 46 | 6 |
| 24311305Fc | 20 | 14 | 16 | 18 | 20 | 6 | 14 | 12 | 10 |
| 2510306Fc | 22 | 18 | 13 | 26 | 12 | 8 | 10 | 12 | 6 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **26**10306Fc | 6 | 12 | 10 | 8 | 30 | 10 | 6 | 16 | 10 |
| **27**10306Fc | 10 | 18 | 12 | 26 | 10 | 10 | 16 | 34 | 8 |
| **28**20306Fc | 26 | 6 | 14 | 16 | 24 | 6 | 10 | 38 | 14 |
| **29**20306Fc | 32 | 10 | 14 | 18 | 18 | 8 | 14 | 32 | 8 |
| **30**20306Fc | 34 | 18 | 16 | 24 | 16 | 10 | 12 | 32 | 9 |
| **31**30306Fc | 36 | 30 | 10 | 24 | 22 | 6 | 12 | 34 | 6 |
| **32**30306Fc | 14 | 14 | 0 | 6 | 16 | 6 | 16 | 12 | 10 |
| **33**30306Fc | 22 | 12 | 18 | 8 | 10 | 12 | 16 | 36 | 6 |
| **34**40306Fc | 22 | 20 | 6 | 10 | 10 | 12 | 10 | 40 | 12 |
| **35**40306Fc | 24 | 18 | 6 | 14 | 6 | 36 | 14 | 44 | 14 |
| **36**40306Fc | 22 | 30 | 36 | 22 | 22 | 6 | 12 | 14 | 10 |
| **37**50306Fc | 24 | 22 | 10 | 10 | 10 | 14 | 10 | 38 | 16 |
| **38**50306Fc | 18 | 20 | 8 | 16 | 6 | 36 | 16 | 40 | 6 |
| **39**50306Fc | 20 | 32 | 34 | 16 | 14 | 6 | 14 | 12 | 12 |
| **40**60306Fc | 18 | 10 | 12 | 22 | 10 | 6 | 10 | 12 | 6 |
| **41**60306Fc | 10 | 14 | 12 | 10 | 30 | 6 | 10 | 16 | 12 |
| **42**60306Fc | 8 | 18 | 12 | 26 | 10 | 8 | 12 | 32 | 6 |
| **43**70306Fc | 32 | 28 | 22 | 26 | 24 | 8 | 14 | 34 | 6 |
| **44**70306Fc | 12 | 14 | 6 | 0 | 16 | 10 | 14 | 12 | 15 |
| **45**70306Fc | 20 | 16 | 10 | 10 | 12 | 6 | 16 | 38 | 6 |
| **46**90306Fc | 24 | 10 | 16 | 16 | 11 | 6 | 12 | 32 | 10 |
| **47**90306Fc | 24 | 14 | 12 | 14 | 9 | 8 | 12 | 36 | 6 |
| **48**90306Fc | 36 | 20 | 10 | 12 | 14 | 10 | 14 | 12 | 10 |
| **49**12707Fc | 18 | 8 | 12 | 16 | 22 | 10 | 10 | 20 | 8 |
| **50**12707Fc | 16 | 16 | 10 | 16 | 24 | 8 | 6 | 38 | 6 |
| **51**12707Fc | 13 | 18 | 16 | 20 | 12 | 10 | 12 | 10 | 8 |
| **52**22707Fc | 30 | 14 | 14 | 20 | 20 | 6 | 6 | 30 | 6 |
| **53**22707Fc | 10 | 12 | 6 | 6 | 12 | 6 | 12 | 10 | 6 |
| **54**22707Fc | 18 | 10 | 8 | 10 | 8 | 6 | 16 | 38 | 8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **55**32707Fc | 20 | 18 | 6 | 8 | 10 | 10 | 10 | 40 | 8 |
| **56**32707Fc | 22 | 14 | 6 | 14 | 6 | 6 | 16 | 46 | 16 |
| **57**32707Fc | 18 | 34 | 11 | 18 | 18 | 8 | 14 | 14 | 10 |
| **58**42707Fc | 20 | 18 | 6 | 6 | 8 | 10 | 10 | 40 | 10 |
| **59**42707Fc | 24 | 18 | 8 | 14 | 6 | 32 | 16 | 42 | 6 |
| **60**42707Fc | 20 | 28 | 32 | 18 | 20 | 6 | 12 | 14 | 10 |
| **61**52707Fc | 22 | 20 | 6 | 10 | 8 | 12 | 10 | 36 | 14 |
| **62**52707Fc | 18 | 22 | 6 | 18 | 6 | 30 | 18 | 36 | 6 |
| **63**52707Fc | 22 | 30 | 32 | 16 | 10 | 10 | 14 | 12 | 10 |
| **64**62707Fc | 16 | 10 | 12 | 20 | 10 | 10 | 8 | 14 | 6 |
| **65**62707Fc | 8 | 12 | 14 | 10 | 26 | 8 | 10 | 16 | 10 |
| **66**62707Fc | 6 | 16 | 16 | 24 | 8 | 10 | 14 | 34 | 6 |
| **67**92707Fc | 10 | 10 | 12 | 14 | 10 | 10 | 10 | 14 | 8 |
| **68**92707Fc | 8 | 14 | 10 | 8 | 12 | 6 | 12 | 16 | 10 |
| **69**92707Fc | 6 | 16 | 12 | 15 | 10 | 10 | 14 | 30 | 8 |
| **70**13108Fc | 18 | 12 | 14 | 18 | 10 | 6 | 8 | 12 | 6 |
| **71**13108Fc | 8 | 10 | 12 | 6 | 22 | 10 | 6 | 10 | 8 |
| **72**13108Fc | 6 | 16 | 12 | 22 | 10 | 6 | 10 | 30 | 6 |
| **73**23108Fc | 22 | 8 | 16 | 20 | 22 | 10 | 11 | 32 | 6 |
| **74**23108Fc | 20 | 12 | 10 | 14 | 24 | 8 | 12 | 28 | 14 |
| **75**23108Fc | 30 | 16 | 15 | 20 | 10 | 6 | 10 | 12 | 6 |
| **76**33108Fc | 20 | 16 | 6 | 6 | 6 | 12 | 10 | 30 | 10 |
