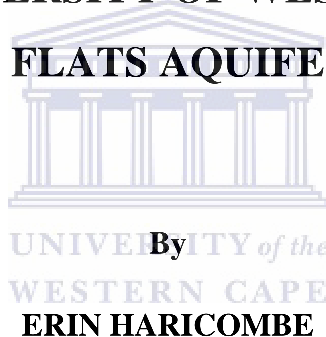
TRANSPORT AND FATE OF CHEMICAL AND MICROBIAL TRACERS AT UNIVERSITY OF WESTERN CAPE (UWC) CAMPUS SITE, CAPE FLATS AQUIFER OF SOUTH AFRICA



By

ERIN HARICOMBE

A thesis submitted in fulfillment of the requirements for the degree of Magister Scientiae (MSc) in the Faculty of Science, Earth Sciences Department, University of the Western Cape, South Africa

Supervisors: Dr. T Kanyerere and Dr Jacobus Martinus Nel

JUNE 2016

Declaration

I declare that: *"Transport and fate of chemical and microbial tracers at University of Western Cape (UWC) campus site, Cape Flats aquifer of South Africa"* is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

Name: Erin Haricombe (Student No.2502281) **Date:** 30th June 2016

Signed: ________________________

UNIVERSITY of the
WESTERN CAPE

Acknowledgements

I would like to thank God for giving me the grace and perseverance to complete what I have started. I would like to dedicate my thesis to my late father, hero and motivator Ernest Salvester Paramoer. This project would not have been a success without the encouragement and support from several people. I believe that through all the stress and despair about finishing the thesis that overcoming the anxiety was all in the name of science.

I gratefully acknowledge the direction and extraordinary patience provided by Dr. Jacobus Nel and Dr Thokozani Kanyerere. Very special thanks to both of them for valued advice, unwavering support and words of encouragement. I also offer my sincerest gratitude to Prof. Jonathon Levy, my mentor for sharing his wisdom providing invaluable guidance, and for his tremendous efforts towards this project in person and via correspondence. My gratitude goes out to Shamiel Davids, Sitembiso Siwawa and Mlamlemi Kolosa for helping me in the lab and field to this project. Thanks to Gaarthier Mahed for his valued help on many occasions.

I would like to acknowledge the Water Research Commission for assistance with bacterial subtyping analysis and supplying the microbes. In particular, I thank Martella Du Preez for making this collaboration possible. I would also like to express my heartfelt thanks to Wouter Le Roux for analysing and growing the microbes. Financial support for this project was provided by the NRF and Water Research Commission. Many thanks to the great friends and bonds I have made over the last few years. In particular, warm thanks to Candice Lasher, Ilse Kotzee, Adolf October, Micah Dominick, Shuaib Dustay, Innocent Muchingami, Sitembiso Siwawa, Mlamleni Kolosa, Robin Petersen and Tercia Strydom.

I would like to express my utmost appreciation to my mother, Belinda Paramoer, for her loving encouragement and unconditional support. To my siblings, Kaelyn and Tarkquin, thank you for driving up and down for me when I had to spend endless nights in the lab. To my aunty, Christina Nomdo, thank you for providing motivation and encouragement to finish what I started. Finally, with much love, thank you to my husband Clement Haricombe for being there through the ups and downs. In empathy, you even offered to help me with my lab experiments to ease my burdens. I sincerely thank my husband for his understanding and constant motivation for keeping me smiling and laughing even in times of difficulties.

UNIVERSITY of the WESTERN CAPE

Abstract

Transport and fate of chemical and microbial tracers at UWC campus site, Cape Flats aquifer of South Africa

Extreme weather events in combination with geographical changes in groundwater utilization, groundwater availability, aquifer recharge, and ultimately changes in the quality of water resources, are expected in the future. As a consequence of changing weather patterns and urbanization the demand for groundwater is likely to increase in certain areas. We know that most waterborne pathogenic health epidemics are associated with contamination of farm water and wastewater. There is however limited understanding of the nature and extent of chemical, physical and biological processes that control the fate and transport of the micro-organisms in primary and secondary aquifers.

In this thesis, transport results are reported, where *E. coli* and PDR1 were selected as the biological tracers transported through a primary aquifer at the University of the Western Cape. In conjunction with the microbes salt and Rhodamine (chemical tracers) were injected to compare their fate and transport mechanism in the primary aquifer medium. A series of controlled Darcy experiments under laboratory and field conditions were conducted. Each provided a different data and information. The results from laboratory studies were used to improve design of the field studies. In both cases, the data collected provided information on fate and transport of microbes in groundwater. The field design phase of the experiment was an up-scaling of the laboratory phase of this project. The amount of chemical tracers injected into the aquifer was increased in proportion to the size of the research site. Tracer tests using chemical and microbial tracers were conducted simultaneously.

Results of laboratory tests demonstrate a 5 times slower transport of microbes, compared to tests with salts during the laboratory phase. The salts at field scale show a breakthrough occurring after 2 days whereas the microbes –did not break through during the 28 days of the observation period. A new borehole was drilled closer to the pumping borehole to eliminate distance or travel time, but this had no effect on field results for the microbes.

UNIVERSITY of the
WESTERN CAPE

CONTENTS

CONTENTS I

LIST OF FIGURES IV

LIST OF TABLES IV

ACRONYMS V

CHAPTER 1: INTRODUCTION 1

1.1 AIMS AND OBJECTIVES 3
1.2 THESIS STRUCTURE 3

CHAPTER 2: LITERATURE REVIEW 5

2.1 INTRODUCTION 5
2.2 MICROBIAL TRANSPORT PROCESSES 5
2.2.1 Physical processes 7
2.2.2 Electrostatic and chemical processes 10
2.2.3 Biological processes 11
2.3 FACTORS AFFECTING FATE 13
2.3.1 Adsorption 14
2.3.2 Temperature 18
2.3.3 Saturation 20
2.4 PATHOGENIC MICROBES OF CONCERN 20
2.4.1 Introduction 20
2.4.2 Escherichia coli *(E. coli)* 24
2.4.3 Salmonella 25
2.4.4 Campylobacter 27
2.4.5 Yersinia 28
2.5 LIMITATIONS OF PREVIOUS WORK DONE ON COLUMN EXPERIMENTS 29
2.6 EXPERIMENTAL SETUP IN PREVIOUS STUDIES 31
2.6.1 Typical laboratory setup 31
2.6.2 Typical Field experimental setup 36
2.6.3 Mathematical models and assumptions used to describe chemical and microbial transport 37

CHAPTER 3: FIELD RESEARCH STUDY SITE 39

3.1 INTRODUCTION 39
3.1.1 Selection of representative microbial tracers 39
3.2 SITE DESCRIPTION 40
3.3 GEOLOGY 41
3.4 DRILLING AND CONSTRUCTION 44
3.5 WATER LEVELS AND FLOW GRADIENTS 47

CHAPTER 4: METHODS AND MATERIALS 49

4.1 PROJECT TEAM AND AFFILIATIONS 49
4.2 INTRODUCTION 49
4.3 LABORATORY TESTS 49
4.3.1 Sand and water collection 49

UNIVERSITY of the
WESTERN CAPE

4.3.2 Disinfection and autoclave ... 49
4.3.3 Column setup and packing ... 50
4.3.4 Tracer test ... 53
4.3.5 Biological tracer preparation ... 53
4.3.6 Sample injection procedure ... 56
4.3.7 Interpretation of laboratory results ... 58
4.4 FIELD STUDIES ... 58
4.4.1 Hydraulic test / conditions ... 58
4.4.2 Tracer tests ... 58
4.4.3 Biological and chemical tracer preparation ... 59
4.4.4 Tracer analyses ... 60

CHAPTER 5: DATA AND DISCUSSION ... 63

5.1 INTRODUCTION ... 63
5.2 DATA AND DISCUSSION ... 63
5.2.1 Column experiments using bacteriophage PDR-1 and *E. coli* ... 63
5.2.2 *E. coli* experiments with sterilized columns ... 65
5.2.2.3 Experiments with uunsterilized columns and water ... 72
5.2.3 Interpretation of laboratory experiments ... 74
5.2.4 Field experiment ... 75
5.2.4.1 PDR-1 experiments (PRFF-01) ... 75
5.2.4.2 *E. coli* experiments (ECF-01) ... 77
5.2.5 Interpretation of field experiments ... 78

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS ... 80

6.1 INSIGHTS FROM THE STUDY ... 80
6.2 FINDINGS AND CCONCLUSIONS ... 81
6.2.1 Factors affecting transport and fate of micro-organisms in groundwater systems ... 81
6.2.2 Microbial contaminants of concern to identify suitable microbial tracers ... 81
6.2.3 Testing the transport and fate of selected chemicals and micro-organisms ... 82
6.2.4 Research results to protect drinking water under real world conditions ... 82
6.3 RECOMMENDATIONS ... 82

CHAPTER 7: REFERENCES ... 84

UNIVERSITY of the WESTERN CAPE

LIST OF FIGURES

Figure 2-1: Image depicting the transport of microbes through the soil and subsurface (McClaulou et al. 1995). 8

Figure 2-2: Map showing groundwater pH distribution across South Africa (DWA National Water quality monitoring points) 16

Figure 2-3: Map showing groundwater temperature distribution across South Africa (Data from DWA national Water Quality network) 20

Figure 2-4: Experimental setup for laboratory column experiments (after Levy et al.'s 2007) 32

Figure 2-5: Images of Dual-Porosity under uniform water flow that only applies to Solute transport. 38

Figure 3-1: Cross section of campus site showing relevant boreholes used for experiment as well as their hydraulic characteristics 43

Figure 3-2: Site photographs of the drilling process 46

Figure 3-3: Logs of newly drilled boreholes 48

Figure 4-1: Darcy upflow experiment setup for laboratory simulation of a sandy aquifer 51

Figure 4-2: Illustration of the ASTM 4874 standard leaching packing method. 52

Figure 5-1: Laboratory biological tracer PRD-1 phage (pfu/ml) results – sterilized (Expt. PRL-01) 64

Figure 5-2: Laboratory salt tracer (µS/cm) results (Expt. PRL-01) 65

Figure 5-3: Laboratory salt tracer (µS/cm) results (Expt. ECL-01) 67

Figure 5-4: Laboratory biological tracer *E. coli* (cfu/ml) results - with ampicillin (Expt. ECL-02) 69

Figure 5-5: Laboratory biological tracer *E. coli* (MPN/ml) results - with ampicillin (Expt. ECL-02) 70

Figure 5-6: Laboratory salt tracer (µS/cm) results (Expt. ECL-02) 71

Figure 5-7: Laboratory biological tracer *E. coli* (MPN/ml) results – unsterilized (Expt. ECL-03) 73

Figure 5-8: Laboratory salt tracer (µS/cm) results (Expt. ECL-03) 73

Figure 5-9: Field salt tracer EC (µS/cm) and Rhodamine (µg/L) results – (Expt. PRF-01) 76

Figure 5-10: Field biological *E. coli* (cfu/ml) results – (Expt. ECF-01). 77

Figure 5-11: Field salt tracer Rhodamine (µg/L) results – (Expt. ECF-01) 78

UNIVERSITY of the
WESTERN CAPE

LIST OF TABLES

Table 3-1: Some international health incidents caused by bacteria and viruses 22

Table 3-2: Some local health incidents caused by bacteria and viruses 23

Table 3-3: Borehole characteristics of UWC campus site 41

Table 4-1: Field biological tracer experiments performed 59

Table 4-2: Chemical and biological tracer preparation, injection and sampling 61

Table 5-1: Laboratory biological tracer experiments performed.................................... 63
Table 5-2: Column parameter values for experiment PRL-01 64
Table 5-3: Column parameter values for experiment ECL-01 66
Table 5-4: Column parameter values for experiment ECL-02 (*E. coli* measured using MFC agar) 68
Table 5-5: Column parameter values for experiment ECL-03 (*E. coli* measured using Colilert) 72
Table 5-6: Laboratory experiment flow parameters and hydraulic conductivity.............. 74
Table 5-7: Laboratory porosity estimates for column tracer tests 75

List of Definitions

Adsorption: The process by which molecules of a substance, such as a gas or a liquid, collect on the surface of another substance, such as a solid. The molecules are attracted to the surface but do not enter the solid's minute spaces as in absorption.

Advection: Is the mass movement of water through the soil or aquifer, it is the primary mechanism transporting micro-organisms from one place to another.

Bacteria: Are single celled micro-organisms which can exist either as independent (free-living) organisms or as parasites (dependent upon another organism for life).

Bacteriophage: Are viruses that have a specific affinity for and infect bacteria.

Borehole: Includes a well, excavation, or any other artificially constructed or improved underground cavity which can be used for the purpose of intercepting, collecting or storing water in or removing water from an aquifer observing and collecting data and information on water in an aquifer or recharging an aquifer.

c. (circa) In dates, to indicate approximate date of origin.

Coliform bacteria: Coliform bacteria are described and grouped, based on their common origin or characteristics, as either Total or Fecal Coliform.

Cyst: A general term used for a specialized microbial cell enclosed in a wall. They may be dormant, resistant to structures formed in response to adverse conditions or reproductive cysts that are a normal stage of the life cycle of protozoa and a few bacteria.

Dispersion: "Spreading" as it moves around soil grains and fractured rock.

Encystment: Means to enter a state of essentially suspended simulation in which the organisms are protected by an outer coating (forming a cyst) and remains immobile and inactive until favorable conditions for growth occur again.

Fimbriae: A proteinaceous attachment in many gram-negative bacteria that is thinner and shorter than a flagellum.

Gram-negative: Gram-negative bacteria in solutions consisting of various chemicals varied between -20 and 80 mV, depending on the chemical used. Gram-negative bacteria have relatively thin cell walls and are generally resistant to the effects of antibiotics or the actions of the body's immune cells.

Lysis: Refers to the breaking down of a cell, often by viral, enzymic, or osmotic mechanisms that comprise its integrity.

Pathogen: An organism that causes disease. Derived from the Greek *Patho* (meaning disease) and *gen* (meaning giving rise to).

Thermophilic: Requiring high temperatures for normal development, such as certain bacteria.

Virus: A micro-organism smaller than bacteria, which cannot grow or reproduce apart from a living cell. Invades living cells and uses their chemical machinery to keep itself alive and to replicate itself.

UNIVERSITY of the
WESTERN CAPE

Chapter 1: Introduction

Knowledge of the relationship between chemical and microbial fate and transport is required to efficiently manage and remediate many environmental contaminants. For example, an accurate description of microbial transport and survival is required to assess contamination potential and to protect drinking water supplies from pathogenic micro-organisms (Bitton et al. 1992), which will give us an overview of the bacterial distribution and the health effects they have on humans. Different methods on how to study the transport and fate of chemical and microbial tracers will be reviewed in an attempt to apply these results to real world field conditions.

The detailed behavior of micro-organisms in groundwater is not well understood due to the complex biophysical and biochemical processes that affect the fate and transport of these micro-organisms. Besides movement of groundwater, there are many processes such as natural die-off, formation of biofilms, adsorption and desorption that influence the spatial and temporal spread of micro-organisms in groundwater. Although having been studied to some extent in international literature, there is no good local understanding of the nature and extent of the related chemical, physical, and biological processes that control the fate and transport of micro-organisms in South African aquifers.

UNIVERSITY of the
WESTERN CAPE

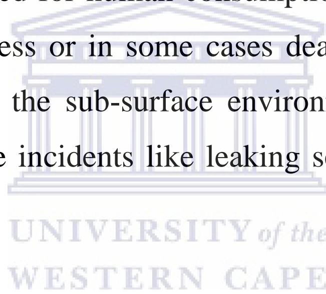
The type of aquifer plays a significant role in determining the transport processes and possible pathways contaminants can follow from a contamination source to a potential receptor. From a water-bearing point of view, geological formations can be divided into different groups based on primary openings (inter-granular) and secondary openings (fractures) (Vegter, 2001). South African aquifers can subsequently be classified into four main types, namely inter-granular aquifers, fractured aquifers, karstic aquifers and inter-granular and fractured rock aquifers.

Primary aquifers form a significant water resource along the Western Cape coastline and are defined as aquifers in which water is stored and where the water moves through the original primary interstices of the geological formation. These aquifers consist primarily of shallow, porous sand and gravel deposits along floodplains and coastal terraces. They range in thickness from about 3 to over 80 meters (Dyson, 2009). Primary aquifers are usually shallow

with soft sediments, it is inexpensive to drill; it also usually has a good yield and good recharge rate (Dyson, 2009).

Primary aquifers are, however, susceptible to contamination and because it is an easy source of water supply it has many competing users. Good examples are the Atlantis, Langebaan and Wadrif Aquifers which are important and significant primary aquifers with well-fields supplying water to agricultural and municipal users (Dyson, 2009).

Groundwater pollution resulting from microbial contamination can pose a significant threat to human health. Micro-organisms collectively comprise a major pollutant of concern — second only to nitrate (Xu and Usher, 2006). Microbial contamination tends to have an immediate impact on human health especially in the elderly, infants and people with susceptible immune systems. There have been reports of cases of water borne diseases resulting from microbial contamination of groundwater used for human consumption in South Africa and other parts of Africa resulting in severe illness or in some cases death (Xu and Usher, 2006). Micro-organisms can exist naturally in the sub-surface environment or can occur as a result of contamination from human prone incidents like leaking sewer lines or animal faecal waste (Xu and Usher, 2006).