| **77**33108Fc | 18 | 12 | 10 | 10 | 8 | 6 | 12 | 32 | 8 |
| **78**33108Fc | 22 | 30 | 14 | 22 | 18 | 10 | 6 | 12 | 10 |
| **79**43108Fc | 30 | 18 | 6 | 10 | 10 | 12 | 10 | 40 | 12 |
| **80**43108Fc | 22 | 16 | 6 | 12 | 6 | 26 | 14 | 34 | 10 |
| **82**43108Fc | 24 | 28 | 26 | 18 | 20 | 6 | 10 | 12 | 10 |
| **83**53108Fc | 22 | 22 | 8 | 6 | 10 | 10 | 8 | 26 | 12 |
| **84**53108Fc | 18 | 24 | 10 | 16 | 10 | 22 | 20 | 26 | 15 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **85**53108Fc | 20 | 26 | 38 | 14 | 12 | 10 | 12 | 10 | 6 |
| **84**63108Fc | 18 | 10 | 12 | 22 | 10 | 6 | 10 | 12 | 8 |
| **85**63108Fc | 8 | 16 | 15 | 6 | 24 | 10 | 6 | 14 | 6 |
| **86**63108Fc | 6 | 20 | 12 | 24 | 12 | 8 | 12 | 30 | 10 |
| **87**93108Fc | 10 | 8 | 12 | 16 | 10 | 6 | 10 | 14 | 8 |
| **88**93108Fc | 10 | 14 | 10 | 8 | 26 | 6 | 8 | 16 | 10 |
| **89**93108Fc | 6 | 16 | 16 | 16 | 10 | 8 | 12 | 28 | 8 |
| **90**32009Fc | 11 | 14 | 6 | 15 | 11 | 10 | 13 | 18 | 12 |
| **91**32009Fc | 12 | 11 | 8 | 11 | 12 | 13 | 14 | 21 | 10 |
| **92**32009Fc | 13 | 10 | 10 | 12 | 14 | 10 | 12 | 20 | 12 |
| **93**62009Fc | 10 | 10 | 14 | 22 | 19 | 10 | 19 | 26 | 17 |
| **94**62009Fc | 7 | 13 | 16 | 18 | 17 | 18 | 17 | 24 | 18 |
| **95**62009Fc | 6 | 10 | 19 | 17 | 18 | 16 | 19 | 21 | 17 |
| **96**72009Fc | 10 | 14 | 6 | 17 | 17 | 15 | 20 | 19 | 17 |
| **97**72009Fc | 11 | 14 | 7 | 18 | 6 | 13 | 17 | 21 | 16 |
| **98**72009Fc | 8 | 10 | 10 | 12 | 18 | 11 | 16 | 22 | 13 |
| **99**92009Fc | 10 | 17 | 9 | 15 | 19 | 10 | 13 | 12 | 10 |
| **100**92009Fc | 7 | 14 | 7 | 13 | 12 | 7 | 10 | 12 | 11 |
| **101**92009Fc | 8 | 11 | 11 | 15 | 10 | 8 | 12 | 13 | 14 |
| **102**31310Fc | 10 | 13 | 6 | 17 | 17 | 15 | 20 | 19 | 17 |
| **104**31310Fc | 11 | 14 | 7 | 18 | 6 | 13 | 17 | 21 | 16 |
| **105**31310Fc | 8 | 11 | 10 | 12 | 18 | 12 | 16 | 22 | 18 |
| **106**61310Fc | 10 | 10 | 14 | 22 | 19 | 10 | 19 | 26 | 17 |
| **107**61310Fc | 7 | 13 | 14 | 18 | 17 | 18 | 17 | 24 | 18 |
| **108**61310Fc | 6 | 10 | 10 | 17 | 18 | 16 | 19 | 21 | 17 |
| **109**71310Fc | 11 | 13 | 9 | 15 | 11 | 11 | 13 | 18 | 12 |
| **110**71310Fc | 12 | 11 | 7 | 11 | 12 | 13 | 14 | 21 | 10 |
| **111**71310Fc | 13 | 10 | 10 | 10 | 14 | 12 | 11 | 20 | 13 |
| **112**91310Fc | 10 | 17 | 9 | 20 | 19 | 10 | 13 | 12 | 10 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **113**91310Fc | 12 | 14 | 7 | 19 | 18 | 7 | 10 | 10 | 12 |
| **114**91310Fc | 8 | 11 | 10 | 20 | 12 | 8 | 12 | 13 | 14 |
| **115**12511Fc | 14 | 10 | 15 | 14 | 10 | 14 | 18 | 22 | 16 |
| **116**12511Fc | 10 | 11 | 13 | 13 | 19 | 15 | 17 | 20 | 17 |
| **117**12511Fc | 9 | 12 | 16 | 12 | 20 | 13 | 19 | 19 | 16 |
| **116**22511Fc | 13 | 8 | 14 | 17 | 18 | 15 | 17 | 21 | 15 |
| **118**22511Fc | 15 | 13 | 12 | 18 | 15 | 14 | 14 | 19 | 13 |
| **119**22511Fc | 10 | 11 | 13 | 12 | 13 | 16 | 15 | 19 | 14 |
| **120**32511Fc | 11 | 13 | 6 | 15 | 9 | 11 | 12 | 18 | 12 |
| **121**32511Fc | 12 | 12 | 6 | 11 | 12 | 13 | 14 | 21 | 11 |
| **122**32511Fc | 13 | 10 | 10 | 10 | 14 | 12 | 11 | 20 | 13 |
| **124**42511Fc | 10 | 14 | 6 | 17 | 17 | 15 | 20 | 19 | 17 |
| **125**42511Fc | 11 | 14 | 7 | 18 | 6 | 13 | 17 | 21 | 16 |
| **126**42511Fc | 8 | 11 | 10 | 12 | 18 | 12 | 16 | 23 | 19 |
| **127**52511Fc | 10 | 10 | 13 | 18 | 16 | 16 | 19 | 18 | 15 |
| **128**52511Fc | 9 | 13 | 15 | 14 | 19 | 14 | 16 | 23 | 14 |
| **129**52511Fc | 11 | 12 | 17 | 19 | 18 | 17 | 18 | 22 | 18 |
| **130**62511Fc | 10 | 10 | 14 | 22 | 19 | 10 | 19 | 26 | 17 |
| **131**62511Fc | 7 | 13 | 16 | 18 | 17 | 18 | 17 | 24 | 18 |
| **132**62511Fc | 6 | 10 | 11 | 17 | 18 | 16 | 19 | 21 | 17 |
| **133**92511Fc | 10 | 17 | 9 | 20 | 19 | 10 | 13 | 12 | 10 |
| **134**92511Fc | 7 | 14 | 7 | 19 | 18 | 7 | 10 | 11 | 12 |
| **135**92511Fc | 8 | 11 | 10 | 20 | 20 | 8 | 12 | 13 | 14 |
| **136**31101Fc | 14 | 8 | 12 | 11 | 16 | 16 | 18 | 7 | 15 |
| **137**31101Fc | 11 | 7 | 6 | 10 | 15 | 14 | 16 | 6 | 7 |
| **138**31101Fc | 10 | 12 | 12 | 17 | 12 | 15 | 13 | 30 | 8 |
| **139**61101Fc | 16 | 14 | 17 | 17 | 18 | 17 | 16 | 28 | 15 |
| **140**61101Fc | 10 | 12 | 16 | 13 | 16 | 16 | 15 | 24 | 13 |
| **141**61101Fc | 11 | 13 | 19 | 14 | 15 | 17 | 16 | 20 | 12 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **142**71101Fc | 14 | 9 | 12 | 11 | 7 | 8 | 18 | 18 | 18 |
| **143**71101Fc | 11 | 10 | 10 | 12 | 9 | 15 | 8 | 17 | 13 |
| **144**71101Fc | 15 | 12 | 13 | 17 | 11 | 14 | 13 | 19 | 12 |
| **145**91101Fc | 10 | 20 | 15 | 6 | 16 | 20 | 12 | 25 | 15 |
| **146**91101Fc | 9 | 16 | 16 | 19 | 14 | 16 | 18 | 20 | 13 |
| **147**91101Fc | 11 | 15 | 11 | 12 | 13 | 15 | 19 | 21 | 14 |
| **148**31502Fc | 14 | 8 | 12 | 11 | 16 | 16 | 18 | 7 | 15 |
| **149**31502Fc | 11 | 7 | 6 | 10 | 15 | 14 | 16 | 6 | 7 |
| **150**31502Fc | 10 | 12 | 13 | 11 | 12 | 15 | 14 | 30 | 8 |
| **151**61502Fc | 16 | 16 | 17 | 17 | 18 | 17 | 16 | 28 | 15 |
| **152**61502Fc | 10 | 15 | 16 | 13 | 16 | 16 | 15 | 24 | 13 |
| **153**61502Fc | 11 | 17 | 19 | 14 | 15 | 19 | 16 | 20 | 12 |
| **154**71502Fc | 23 | 16 | 12 | 11 | 18 | 19 | 17 | 18 | 18 |
| **155**71502Fc | 11 | 10 | 10 | 12 | 9 | 10 | 8 | 17 | 17 |
| **156**71502Fc | 15 | 17 | 17 | 17 | 11 | 14 | 17 | 19 | 13 |
| **157**91502Fc | 10 | 20 | 15 | 6 | 12 | 20 | 19 | 25 | 15 |
| **158**91502Fc | 9 | 16 | 16 | 13 | 14 | 16 | 13 | 20 | 13 |
| **159**91502Fc | 11 | 14 | 12 | 12 | 13 | 15 | 19 | 21 | 13 |
| **160**10103Fc | 15 | 13 | 15 | 14 | 18 | 16 | 16 | 30 | 18 |
| **161**10103Fc | 20 | 12 | 16 | 13 | 17 | 15 | 17 | 25 | 19 |
| **162**10103Fc | 17 | 13 | 11 | 14 | 16 | 14 | 13 | 23 | 12 |
| **163**20103Fc | 16 | 10 | 10 | 12 | 17 | 15 | 19 | 22 | 20 |
| **164**20103Fc | 17 | 12 | 15 | 13 | 16 | 14 | 18 | 24 | 21 |
| **165**20103Fc | 15 | 11 | 14 | 12 | 17 | 13 | 15 | 21 | 15 |
| **166**30103Fc | 14 | 8 | 12 | 11 | 16 | 16 | 18 | 7 | 15 |
| **167**30103Fc | 11 | 7 | 6 | 10 | 15 | 14 | 16 | 6 | 7 |
| **168**30103Fc | 10 | 12 | 13 | 11 | 14 | 15 | 17 | 30 | 8 |
| **169**40103Fc | 20 | 7 | 17 | 17 | 6 | 16 | 7 | 19 | 10 |
| **170**40103Fc | 8 | 6 | 8 | 7 | 7 | 7 | 6 | 22 | 15 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **171**40103Fc | 15 | 15 | 10 | 14 | 17 | 15 | 14 | 21 | 17 |
| **172**50103Fc | 9 | 16 | 12 | 11 | 7 | 16 | 9 | 18 | 18 |
| **173**50103Fc | 11 | 10 | 10 | 12 | 9 | 10 | 8 | 17 | 17 |
| **174**50103Fc | 15 | 12 | 17 | 16 | 11 | 19 | 13 | 19 | 18 |
| **175**60103Fc | 16 | 14 | 17 | 17 | 18 | 17 | 16 | 28 | 15 |
| **176**60103Fc | 10 | 12 | 16 | 13 | 16 | 16 | 15 | 24 | 13 |
| **177**60103Fc | 11 | 13 | 19 | 14 | 15 | 15 | 16 | 20 | 12 |
| **178**90103Fc | 10 | 20 | 15 | 6 | 16 | 20 | 12 | 25 | 15 |
| **179**90103Fc | 9 | 16 | 16 | 13 | 14 | 14 | 18 | 20 | 13 |
| **180**90103Fc | 14 | 15 | 15 | 12 | 13 | 15 | 19 | 11 | 14 |