UNIVERSITY of the
WESTERN CAPE

Unlike most chemicals, one low-dose exposure to some microbes can cause serious ill effects (Adamson et al. 1993). There is a wide range of illnesses that can result from water contaminated with pathogenic microbes. There is therefore a need to understand how these micro-organisms behave in groundwater systems. Bradbury et al. (2006) conveys the occurrence of human pathogenic viruses that have the greatest potential to move deep into the sub-surface environment, penetrate an aquitard and reach a confined aquifer (Adamson et al. 1993).

Viruses are thought to pose a greater health risk than bacteria. Their small size enables them to move more effectively than larger micro-organisms. They also have a high infectivity rate and prolonged persistence in the environment (Jiwan and Gates, 1998; Schijven, 2001). Moreover, the presence of the traditional indicators such as faecal coliforms and *E. coli* does not correlate with the presence of microbes or parasites. The behavior of the micro-organisms in particular needs to be investigated in groundwater.

Aims and objectives

This project hopes to contribute towards the knowledge of transport and fate of micro-organisms in South African primary aquifers. The overall aim of this thesis is to investigate the transport and fate of chemical and microbial tracers in the Cape flats aquifer media. The specific aims are to:

- Conduct an international and local literature study on factors affecting the transport and fate of micro-organisms in groundwater systems;
- Review microbial contaminants of concern to identify suitable microbial tracers;
- Conduct experiments to investigate the transport and fate of selected chemicals and micro-organisms in:
    - Simulated aquifer conditions in the laboratory; and
    - Field studies in Cape Flats aquifer.
- Evaluate the results of the experiments for application to protect drinking water in real world conditions.

UNIVERSITY of the
WESTERN CAPE

Thesis structure

This thesis is organized into seven chapters. Experimental findings are summarized in chapter 5, which will be refined and submitted as papers.

A literature review is provided in chapter 2. The review addresses four key points:

(1) Microbial transport processes,

(2) Factors affecting fate

(3) , Limitations of previous work done on column experiments, and

(4) Experimental setup in previous studies

The site description study is outlined in chapter 3. This will evaluate the geology of the experimental site, drilling construction and water levels, and flow gradients as well as microbes of concern to groundwater.

Laboratory and field tests studies are outlined in chapter 4. Tracer tests were performed on small scale, in the laboratory, and on larger scale, in the field. Microbial and chemical tracers

were used at both sites to compare the difference between natural and controlled environments. The laboratory environment was mimicked as far as possible to provide an understanding of conditions in natural uncontrolled environments. Upflow methods were used in the laboratory to eliminate any air pockets from entering the system. Key results and interpretation are summarized in chapter 5.

The thesis concludes with Chapter 6, which outlines some general remarks and recommendations for future work.

UNIVERSITY of the
WESTERN CAPE

Chapter 2: Literature Review

Introduction

Waterborne diseases due to faecal pollution of human and animal origin are responsible for approximately 2.2 million deaths annually in children under the age of 5 years old in developing countries (WHO, 2002a). Most of these deaths are due to inadequate potable water supplies, poor hygiene practices and insufficient sanitation infrastructure (Sobesy, 2002). In South Africa, intestinal infections and viral hepatitis deaths have accounted for about 3% of deaths in children six years old (Bourne and Coetzee, 1990). It is the pathogenic micro-organisms that are a major source of concern in groundwater with bacteria responsible for the transmission of water borne diseases such as typhoid, dysentery and cholera. Such bacteria become a major threat to humans when present in groundwater used for drinking purposes (Bourne and Coetzee, 1990).

Waterborne transmission of diseases and their impact on public health are greatly under-estimated (Grabow, 1996). The most common manifestation of waterborne illness is gastro-intestinal upset like vomiting, and this is usually of short duration (Health Canada, 2006). In susceptible individuals, the effects may be more severe, chronic, or even fatal (Grabow, 1996). Grabow (1996) estimated that approximately 50 000 adults die daily due to water-related diseases throughout the world.

UNIVERSITY of the
WESTERN CAPE

Microbial transport processes

Movement of microbes through soils and aquifers is primarily facilitated by water flow. Without water there is not likely to be movement. However, microbial transport involves a host of complex and interacting processes that can be highly site-specific. It is thus not surprising that the literature contains many apparent inconsistencies regarding the effects of bacterial variables such as size, shape, hydrophobicity, and electrostatic charge on their fate and transport (Lawrence and Hendry, 1996). According to Ginn et al. (2002) it is not possible to state definitive general correlations between bacterial properties and transport.

Because microbes are living organisms, their transport in groundwater is more complex than is the case for abiotic colloids. This complexity is partly responsible for the lack of

understanding of transport processes of micro-organisms in groundwater (Flynn et al. 2004). Not only are they subject to the same physicochemical phenomena as are colloids, but there are also a number of strictly biological processes that affect their transport (for example, temporal changes in surface properties due to changes in metabolic state or predation by other subsurface organisms) (Ginn et al. 2002).

Controversy exists on exactly how long human viruses can survive in soil and groundwater. One study found that after approximately 29 days, poliovirus type 1 underwent a 1 log reduction in concentration (Yates et al. 1985), whereas, another study found the same reduction after 300 days (Rao and Melnick, 1986).

The inactivation rates (natural log per day) of faecal indicator bacteria in groundwater at groundwater temperatures have been found to be as low as 0.53 day^{-1} (faecal streptococci); 0.82 day^{-1} (faecal coliform); and 0.04 - 0.73 day^{-1} (*E. coli*) (Schijven, 2001). For pathogenic bacteria, inactivation rates have been measured as low as 1.42 day^{-1} (*Shigella* spp.) and 0.33 day^{-1} (*E. coli* 0157:H7) (Schijven, 2001). Of further concern is the fact that some pathogenic bacteria enter viable but non-culturable dormant states (Schijven, 2001). This could further prolong bacterial persistence in groundwater. Spores of pathogens such as *Clostridium* spp. may survive in the environment for several years while remaining viable (Schijven, 2001).

UNIVERSITY of the
WESTERN CAPE

While it would be convenient to be able to deal with microbial transport and survival separately, many of the above processes also contribute directly to the inactivation of micro-organisms. Inactivation renders micro-organisms 'inactive' — unable to infect other organisms (Flynn et al. 2004). These important processes provide a framework of bacterial transport and reaction in porous media. Indeed, the impact of biological processes in a flowing groundwater system can only be evaluated within this physicochemical framework (McDowell-Boyer et al. 1986).

The focus of this section is on the physical, electrostatic and chemical, and biological processes involved in the fate and transport of micro-organisms in the saturated subsurface (which are described in the subsequent sections):

1.1.1 Physical processes

Most reactive transport models incorporate a variety of physical processes, such as advection, dispersion, straining, and physical filtration. Unlike the biological processes (subjected to limited research), physical processes affecting microbial transport have been the focus of numerous experimental and numerical modelling studies.

1.1.1.1 Transport

The major factors controlling microbial transport in subsurface media are attachment to and detachment from the medium surfaces, growth and inactivation, and advection and dispersion seen in Figure 0-1 (McCaulou et al. 1995; Gerba et al. 1975; Harvey, 1991). In porous media the size of the micro-organisms may be comparable to length-scale associated with pores (Buchan and Flury, 2004). Under these conditions the microbe-solid surface interactions become important for describing the microbial transport phenomena at the sub-pore scale (Ginn et al. 2002).

Convective transport in porous media is associated with hydrodynamic dispersion, the mixing process arising from the tortuosity of the convective paths compounded by molecular-scale (diffusional) or particle-scale (Brownian) mixing (Ginn et al. 2002). Advection depends on groundwater velocity (Ginn et al. 2002). Dispersion depends on velocity and aquifer heterogeneity and is scale dependent. Inactivation of viruses is typically slow compared to the rates of advection, attachment, and detachment (Lehman et al. 2001). Accurate prediction of microbe transport through porous media near their source therefore often depends solely on the correct evaluation of the rates at which microbes attach to or detach from the porous medium surfaces (Ginn et al. 2002).

Microbes also undergo convective transport as a particulate or a dissolved species moving with the pore-water whose velocity is governed by the hydraulic pressure gradient, porosity, and permeability distribution (Ginn et al. 2002).

UNIVERSITY of the
WESTERN CAPE

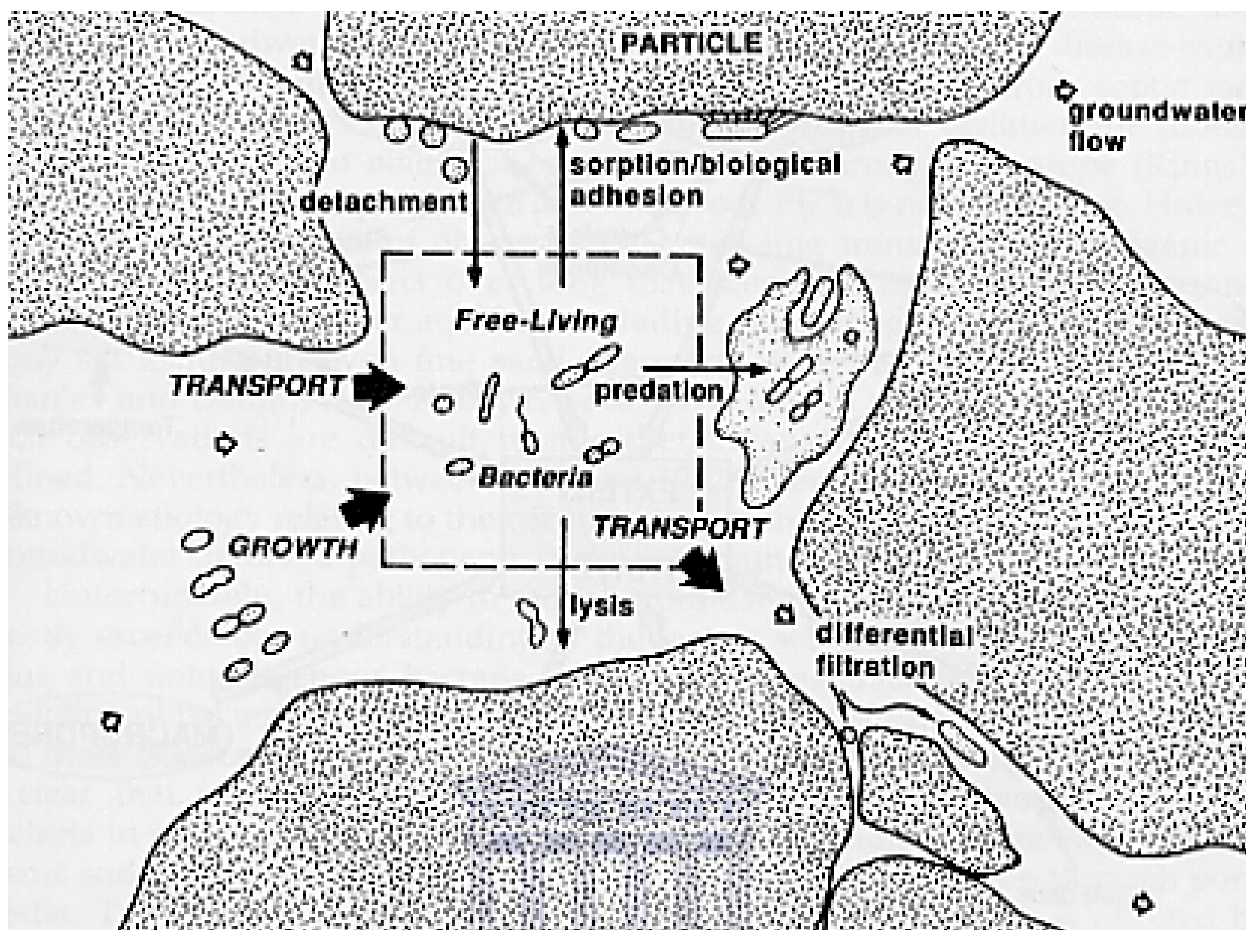


Figure 0-1: Image depicting the transport of microbes through the soil and subsurface (McClaulou et al. 1995).

UNIVERSITY of the
WESTERN CAPE

1.1.1.2 Straining and filtration

Straining and physical filtration represent the removal of microbes from solution by physical forces (Foppen and Schijven, 2005). It includes mechanisms such as straining and sedimentation (Foppen and Schijven, 2005).

Straining is a microbial removal mechanism that occurs when particles in suspension (in this case viruses and bacteria) in a porous matrix cannot pass through a smaller pore opening resulting in a stoppage in transportation (Gerba et al. 1975; Corapcioglu and Haridas, 1984). However, for small micro-organisms like viruses this process can be negligible in coarse-grained aquifer material as the pores would be larger than the viruses (Gerba et al. 1975; Corapcioglu and Haridas, 1984).

Sedimentation occurs as a result of density differences between micro-organisms and the groundwater (Corapcioglu and Haridas, 1984). Therefore, filtration by sedimentation could

be one removal mechanism for viruses and some bacteria from groundwater (Gerba et al. 1975).

Estimates based on purely geometric relations between the effective diameter of bio-colloids and the diameter and packing of grains suggest that mass removal by straining is not significant where the colloid diameter is less than 5% of the porous media grain diameter (Corapcioglu and Haridas, 1984; Harvey, 1991; Herzig et al. 1970; McDowell-Boyer et al. 1986; Sakthivadivel, 1966; Sakthivadivel, 1969). Therefore, aquifers comprised of silt or clay material would have the best microbial removal mechanisms through filtration. Attachment of bacteria in the natural subsurface via filtration is often, but not always (Murphy et al. 1997) treated using colloid filtration theory (CFT) (Rajagopalan and Tien, 1976; Ginn et al. 2002).

1.1.1.3 Size exclusion

Size exclusion is evident from laboratory columns and in field experiment results in bacterial and ion tracer breakthrough times that are different than those of a non-reacting tracer (Ginn et al. 2002). Exclusion is a phenomenon where transported particles move faster than the mean pore-water velocity, and involves an increase in the transport rate due to the size or charge of the material conveyed (Ginn et al. 2002). Identification of exclusion from observations of tracer and particle breakthrough is not straightforward and has led to some confusion in the literature (Ginn et al. 2002).

UNIVERSITY of the
WESTERN CAPE

Often, the time to arrival of the peak breakthrough concentration or centre of mass of the breakthrough curve are used as indicators of exclusion (Ginn et al. 2002). However, attenuation by kinetic attachment with minimal detachment can have the effect of shifting the peak (and centre of mass) to earlier times even in the absence of enhanced velocity, rendering these indicators unreliable (DeBorde et al. 1999; Zhang et al. 2001).

With regard to processes causing exclusion, one may distinguish between anionic and size effects, and further divide size effects into classical chromatographic and ‘‘pore exclusion’’ processes (Ginn et al. 2002). Anion-exclusion involves velocity enhancement by channeling of anionic molecular-scale solute particles in finer-grained porous media away from pore walls due to electrostatically-repulsive forces that act on nanometer scales, and as such are

not generally significant for bacterial transport (although it may amplify size-exclusion when bacteria are like-charged to fine-grained media) (Levy et al.'s 2007).

Microbes and large colloids, by virtue of their size (range from ten to several hundred nanometers), preferentially experience the higher velocities near pore centre-lines, yielding an average velocity that is higher than that of a dissolved tracer (Levy et al.'s 2007). Thus microbes can precede the tracers down gradient, for example, as observed by Rossi et al. (1998). The occurrence of exclusion typically requires the bacterial diameter be less than 1% of the media mean grain diameter, which is common for transport in sandy aquifers (de Marsily, 1986; Dodds, 1982).

The mechanics and modeling of pore exclusion has been recently debated (Ginn, 2000; Rehmann et al. 2000). In coarse-grained media size exclusion is a larger factor than pore exclusion (Harter et al. 2000) because the excluded particle is far smaller than almost all pores. However, exclusion is not consistently manifested in larger-scale field studies (Harvey et al. 1995) even under consistent conditions and so the final impact over long-term transport is unknown (Harter et al. 2000).

UNIVERSITY of the
WESTERN CAPE

Micro-organisms can be transported almost entirely along preferential flow paths while inert tracers also spread along less permeable pathways (Mallén et al. 2005). This can result in the inert tracers breaking through over longer time periods than microbes (Mallén et al. 2005).

1.1.2 Electrostatic and chemical processes

Although the physical processes described above are well understood by most hydrogeologists, the influence of electrostatic and chemical interactions between micro-organisms and solid surfaces are not as familiar (Ginn et al. 2002). These forces may act over characteristic lengths that are only fractions of nanometers to microns, but ultimately they determine how micro-organisms adsorb and desorbs from the solid surface, and thus can dramatically affect microbial transport at the largest scales (Ginn et al. 2002). Adsorption is a process by which both chemicals and micro-organisms 'stick' to the surfaces of solids. It can be reversible or irreversible (Ginn et al. 2002).

Colloid filtration theory assumes that attachment is a two-step process. First the bacterium must be transported to the porous media grain (the ''collector'') (Ginn et al. 2002). Secondly

the physicochemical interactions that occur upon contact of the two surfaces determine if the bacterium attaches to the surface of the collector (Ginn et al. 2002).