**Table C.3:** Average per site for the inhibtion zone diameter measured for all enteroccoci isolates during the determination of antibiotic resistance

| CODE | VAN | CIP5 | KAN | OXY | AMO | NEO | AMP | KAN | CHL | ERY |
|---|---|---|---|---|---|---|---|---|---|---|
| **S7200405Ent** | 16.3 | 18.3 | 11 | 22.3 | 13 | 10 | 13.67 | 9 | 19.3 | 15.67 |
| **S6200405Ent** | 13.7 | 19.7 | 13.33 | 23 | 14.3 | 7.67 | 11 | 10 | 22.7 | 21 |
| **S5200405Ent** | 13 | 17 | 19.67 | 22.3 | 11.3 | 18.7 | 11.67 | 7.33 | 20.3 | 19.33 |
| **S3200405Ent** | 14.5 | 16 | 9.5 | 18 | 13 | 10.5 | 15.5 | 7.5 | 18.5 | 24 |
| **S7130505Ent** | 16 | 11.3 | 17.33 | 10.7 | 28 | 15.3 | 10 | 14 | 16 | 18 |
| **S6130505Ent** | 30 | 9.33 | 7 | 13.3 | 20 | 10 | 14 | 11.3 | 12 | 18.67 |
| **S5130505Ent** | 21.3 | 7.33 | 19.33 | 7.67 | 21.3 | 19.7 | 13.67 | 13.7 | 11.3 | 14.67 |
| **S8130505Ent** | 15.3 | 6 | 11.33 | 8.33 | 25.3 | 14.7 | 10.33 | 7 | 10.7 | 11.33 |
| **S3130505Ent** | 19 | 8 | 16 | 6 | 25 | 8 | 15 | 6.5 | 18 | 8 |
| **S1130505Ent** | 16.7 | 12.7 | 7.333 | 8 | 25.3 | 15.3 | 11.67 | 10.3 | 12 | 11.33 |
| **S2130505Ent** | 16 | 14 | 9.333 | 7.33 | 24.7 | 10 | 12 | 7.33 | 9 | 11.33 |
| **S7030605Ent** | 20.7 | 12 | 18 | 12 | 31.3 | 16.7 | 12 | 15.3 | 16 | 18.67 |
| **S6030605Ent** | 31.3 | 9.33 | 7.333 | 12.7 | 18 | 10 | 12 | 7.33 | 9.33 | 19.33 |
| **S5030605Ent** | 23.3 | 7.33 | 8 | 6 | 20.7 | 8.67 | 14 | 8.67 | 15.3 | 16 |
| **S3030605Ent** | 16.7 | 8.67 | 14 | 8 | 26 | 8 | 10.33 | 9.33 | 12.7 | 14 |
| **S2030605Ent** | 22 | 6.67 | 16.67 | 9.33 | 26.7 | 10 | 17.33 | 12 | 14 | 10 |
| **S8030605Ent** | 18 | 14 | 7.333 | 12.7 | 26 | 16 | 13.33 | 10.7 | 12 | 12.67 |
| **S1030605Ent** | 18 | 15.3 | 12.33 | 8.67 | 26.7 | 10.7 | 10 | 10 | 8 | 12 |
| **S4030605Ent** | 28 | 12.7 | 17.33 | 7.33 | 26 | 8.67 | 22 | 8.67 | 16.7 | 10.67 |
| **S7270705Ent** | 18 | 11.3 | 14 | 10 | 22 | 15.3 | 13.33 | 12 | 16.7 | 16 |
| **S6270705Ent** | 21.3 | 7.33 | 6.667 | 9.33 | 20.7 | 8.67 | 13.33 | 7.33 | 13.3 | 18 |
| **S5270705Ent** | 15.3 | 7.33 | 10 | 8.33 | 24 | 9 | 14 | 9 | 8.67 | 14 |
| **S3270705Ent** | 22.7 | 9.33 | 16 | 10 | 24.7 | 12 | 20.67 | 9.33 | 14 | 12 |
| **S5270705Ent** | 15.3 | 10.7 | 8 | 8 | 23.3 | 14.7 | 21.33 | 6.67 | 8.67 | 9.333 |
| **S8270705Ent** | 14.7 | 13.3 | 10 | 8.33 | 22 | 19 | 10 | 10 | 7.33 | 12 |
| **S4270705Ent** | 16 | 8 | 12 | 8.67 | 22.7 | 8.67 | 13.67 | 6 | 10.7 | 9.333 |
| **S7310805Ent** | 12 | 12.7 | 13.33 | 10 | 27.3 | 13.3 | 13.33 | 14.7 | 14.7 | 16.67 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **S6310805Ent** | 20 | 8.67 | 8.333 | 7.67 | 18.7 | 10 | 12 | 8 | 16.7 | 12.67 |
| **S5310805Ent** | 14 | 7.33 | 12 | 8 | 19.3 | 8 | 10 | 8 | 10 | 10.67 |
| **S3310805Ent** | 14 | 8.67 | 14.67 | 7.33 | 21.3 | 8.67 | 19.33 | 6.67 | 15.3 | 12 |