Once a bacterium is transported to within a separation distance of the order of fractions of its own radius away from a collector, a complex set of interactions occur that dictates the outcome of the possible attachment (Ginn et al. 2002). The Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability has been widely employed as a model for describing the interaction forces between a microbe and a solid surface (Ginn et al. 2002).

The DLVO force is the sum of the London–van der Waals force and the electrostatic force (i.e., the 'double layer' force which may be repulsive or attractive depending on the charges of the two interacting surfaces) (Ginn et al. 2002). The theory has been extended to cover a host of other possible interactions including so-called Lewis acid–base forces, and steric interaction forces (Azeredo et al. 1999; Camesano and Logan, 2000; Rijnaarts et al. 1995).

These forces are not pH dependent. Ninham, 1999 has pointed out that the DLVO theory applies only for certain well-defined conditions that are not in general met for microbial cells (Ninham, 1999). The assumptions most evidently violated are that the colloidal surface is molecularly smooth, solid, and inert essentially having no ability to react (Ninham, 1999). Detailed critical assessment of assumption validity can be found in (Ninham, 1999).

UNIVERSITY of the
WESTERN CAPE

1.1.3 Biological processes

Processes relating directly to the fact that living organisms are involved also affect survival (Schijven, 2001). Bacterial inactivation of viruses may additionally play a role in the survival time of viruses in groundwater (Rao and Melnick, 1986). This has especially been found to be the case under aerobic conditions, suggesting that specifically aerobic bacteria inactivate viruses (Schijven, 2001). Predatory eukaryotic organisms have been found in groundwater and may contribute to the removal of both viruses and bacteria in groundwater (Kinner et al. 1998).

Bacteria reproduce and flourish under favorable conditions of temperatures ranging between 10˚ and 20˚C, sufficient oxygen, and pH between 4.0 and 4.8 (Schijven, 2001). Conversely, sulphate reducing bacteria are capable of growing and reproducing at temperatures within the range of 47° to 54° C (Chapelle, 2001). This suggests that microbes are capable of adapting to

temperatures of an aquifer and hence they become indigenous to it (Chapelle, 2001). While bacteria survival can be supported in the groundwater, conditions to support their growth rarely exist (Chapelle, 2001). However, when it occurs, it tends to happen during the first 7 days after entry to groundwater due to contamination (Corapcioglu and Haridas, 1984). This depends on nutrient availability (Corapcioglu and Haridas, 1984) and the absence of predators.

Another biological factor that increases survival rate is the ability to form cysts (Chapelle, 2001). A microbial cyst is a resting stage of a microbe, usually a bacterium or a protist (diverse group of eukaryotic microbes) that helps the organism to survive in unfavorable environmental conditions. It can be thought of as a state of suspended animation (is the slowing of life processes by external means without termination) in which the metabolic processes of the cell are slowed down and the cell ceases all activities like feeding and locomotion (Nester, 2007).

Encystment also helps the microbe to disperse easily, from one host to another or to a more favorable environment. When the encysted microbe reaches an environment favorable to its growth and survival, the cyst wall breaks down by a process known as excystation (Nester, 2007). Unfavorable environmental conditions such as lack of nutrients or oxygen, extreme temperatures, lack of moisture and presence of toxic chemicals, which are not conducive for the growth of the microbe, trigger the formation of a cyst (Nester, 2007).

UNIVERSITY of the
WESTERN CAPE

Experimental work carried out by Kudryavtseva (1972) showed that some strains of *E. coli* in groundwater kept in the dark are capable of surviving up to 5½ months. Some virus types are more resistant to environmental changes and this means that they may survive longer than their bacterial counterparts (Corapcioglu and Haridas, 1984). This implies that in this research project, it can be expected that viruses will survive for a longer period in the Cape Flats Aquifer (CFA) because it is relatively shallow.

1.1.3.1 Active adhesion/detachment

Active adhesion and detachment can be treated as biologically driven processes (Ginn et al. 2002). Several studies have reported that micro-organisms exhibit active adhesion and detachment processes that may be a response to local nutrient availability, survival mechanisms, and growth. No generally accepted quantitative treatment of dynamic

biologically mediated adhesion and detachment processes exists. Smets et al. (1999) reported experimental results indicating the adhesion of a pseudomonad (bacteria usually producing greenish fluorescent water-soluble pigment) to glass was significantly more favorable in the exponential growth phase than in the stationary or decay phase.

They hypothesized that differences in the cell surface structure, the cell physicochemistry, or the hydrodynamic behavior of the cells were the most likely reasons for the enhanced adhesion of exponential phase cells (Ginn et al. 2002). Thus the distinction between a micro-organisms response to nutrient availability, survival stress, and growth are neither necessarily separable nor independent processes.

1.1.3.2 Chemotaxis

Some micro-organisms have the capability to move in response to a chemical gradient and are termed chemotactic (Ginn et al. 2002). Both random motility (taxis in the absence of a chemical gradient) and chemotaxis have been cited as potential means of transport for subsurface organisms. Quantitatively, random motility is an effective diffusive flux for micro-organisms that depends on the local spatial gradient in aqueous micro-organism concentration. Chemotaxis is a flux of micro-organisms associated with the gradient in nutrient supply (Buchan and Flury, 2004).

UNIVERSITY of the
WESTERN CAPE

Chemotaxis requires energy and is closely linked to growth processes in porous media (Ginn et al. 2002). In oligotrophic (a poor nutrient and plant life and rich in oxygen) environments nutrient gradients will be quite small and will likely be associated with either preferential flow paths (if the nutrients arise from recharge) or solid-phase chemical heterogeneity. Chemotaxis may be a very important transport mechanism in these (oligotrophic) low-nutrient environments (Buchan and Flury, 2004).

Factors affecting fate

Once micro-organisms have entered the soil a wide variety of factors affect their ability to survive long enough to enter groundwater and hence pose a health risk to humans who use the water (Murray et al. 2004). Microbes are inactivated as they pass through the soil by adsorption to soil particles and dying off (Ganzter et al. 2001; Harris et al. 1997; Pedley and Howard, 1997; Schijven, 2001).

There are several factors that influence the fate of microbes in groundwater. These factors may also be physical, chemical or biological (Gerba, 1984; Bales et al. 1993). The physical factors include light, temperature, adsorption and aggregation processes while the chemical factors include pH, ionic strength and redox processes (Gerba, 1984; Bales et al. 1993). Some of the biological factors that affect the fate of microbes in groundwater include the types of viruses, bacterial growth and inactivation and protozoan activity (Bales et al. 1991; Gerba et al. 1991; Harvey, 1991; Chapelle, 2001).

The factors influencing the inactivation of micro-organisms in soil and water environments include adsorption, temperature and saturation.

1.1.4 Adsorption

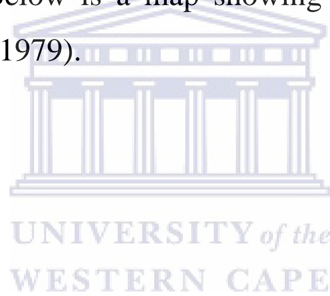
Adsorption is a complex mechanism mainly driven by the relative electrical charges on the surfaces of the microbe and the soil particles (Schijven, 2001; Dowd et al. 1998). The greater the charge difference, the greater the electrostatic attraction between them. Microbial inactivation and adsorption, resulting from interactions with minerals, constitute important aspects of an aquifer's disinfection capacity (Flynn et al. 2004). The degree of inactivation caused by adsorption depends on the strength of attachment of the microbe to the mineral surface and the virus type (Schijven and Hassanizadeh, 2001). The process of microbial adsorption is kinetically limited relative to flow velocity so no immediate equilibrium partitioning between attached and free microbes is achieved (Schijven, 2001; Dowd et al. 1998).

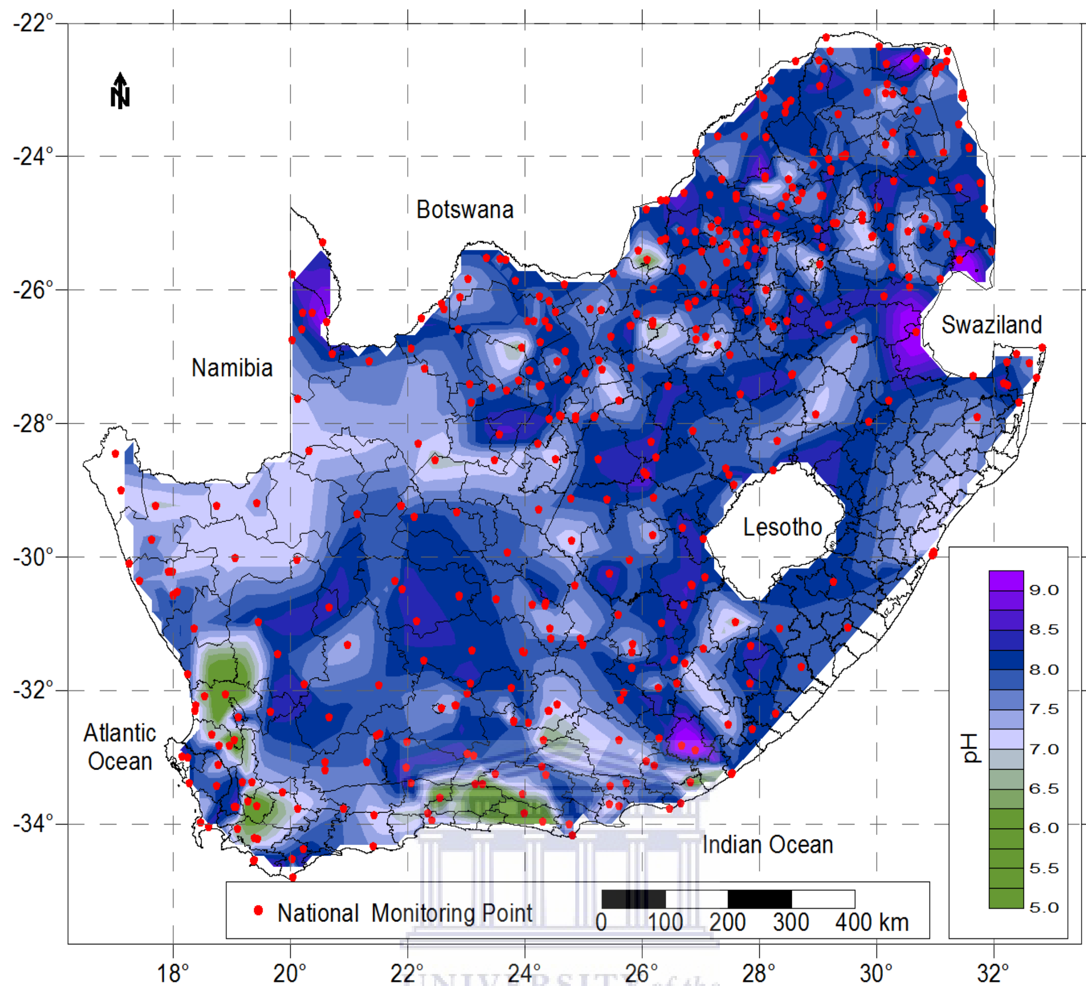
UNIVERSITY of the
WESTERN CAPE

Schijven (2001) suggested that a substantial fraction of microbes attach to colloidal particles smaller than 0.3 μm. These particles include clays, cell fragments, solids in wastewater and effluents and other miscellaneous debris. It is thought that this is due to the stabilizing effect the attachment has on the viral envelope (Schijven, 2001; Dowd et al. 1998). This effect appears to be pronounced with stronger adhesion. At very low adhesion rates viral inactivation is not influenced. In some instances, however, adhesion has been shown to increase inactivation. This is limited to certain bacteriophage types such as PRD1 (Schijven, 2001; Dowd et al. 1998).

The following factors affect adsorption of micro-organisms:

- **pH**: Adsorption decreases with increasing pH (Schijven, 2001). Lower pH generally favors attachment by decreasing the negative charge density on bacteria particles. Soils having a saturated pH of less than 5 are generally good adsorbers (Goyal and Gerba, 1979). Virus surface charge is often characterized by an isoelectric point (pH_{iep}), the pH at which the net surface charge is zero in an aqueous solution of specified ionic strength and composition (Abudalo et al. 2005). Mineral phases characterized by positive surface charge at pH values typical of most drinking water aquifers also promote extensive microbe attachment through electrostatic attraction because the surfaces of most microbes are negatively charged in natural waters (Murray and Parks, 1980; Park et al. 1980; Loveland et al. 1996). In South Africa, the pH of natural groundwater varies between 7 and 8 (Figure 0-2) for most of the country. Studies have proved that microbes are less likely to attach at higher pHs (Goyal and Gerba, 1979). Western Cape has low ranging pH, therefore, a higher adsorption is expected. Below is a map showing the pH distribution across South Africa (Goyal and Gerba, 1979).


UNIVERSITY of the
WESTERN CAPE



UNIVERSITY of the WESTERN CAPE

Figure 0-2: Map showing groundwater pH distribution across South Africa (DWA National Water quality monitoring points)

- **Microbe type**: The type and even strain of microbes affects the adsorption rate. Adsorption onto solid surfaces is a factor that can increase or decrease the survival rate depending on the type of organism and the specific site (Schijven, 2001). These differences are believed to be due to the differences in surface charge and hydrophobicity (Schijven, 2001). At pH's between 6 and 8, microbes with a net negative surface charge will generally attach less successfully to soil particles due to greater electrostatic repulsion (Schijven, 2001). Examples include Coliform bacteria that can not only survive, but multiply in a water environment. *E. coli* reportedly cannot multiply (Grabow, 1996). Some microbes have special mechanisms that facilitate survival and transport viability for long periods in a dormant state. Parasitic protozoa like *Cryptosporidium* can survive in the environment for long periods in a cyst form (Grabow, 1996). They can only multiply when ingested by a suitable host.

Attachment appears to play an important role in microbe inactivation, but the effect of attachment on surface inactivation is not entirely clear (Ryan et al. 2002).

- **Soil type and composition**: Soil grains have patch-wise distributed surface charges that result from the inherent differences in properties of crystal faces of mineral grains and from minerals having bulk or surface-bound chemical impurities (Schijven, 2001). Oxides of iron, aluminum and manganese are the most common sources of charge heterogeneity in the environment (Schijven, 2001). These oxides carry a positive charge at a neutral pH and are present in minor amounts as surface coatings on mineral grains. These are favourable adsorption sites for negatively charged viruses (Schijven, 2001). Heterogeneous surfaces have attachment rates several orders of magnitude greater than homogenous surfaces. This causes granular soils to be weaker adsorbents than clays and minerals (Schijven, 2001). The water content of soil also regulates grazing and predation by the soil micro fauna and associated nutrient transformations (Elliott et al. 1980, Görres et al. 1999; Savin et al. 2001).

- **Hydrophobic interactions**: Hydrophobic interactions may be seen as a consequence of the thermodynamically unfavorable interaction of hydrophobic substances with water molecules and is not due to interactions among hydrophobic particles themselves (Wait and Sobsey, 1995). At high pH, electrostatic repulsion dominates the charge-specific interactions on the virus and soil surfaces (Shields and Farrah, 1983; Gerba, 1984; Bales et al. 1991). The interaction of hydrophobic groups on the surface of microbes and solids become more apparent under high pH conditions and becomes the major mechanism for virus attachment (Schijven, 2001). Bales et al. (1991) concluded that hydrophobic effects are important for adsorption of even relatively hydrophilic microbes.

- **Ionic strength (salinity)**: Deionization of water increases viral detachment, typically happening during a rainfall event (Schijven, 2001). Ionic strength affects the distance at which the electrostatic double layers of the virus and geologic media begin to overlap (Abudalo et al. 2005). At low ionic strength, double layers extend further into the bulk solution because fewer ions are available to balance the charge of the surfaces. At high ionic strengths, double layers are compressed and the electrostatic

UNIVERSITY of the
WESTERN CAPE

repulsion between surfaces of the same charge is reduced, increasing the degree of adsorption (Abudalo et al. 2005).

- **Multivalent cations**: Multivalent cations can link virus and adsorbents of like charge by forming salt bridges between them or by charge reversal (Schijven, 2001). The most common multivalent cation of concern is calcium. Increases in ionic strength and bivalent cation concentrations generally increase the extent of virus attachment through double-layer compression and shielding of repulsive interactions (Abudalo et al. 2005).

- **Organic matter**: Organic substances compete for adhesion sites on the soil particles and therefore reduce microbial attachment (Schijven, 2001). Dissolved organic matter, like surfactants, may also disrupt hydrophobic bonds between soil and microbes, resulting in an increased detachment rate. The presence of sewage organic matter both dissolved and adsorbed to the geologic medium has been observed to enhance transport (Abudalo et al. 2005). Organic matter, both in the aquatic and mineral-bound phases, plays an important role in microbe transport by altering the interaction between microbes and aquifer grains (Ryan, 1997). Some researchers have concluded that organic matter enhances microbial transport by blocking microbe attachment to mineral surfaces (Burge, 1978 and Powelson et al. 1991).