| **S2310805Ent** | 20.7 | 7.33 | 15.33 | 8.67 | 22 | 10 | 14.67 | 12 | 13.3 | 8 |
| **S8310805Ent** | 15.3 | 11 | 8.667 | 7.33 | 19.3 | 8.67 | 10 | 9.33 | 7.33 | 12.67 |
| **S4310805Ent** | 21.3 | 7.33 | 8 | 7.33 | 17.3 | 11.3 | 13.33 | 7.33 | 14 | 16 |
| **S5200905Ent** | 7 | 6.67 | 7 | 6.33 | 7 | 7 | 6.333 | 9.67 | 6.33 | 7 |
| **S8200905Ent** | 15.3 | 19 | 13 | 20 | 10 | 9.67 | 21 | 7 | 16.7 | 17.33 |
| **S1200905Ent** | 15.3 | 18.7 | 13.67 | 20 | 10.3 | 14.3 | 13.33 | 7 | 18 | 15 |
| **S4200905Ent** | 17.3 | 22 | 11 | 21.3 | 12.7 | 7.33 | 13.67 | 6.67 | 20 | 19.67 |
| **S5131005Ent** | 7 | 6.67 | 7 | 6.33 | 7 | 7 | 6.333 | 9 | 6.33 | 7 |
| **S8131005Ent** | 15.3 | 19 | 13 | 20 | 10 | 9.67 | 21 | 7 | 16.7 | 17.33 |
| **S1131005Ent** | 15.3 | 18.7 | 13.67 | 20 | 10.3 | 14.3 | 18 | 7 | 18 | 15 |
| **S4131005Ent** | 17.3 | 22 | 11 | 21.3 | 12.7 | 7.33 | 13 | 6.67 | 20 | 19.67 |
| **S7251105Ent** | 15.3 | 18.3 | 9 | 18.3 | 10 | 8 | 18.67 | 6 | 18 | 20 |
| **S6251105Ent** | 16 | 19.7 | 9 | 20 | 13.3 | 17.7 | 17 | 6.33 | 18 | 20.67 |
| **S5251105Ent** | 10.3 | 7.67 | 8.333 | 6.33 | 8.33 | 7 | 8 | 11 | 7.33 | 8.667 |
| **S3251105Ent** | 15.7 | 19.3 | 15.33 | 23 | 11 | 15 | 18.33 | 6.67 | 19 | 15.67 |
| **S2251105Ent** | 15.3 | 18.7 | 13.67 | 20 | 10.3 | 14.3 | 18 | 11.3 | 18 | 15 |
| **S8251105Ent** | 15.3 | 19 | 13 | 20 | 10 | 9.67 | 16 | 7 | 16.7 | 17.33 |
| **S4251105Ent** | 17.3 | 22 | 11 | 21.3 | 12.7 | 7.33 | 12.33 | 8 | 20 | 19.67 |
| **S5110106Ent** | 13 | 16.3 | 11 | 19 | 11.3 | 6.67 | 14 | 7.33 | 18 | 19.33 |
| **S8110106En** | 15 | 15.7 | 12.67 | 20.7 | 14.3 | 10 | 14.33 | 6.67 | 21 | 15.67 |
| **S1110106Ent** | 13.7 | 15.7 | 10.67 | 16 | 11 | 7.67 | 14.67 | 9.67 | 16.7 | 20.33 |
| **S4110106Ent** | 13 | 16.3 | 12 | 18 | 14.3 | 9.67 | 13.33 | 11.3 | 19.3 | 13 |
| **S5150206Ent** | 13.7 | 17 | 11 | 18.7 | 13 | 6.67 | 13 | 7.33 | 17.7 | 16.67 |
| **S8150206Ent** | 15 | 15.7 | 12.67 | 20.7 | 12.7 | 9 | 14.67 | 8 | 21 | 15.67 |
| **S1150206Ent** | 13.7 | 15.7 | 10.67 | 16 | 11 | 9 | 12.33 | 8 | 16.7 | 20.33 |
| **S4150206Ent** | 13 | 16.3 | 12 | 18 | 14.3 | 8.33 | 14.33 | 10.7 | 19.3 | 13 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **S7010306Ent** | 14.7 | 17.7 | 11 | 22.3 | 13 | 10 | 13.33 | 9 | 16.7 | 15.67 |
| **S6010306Ent** | 15.7 | 19.7 | 13.33 | 23 | 14.3 | 7.67 | 10 | 10 | 22.7 | 18.67 |
| **S5010306Ent** | 13 | 17 | 11 | 22.3 | 11.7 | 6.67 | 13 | 7.33 | 20.3 | 19.33 |
| **S3010306Ent** | 14.7 | 16.3 | 11.67 | 17.7 | 14 | 10.7 | 11.33 | 8.67 | 17.7 | 23 |
| **S2010306Ent** | 13.7 | 15.7 | 11.33 | 16 | 13 | 7.67 | 15.67 | 6.33 | 16.7 | 20.33 |
| **S8010306Ent** | 15 | 15.7 | 12.67 | 20.7 | 12.7 | 10 | 14.67 | 9.33 | 21 | 15.67 |
| **S4010306Ent** | 13 | 16.3 | 12 | 18 | 14 | 7 | 17.33 | 9 | 19.3 | 13 |