UNIVERSITY of the
WESTERN CAPE

1.1.5 Temperature

The most important factor influencing micro-organism survival appears to be temperature (Yates et al. 1985; Gantzer et al. 2001; Schijven, 2001). Survival decreases with increased temperature. Below 10°C, many microbes can survive for months or even years (Metge, 2002). In contrast, at temperatures greater than 20°C, inactivation of most microbes occurs rapidly (Metge, 2002). Temperature appears to have a minor effect on micro-organisms attached to soil particles. At temperatures from 12 to 20°C, faecal coliforms have an inactivation rate coefficient of about 0.83. For *E. coli* at temperatures between 3 and 15°C the coefficient is 0.74 (decay rate) (Keswick et al. 1982; Yates et al. 1985).

Many bacteria of concern flourish at temperatures ranging between 10° and 20°C (Metge, 2002), as found in most of South Africa, while sulphate reducing bacteria are capable of

growing and reproducing at temperatures from 47° to 54°C in hot water springs (Chapelle, 2001).

Notwithstanding the importance of temperature for microbial survival generally, it has been suggested that temperature is of limited concern in soil and groundwater because extremes of temperature are seldom achieved (Yates et al. 1985). Below is a map

Figure 0-3 showing the groundwater temperature distribution across South Africa which gives us an indication of locations in which bacteria are likely to flourish according to criteria determined by Metge, (2002) and Chapelle, (2001). This range of temperatures can be anticipated, considering the difference in lithostratigraphic and climatic conditions we experience in South Africa (Vegter, 2001).

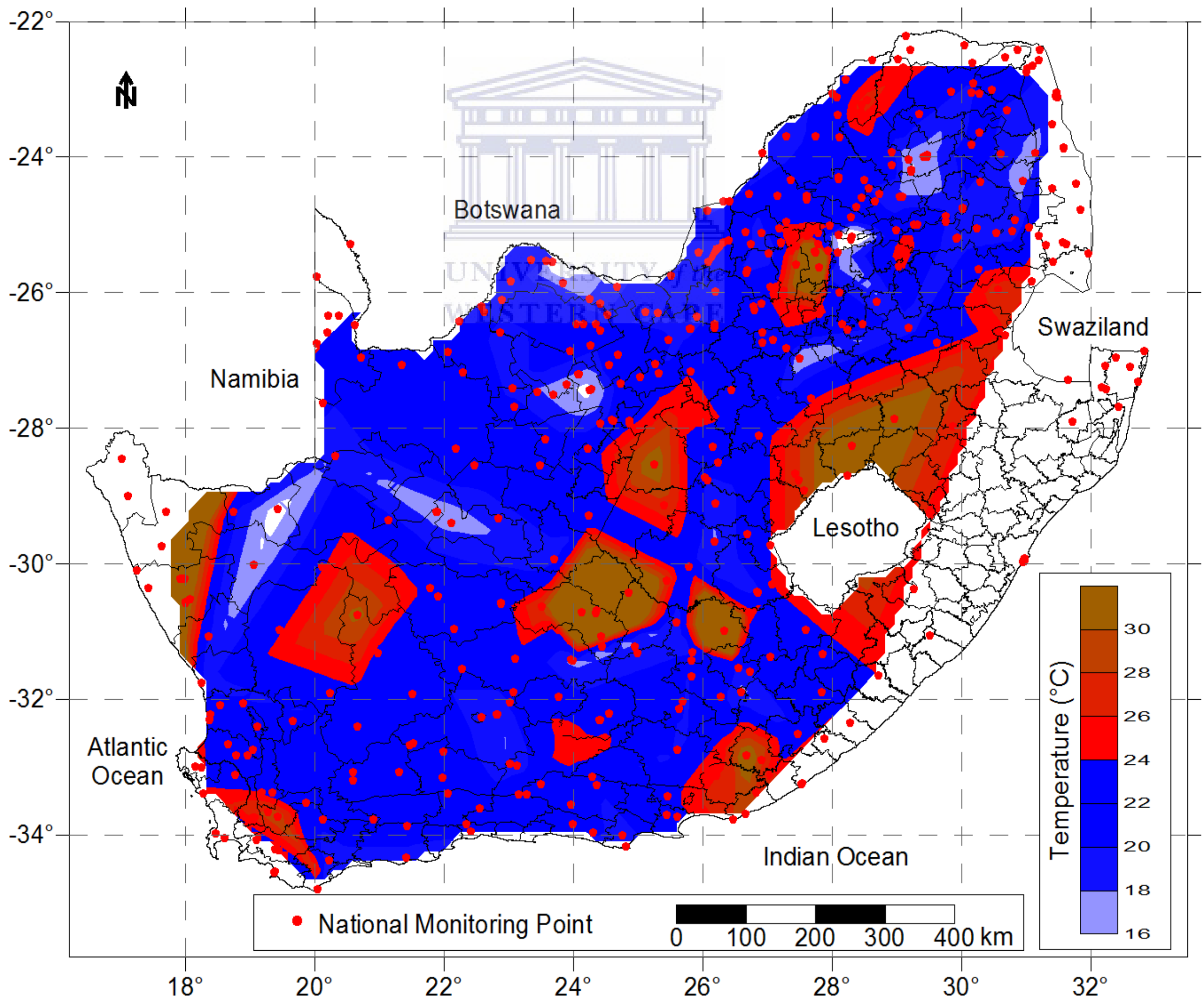


Figure 0-3: Map showing groundwater temperature distribution across South Africa (Data from DWA national Water Quality network)

1.1.6 Saturation

Inactivation of micro-organisms is usually much higher under unsaturated conditions than saturated conditions (Schijven, 2001; Corapcioglu and Haridas, 1984). The effect of unsaturated conditions is highly dependent on the organism type (Schijven, 2002). Viruses on a water air interface are inactivated through oxidative processes and physical forces acting on the viral capsid or envelope. This causes viruses to be inactivated at a higher rate when exposed to a water air interface (Schijven, 2001), such as found in unsaturated aquifer conditions. Hydrophobic viruses tend to have increased inactivation with the water air interface, whereas hydrophilic viruses tend not to enter the interface (Schijven, 2001).

Soil water content also regulates ecological processes involved in nutrient cycling. The availability of nutrients affects the rate and pathways for microbial transformations of nitrogen (Linn and Doran, 1984) and carbon, which can affect microbial survival.

Pathogenic microbes of concern

UNIVERSITY of the
WESTERN CAPE

1.1.7 Introduction

The health effects of exposure to disease-causing bacteria, viruses, and protozoa in drinking water are varied. There are common pathogenic micro-organisms in groundwater which include the *Escherichia coli* O157:H7 (*E. coli*), *Salmonella* and *Shigella*. Bacteria such as *Campylobacter*, viruses such as norovirus, coxsackie, rota and hepatitis A and protozoa such as *Giardia* and *Cryptosporidium* can be responsible for severe gastrointestinal illness (Health Canada, 2006). Other pathogens may infect the lungs, skin, eyes, the central nervous system or liver (Grabow, 1996). Local and international literature has identified the most important viruses, bacteria, and protozoa of concern for human health (WHO, 2002a) are *E. coli*, *Salmonella* and *Shigella*, *Campylobacter* and *Yersinia* (Health Canada, 2006) (Bourne and Coetzee, 1990).

Many of several thousand types of bacteria (both non-pathogenic and pathogenic) can contaminate a water supply (Jemison, 2002). Some of these have caused significant problems internationally and listed in Table 0-1, while others are known to have caused significant local health problems (Table 0-2).

UNIVERSITY of the
WESTERN CAPE

Table 0-1: Some international health incidents caused by bacteria and viruses

Bacterium or virus	Health Effects	Year	Country	Causes
Coxsackieviruses	Foot and mouth	1980–1981	Taiwan	Unwashed hands and surfaces contaminated by feaces.[2]
Vibrio cholera	Watery diarrhea, vomiting, death	1991	Peru	Poor sanitation conditions, poor separation of water and waste streams and inadequate water treatment and distribution systems.[1]
Cryptosporidium, the "hidden germ"	Diarrhea and death	1993	Milwaukee, Wisconsin	DNA evidence shows that the outbreaks are human induced.[2]
E. coli O157:H7	Gastroenteritis (arthritis, pneumonia, Guillain-Barre syndrome)	2007	America	Consumption of premade, frozen ground beef patties.[3]
E. coli O157:H7	Gastroenteritis (arthritis, pneumonia, Guillain-Barre syndrome)	2008	Minnesota	Consumption of premade, frozen ground beef patties.[3]

[1](Tickner, 1991), [2](Ryan, 2000), [3](Magnan, 2008)