**Table C.4:** Average per site for the inhibtion zone diameter measured for all faecal coliform isolates isolates during the determination of antibiotic resistance

| CODE | ERY | KF30 | AMP | AMO | CHL | OXY | NEO | CIP5 | KAN |
|---|---|---|---|---|---|---|---|---|---|
| **S7200405Fc** | 17.3 | 12.67 | 14.33 | 13.67 | 17 | 16 | 16 | 26 | 18.7 |
| **S6200405Fc** | 16 | 11 | 14.33 | 12.33 | 17 | 14 | 17.33 | 22.33 | 18.7 |
| **S5200405Fc** | 11.7 | 9 | 10.33 | 10.67 | 15 | 15 | 17 | 14.33 | 10 |
| **S3200405Fc** | 10 | 9.333 | 8 | 9 | 10 | 9.333 | 9 | 20.67 | 14 |
| **S3130505Fc** | 27.3 | 12.67 | 15.33 | 18.67 | 22 | 7.333 | 11.33 | 30 | 6 |
| **S6130505Fc** | 20.7 | 18 | 7.333 | 12.67 | 15 | 8 | 12.67 | 27.33 | 11.7 |
| **S5130505Fc** | 18.3 | 16 | 9.333 | 12 | 11 | 8.667 | 12 | 34 | 7.33 |
| **S7030605Fc** | 14 | 15 | 11.5 | 17 | 21 | 9 | 8 | 14 | 8 |
| **S6030605Fc** | 30.7 | 11.33 | 14.67 | 19.33 | 19 | 8 | 12 | 34 | 10.3 |
| **S5030605Fc** | 24 | 18.67 | 9.333 | 12.67 | 16 | 8 | 14.67 | 27.33 | 7.33 |
| **S3030605Fc** | 22.7 | 22.67 | 16 | 15.33 | 13 | 18 | 12 | 32.67 | 12 |
| **S2030605Fc** | 21 | 21 | 9 | 13 | 8 | 25 | 13 | 39 | 11 |
| **S8030605Fc** | 12 | 14 | 12 | 19.33 | 17 | 6.667 | 10.67 | 20 | 8 |
| **S1030605Fc** | 21.3 | 19.33 | 12.67 | 12 | 17 | 8 | 14.67 | 28 | 9 |
| **S4030605Fc** | 24 | 12 | 14 | 15 | 10 | 7 | 12 | 34 | 8 |
| **S7270705Fc** | 15.7 | 14 | 12.67 | 17.33 | 19 | 9.333 | 9.333 | 22.67 | 7.33 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **S6270705Fc** | 19.3 | 12 | 9.333 | 12 | 13 | 6 | 11.33 | 26 | 6.67 |
| **S5270705Fc** | 20 | 22 | 7.667 | 13.33 | 11 | 8 | 13.33 | 33.33 | 11.3 |
| **S3270705Fc** | 21.3 | 21.33 | 15.33 | 12.67 | 11 | 16 | 12.67 | 32 | 8.67 |
| **S2270705Fc** | 20.7 | 24 | 14.67 | 14.67 | 8 | 17.33 | 14 | 28 | 10 |
| **S8270705Fc** | 10 | 12.67 | 14 | 18 | 15 | 9.333 | 10.67 | 21.33 | 7.33 |
| **S4270705Fc** | 8 | 13.33 | 11.33 | 12.33 | 11 | 8.667 | 12 | 20 | 8.67 |
| **S7310805Fc** | 10.7 | 12.67 | 12.67 | 15.33 | 14 | 7.333 | 8 | 17.33 | 6.67 |
| **S6310805Fc** | 24 | 12 | 13.67 | 18 | 19 | 8 | 11 | 24 | 8.67 |
| **S2310805Fc** | 20 | 19.33 | 10 | 12.67 | 11 | 9.333 | 9.333 | 24.67 | 9.33 |
| **S3310805Fc** | 25.3 | 20.67 | 12.67 | 13.33 | 12 | 14.67 | 11.33 | 28.67 | 10.7 |
| **S2310805Fc** | 20 | 24 | 18.67 | 12 | 11 | 14 | 13.33 | 20.67 | 11 |
| **S8310805Fc** | 10.7 | 15.33 | 13 | 17.33 | 15 | 8 | 9.333 | 18.67 | 8 |
| **S4310805Fc** | 8.67 | 12.67 | 12.67 | 13.33 | 15 | 6.667 | 10 | 19.33 | 8.67 |
| **S5200905Fc** | 12 | 11.67 | 8 | 12.67 | 12 | 11 | 13 | 19.67 | 11.3 |
| **S8200905Fc** | 7.67 | 11 | 16.33 | 19 | 18 | 14.67 | 18.33 | 23.67 | 17.3 |
| **S1200905Fc** | 9.67 | 12.67 | 7.667 | 15.67 | 14 | 13 | 17.67 | 20.67 | 15.3 |
| **S4200905Fc** | 8.33 | 14 | 9 | 14.33 | 14 | 8.333 | 11.67 | 12.33 | 11.7 |
| **S5131005Fc** | 9.67 | 12.67 | 7.667 | 15.67 | 14 | 13.33 | 17.67 | 20.67 | 17 |
| **S8131005Fc** | 7.67 | 11 | 12.67 | 19 | 18 | 14.67 | 18.33 | 23.67 | 17.3 |
| **S1131005Fc** | 12 | 11.33 | 8.667 | 12 | 12 | 12 | 12.67 | 19.67 | 11.7 |
| **S4131005Fc** | 10 | 14 | 8.667 | 19.67 | 16 | 8.333 | 11.67 | 11.67 | 12 |
| **S7251105Fc** | 11 | 11 | 14.67 | 13 | 16 | 14 | 18 | 20.33 | 16.3 |
| **S6251105Fc** | 12.7 | 10.67 | 13 | 15.67 | 15 | 15 | 15.33 | 19.67 | 14 |
| **S5251105Fc** | 12 | 11.67 | 7.333 | 12 | 12 | 12 | 12.33 | 19.67 | 12 |
| **S3251105Fc** | 9.67 | 13 | 7.667 | 15.67 | 14 | 13.33 | 17.67 | 21 | 17.3 |
| **S2251105Fc** | 10 | 11.67 | 15 | 17 | 18 | 15.67 | 17.67 | 21 | 15.7 |
| **S8251105Fc** | 7.67 | 11 | 13.67 | 19 | 18 | 14.67 | 18.33 | 23.67 | 17.3 |
| **S4251105Fc** | 8.33 | 14 | 8.667 | 19.67 | 19 | 8.333 | 11.67 | 12 | 12 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **S5110106Fc** | 11.7 | 9 | 10 | 12.67 | 14 | 15 | 15.67 | 14.33 | 10 |
| **S8110106Fc** | 12.3 | 13 | 17.33 | 14.67 | 16 | 16.67 | 15.67 | 24 | 13.3 |
| **S1110106Fc** | 13.3 | 10.33 | 11.67 | 13.33 | 9 | 12.33 | 13 | 18 | 14.3 |
| **S4110106Fc** | 10 | 17 | 14 | 12.33 | 14 | 17 | 16.33 | 22 | 14 |
| **S5150206Fc** | 11.7 | 9 | 10.33 | 10.67 | 14 | 15 | 16 | 14.33 | 10 |
| **S8150206Fc** | 12.3 | 16 | 17.33 | 14.67 | 16 | 17.33 | 15.67 | 24 | 13.3 |
| **S1150206Fc** | 16.3 | 14.33 | 13 | 13.33 | 13 | 14.33 | 14 | 18 | 16 |
| **S4150206Fc** | 10 | 16.67 | 14.33 | 10.33 | 13 | 17 | 17 | 22 | 13.7 |
| **S7010306Fc** | 17.3 | 12.67 | 14 | 13.67 | 17 | 15 | 15.33 | 26 | 16.3 |
| **S6010306Fc** | 16 | 11 | 13 | 12.33 | 17 | 14 | 17.33 | 22.33 | 18.7 |
| **S5010306Fc** | 11.7 | 9 | 10.33 | 10.67 | 15 | 15 | 17 | 14.33 | 10 |
| **S3010306Fc** | 14.3 | 9.333 | 11.67 | 12.67 | 10 | 12.67 | 9 | 20.67 | 14 |
| **S2010306Fc** | 11.7 | 12.67 | 13 | 13 | 9 | 15 | 10 | 18 | 17.7 |
| **S8010306Fc** | 12.3 | 13 | 17.33 | 14.67 | 16 | 16 | 15.67 | 24 | 13.3 |
| **S4010306Fc** | 11 | 17 | 15.33 | 10.33 | 14 | 16.33 | 16.33 | 18.67 | 14 |

**Table C.5:** Table indicating antibiotic resistance profiles for individual enteroccoci isolates

| CODE | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Van | Cip | Ery |
|---|---|---|---|---|---|---|---|---|---|---|
| 312004Ent | Amp | Amo | Cep | Kan | Neo | | | | | |
| 422004Ent | Amp | Amo | Cep | Kan | Neo | | | | | |
| 622004Ent | Amp | Cep | Kan | Neo | | | | | | |
| 1042004Ent | Amp | Amo | Cep | Kan | Neo | | | | | |
| 2741305Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 29711305Ent | Amp | Cep | Kan | Neo | Oxy | Cip | | | | |
| 3820306Ent | Amp | Amo | Cep | Kan | Neo | Cip | | | | |
| 4130306Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 5370306Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 6122707Ent | Amp | Amo | Cep | Kan | Neo | Cip | | | | |
| 6222707Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 6322707Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 6432707Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 7692707Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 8323108Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 8533108Ent | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Van | Cip | Ery |
| 8633108Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 8843108Ent | Amo | Cep | Kan | Neo | Oxy | Chl | Cip | | | |
| 9353108Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 9463108Ent | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Van | Cip | Ery |
| 9563108Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | Ery | | |
| 9893108Ent | Amp | Cep | Kan | Neo | Oxy | Chl | Cip | | | |

**999**3108Ent Amp Cep Kan Neo Oxy Chl Cip

**100**32009Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**102**32009Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**103**62009Ent Amo Cep Kan Neo

**104**62009Ent Amo Cep Kan Neo

**105**62009Ent Amo Cep Kan Neo

**108**72009Ent Amp Amo Cep Kan Neo Van

**108**92009Ent Amp Amo Cep Kan Neo

**109**92009Ent Amp Amo Cep Kan Neo

**111**31310Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**112**31310Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**113**61310Ent Amo Cep Kan Neo

**115**61310Ent Amo Cep Kan Neo

**119**91310Ent Amp Amo Cep Kan Neo

**120**91310Ent Amp Amo Cep Kan Neo

**121**91310Ent Amp Amo Cep Kan Neo

**122**12511Ent Amo Cep Kan Neo

**123**12511Ent Amp Amo Cep Kan Neo

**124**12511Ent Amo Cep Kan Neo

**128**32511Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**130**32511Ent Amp Amo Cep Kan Neo Oxy Chl Van Cip Ery

**136**52511Ent Amo Cep Kan Neo

**137**62511Ent Amp Amo Cep Kan Neo

**138**62511Ent Amp Amo Cep Kan Neo

**1396**2511Ent Amo Cep Kan Neo

**1409**2511Ent Amp Amo Cep Kan Neo

**1419**2511Ent Amp Amo Cep Kan Neo

**1429**2511Ent Amp Amo Cep Kan Neo

**1433**1101Ent Amp Amo Cep Kan Neo Van

**1466**1101Ent Amp Amo Cep Kan Neo Cip

**1476**1101Ent Amp Amo Cep Kan Neo Van

**1497**1101Ent Amp Amo Cep Kan Neo Cip

**1517**1101Ent Amp Amo Cep Kan Neo Van

**1549**1101Ent Amp Amo Cep Kan Neo Van

**1596**1502Ent Amp Amo Cep Kan Neo Van

**1617**1502Ent Amp Amo Cep Kan Neo Cip

**1627**1502Ent Amp Amo Cep Kan Neo Van

**1637**1502Ent Amp Amo Cep Kan Neo Van

**1713**0103Ent Amp Amo Cep Kan Neo Van

**1733**0103Ent Amp Amo Cep Kan Neo Van

**1775**0103Ent Amp Cep Kan Neo Cip

**1816**0103Ent Amp Amo Cep Kan Neo Van

**1839**0103Ent Amp Amo Cep Kan Neo Van

**1849**0103Ent Amp Amo Cep Kan Neo Van Cip Ery

**Table C.6:** Table indicating antibiotic resistance profiles for individual faecal coliform isolates.

| CODE | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Cip | Ery |
|---|---|---|---|---|---|---|---|---|---|
| 522004Fc | Amp | Amo | Cep | Oxy | | | | | |
| 622004Fc | Amp | Amo | Cep | Oxy | | | | | |
| 732004Fc | Amp | Amo | Cep | Cip | | | | | |
| 832004Fc | Amp | Amo | Cep | Kan | Oxy | Cip | Ery | | |
| 932004Fc | Amp | Amo | Cep | Kan | Ery | | | | |
| 1042004Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Ery | |
| 21211305Fc | Amp | Amo | Cep | Neo | Oxy | Chl | | | |
| 22311305Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 3020306Fc | Amp | Kan | Neo | Oxy | | | | | |
| 3130306Fc | Amp | Kan | Neo | Oxy | | | | | |
| 3230306Fc | Amo | Cep | Kan | Oxy | Cip | | | | |
| 4160306Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 4690306Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 5012707Fc | Amp | Kan | Neo | Oxy | | | | | |
| 5222707Fc | Amp | Kan | Neo | Oxy | | | | | |
| 5322707Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Ery | |
| 5422707Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | | |
| 5532707Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 5842707Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 6792707Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Cip | Ery |
| 7633108Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 7733108Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 7833108Fc | Amp | Kan | Neo | Oxy | | | | | |
| 7943108Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |
| 8353108Fc | Amp | Amo | Kan | Neo | Oxy | Chl | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8563108Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 8793108Fc | Amp | Amo | Cep | Kan | Neo | Chl | Oxy | Ery | |
| 9232009Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 9992009Fc | Amp | Amo | Kan | Neo | Oxy | Chl | Ery | | |
| 10092009Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Cip | Ery |
| 10531310Fc | Amp | Amo | Cep | Oxy | Ery | | | | |
| 10971310Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Ery | |
| 11171310Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 11512511Fc | Amp | Amo | Cep | Oxy | | | | | |
| 11712511Fc | Amp | Amo | Cep | Oxy | Ery | | | | |
| 12032511Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Chl | Ery | |
| 12232511Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 12642511Fc | Amp | Amo | Cep | Oxy | Ery | | | | |
| 12852511Fc | Amp | Amo | Cep | Oxy | Ery | | | | |
| 13631101Fc | Amp | Amo | Cep | Cip | | | | | |
| 13731101Fc | Amp | Amo | Cep | Kan | Oxy | Cip | Ery | | |
| 14061101Fc | Amp | Amo | Cep | Kan | Ery | | | | |
| 14831502Fc | Amp | Amo | Cep | Cip | | | | | |
| 14931502Fc | Amp | Amo | Cep | Kan | Oxy | Cip | Ery | | |
| 16210103Fc | Amp | Amo | Cep | Kan | Neo | Oxy | Ery | | |
| 16420103Fc | Amp | Amo | Cep | Oxy | | | | | |
| 16520103Fc | Amp | Amo | Cep | Neo | Oxy | | | | |
| 16630103Fc | Amp | Amo | Cep | Cip | | | | | |
| 16730103Fc | Amp | Amo | Cep | Kan | Oxy | Cip | Ery | | |
| 16830103Fc | Amp | Amo | Cep | Kan | Ery | | | | |
| 17250103Fc | Amp | Amo | Oxy | Chl | Ery | | | | |
| 17350103Fc | Amp | Amo | Cep | Neo | Oxy | Chl | Ery | | |

# APPENDIX D

**Table D.1:** Microbial categorization of the three segments of the Mooi River in terms of health category and water use of the sampled sites (DWAF, 1999).

| **RIVER HEALTH CATEGORIZATION** | | | |
|---|---|---|---|
| **Site no's & river segment** | **Category** | **Description** | **Water Use** |
| (1,2,&3) Upstream segment | Good Water Quality | Least numbers of faecal coliforms and enterococci present. | Agricultural and recreational use |
| (7& 8) Downstream segment | Fair Water Quality | Low numbers of faecal coliforms and Enteroccoci indicators present. | Agricultural |
| (4,5 & 6) Potchefstroom middle segment | Poor Water Quality | Highest faecal contaminated area. | Agricultural further downstream |

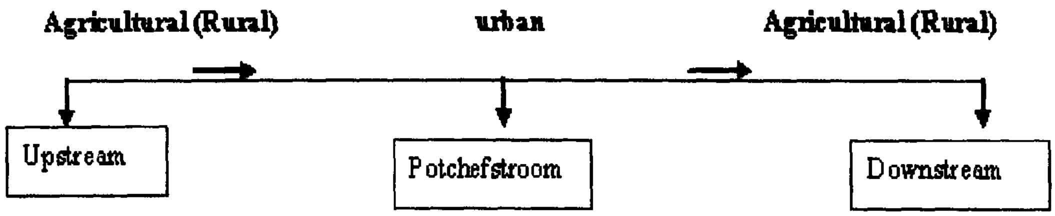



**Figure D.1:** Schematic representations of the segments of the Mooi River along urban-rural gradients of faecal pollution from upstream, Potchefstroom and downstream sites.