UNIVERSITY of the
WESTERN CAPE

Table 0-2: Some local health incidents caused by bacteria and viruses

Bacterium or virus	Health Effects	Year	Province	Causes
Vibrio chlorea	Watery diarrhea, vomiting, ultimately death	2009	Zimbabwe border	General living conditions and unsafe drinking water supplies[4]
Vibrio chlorea	Watery diarrhea, vomiting, ultimately death	2009	Limpopo	General living conditions and unsafe drinking water supplies[4]
Vibrio chlorea	Watery diarrhea, vomiting, ultimately death	2009	Western Cape	General living conditions and unsafe drinking water supplies[4]
Salmonella Typhi	Enterocolitis (heart disease, meningitis, arthritis, pneumonia), diarrhea and death	2005 & 2007	Mpumalanga (Delmas)	Consuming water contaminated with the faeces or urine of infected people.[5]
E. coli O157	Gastroenteritis (arthritis, pneumonia, Guillain-Barre syndrome)	1988	Pretoria	Eating a hamburger from a fast-food outlet[6]

[4](Maponya, 2009), [5](Lang, 2005), [6](WHO, 2001)

UNIVERSITY of the
WESTERN CAPE

The most common microbes associated with waterborne disease outbreaks are the enteric viruses (Abbaszadegan et al. 1999; Ford, 1999). Human enteric viruses are excreted in large numbers (about 100 to 1000 particles/g of faeces) (Abbaszadegan et al. 1999; Sinton, 2001) by infected individuals and may contaminate groundwater sources through soil seepage (Abbaszadegan et al. 1999; Scjihven, 2001; Sinton, 2001). With viral levels as high as this, the chances of some infectious particles reaching the water table is significant even if soil filtration removes large numbers (Yates et al. 1985). Enteroviruses have been shown to be hardy in water environments and especially so in groundwater (Yates et al. 1985; Rao and Melnick, 1986; Schijven, 2001).

Microbial pathogens frequently contaminating groundwater systems include *Vibrio cholerae*, *Shigella dysenteriae*, *Escherichia coli*, *Campylobacter* spp., *Salmonella paratyphi* and *Salmonella typhi* (Dzeda et al. 1997; Stanley et al. 1998) and *Arcobacter butzleri* (Rice et al. 1999). Due to the relatively large sizes of bacteria (compared to viruses), they are removed

much more efficiently by soil filtration (Schijven, 2001). It has been suggested that the lower incidence of waterborne disease outbreaks due to bacteria compared to that of viruses substantiates this hypothesis (Schijven, 2001).

The following sections summarize studies relating to selected bacteria of particular concern.

1.1.8 Escherichia coli *(E. coli)*

Escherichia coli is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms.

While most strains of *E. coli* are non-pathogenic, some can cause serious diarrheal infections in humans (Health Canada, 2006). The O157:H7 strain produces a powerful toxin and can cause severe illness. Infection often causes severe bloody diarrhea and abdominal cramps. Sometimes the infection causes non-bloody diarrhea (Moe, 1997).

The incubation period is 3–4 days, and the symptoms occur for 7–10 days (Moe, 1997; Rice, 1999). Hemolytic uremic syndrome (HUS) is caused by *E. coli*. It is estimated that 2 per cent of *E. coli* O157:H7 infections result in hemolytic uremic syndrome, in which the destruction of erythrocytes leads to acute renal failure (Moe, 1997).

UNIVERSITY of the
WESTERN CAPE

Studies have shown that the dose of *E. coli* required to produce symptoms is lower than that for most other enteric pathogenic bacteria. The probability of becoming ill depends on the number of organisms ingested, the health status of the person, and the resistance of the person to the organism or toxin (AWWA Committee Report, 1999).

The World Health Organization (WHO, 2002a) guidelines for drinking water quality include testing for *E. coli* as an indicator of faecal pollution. The South African primary drinking water regulations give a set of recommended amounts of microbial organisms that should be in our drinking water (WHO, 2002a). The maximum contaminant level should be zero for all the specified micro-organisms. They should not be present in drinking water.

Sources of environmental contaminations are thought to be animal faeces or sewage. *E.coli* has been detected in a variety of water environments: raw water (Gannon, 2004) and surface water (Johnson, 2003). *E. coli* does not multiply in any natural water environment and is

therefore used as specific indicators of faecal pollution (Grabow, 1996; WHO, 1996). The presence of faecal coliforms in borehole water may indicate recent contamination of the groundwater by human sewage or animal droppings which could contain other micro-organisms. Levels of contamination and distribution are not well documented, particularly in developing countries.

The lifecycle of *E. coli* has three major stages: a free swimming stage spent searching for prey in surface water and groundwater or soil, a growth stage spent inside the periplasm of the prey bacterium, and a lysis stage when getting insufficient nutrition from its environment. Die-off times in groundwater for some displaced bacteria appear to be slow and a reduction in abundance by one order of magnitude can take several weeks or longer (Crane and Moore, 1984; Bitton *et al.* 1983).

Few studies have evaluated the ability of *E. coli* to persist in environmental water. Limited observations, however reported that *E. coli* is particularly well adapted for surviving in the aquatic environment (Crane and Moore, 1984; Bitton et al. 1983). It survives for long periods of time in water, especially in cold temperatures (<10°C) (Porter, 1997).

UNIVERSITY of the
WESTERN CAPE

1.1.9 Salmonella

The genus *Salmonella* is mostly comprised of facultatively anaerobic, oxidase-negative, catalase-positive, gram-negative, rod-shaped bacteria. Most stains are motile and ferment glucose with production of both acid and gas (Thomason et al. 1975).

Salmonella can cause four different kinds of illness: gastroenteritis, typhoid fever, bacteremia and dysentery. Symptoms include diarrhea, abdominal cramps, fever, nausea, and/or vomiting. The infection and life cycle begins with ingestion of the bacteria. *Salmonella* travels through the stomach and adheres to the lining of the small intestine. Within 12 to 72 hours, the infection will cause diarrhea, stomach cramps and fever. These symptoms usually last from four to seven days (DiRita, 2001).

The ever-present nature of *Salmonella* may facilitate a cyclic lifestyle consisting of passage through a host into the environment and back into a new host (Thomason et al. 1975). In the USA, there are approximately 40,000 cases of *Salmonella* infection reported each year. According to the World Health Organization (2002a), over 16 million people worldwide are

infected with typhoid fever each year, with 500,000 to 600,000 of these cases proving to be fatal.

Salmonellae are scattered in the natural environment (water, soil, sometimes plants used as food) and through human or animal excretion. *Salmonella* species are often detected in sewage, river and irrigation water, freshwater, marine coastal water and groundwater (Gannon, 2004).

Environmental factors such as temperature, pH, and water activity affect pathogen survival. *Salmonella* grow in foods within the range of 7-54°C. The optimum temperature for growth is 37°C. *Salmonella* survival characteristics in water and their susceptibility to disinfection have been demonstrated to be similar to those of a similar strain of *E. coli* (McFeters et al. 1974; Mitchell and Starzyk, 1975). *Salmonella* have high survival rates in aquatic environments (Chao et al. 1987). It outlives both *Staphylococcus aureus* and the waterborne *Vibrio cholerae* in groundwater and in heavily eutrophied river water (DiRita, 2001). Compared to *E. coli*, *Salmonella* appears to withstand a wider variety of stresses associated with environmental fluctuations and may persist in water environments for some time *Shigella.*

UNIVERSITY of the
WESTERN CAPE

Shigella are gram-negative (resistant to the effects of antibiotics), non-spore-forming, non-motile bacilli, which grow in the presence or absence of oxygen. Through plasmid transfer, these organisms can resist a large-spectrum of antibiotics which currently leads to a resurgence of shigellosis (Guillot, 2010).

Shigella is known to cause disease only in humans and primates. The symptoms of shigellosis include diarrhea and or dysentery with frequent blood stools and abdominal cramps. The incubation period is usually 24 – 72 hours (Guillot, 2010). All species can produce severe disease, but illness due to *Sh. Sonnei* is usually relatively mild and self-limiting (Guillot, 2010).

No group of individuals is immune to *Shigella* but certain individuals are at increased risk, like small children and adults with HIV that acquire *Shigella* at high rates (Baer et al. 1999). A case study that highlights the magnitude of potential pathogenic outbreaks is the Delmas case in 2007. Many diarrhea cases were received by the hospitals, clinics and general

practitioners with the highest rate being 90 patients per day. In total 1003 cases were reported (UKhanlamba Municipality, 2008). *Shigella* infections may be acquired from eating food that has become contaminated by infected food handlers. Vegetables can become contaminated if they are harvested from a field with contaminated sewage or wherein infected field workers defecate. Flies can breed in infected feaces and then contaminate food. *Shigella* infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it or even if someone with shigellosis swims, bathes or defecates in it (UKhanlamba Municipality, 2008).

Available data on prevalence in water supplies may be underestimated, because detection techniques generally used can have relatively low sensitivity and reliability (WHO, 2004). The distribution of *Shigella spp*. in the environment reflects human faecal contamination. *Shigella spp*. has been isolated from some water supply systems in tropical countries (Guillot, 2010).

It is able to survive the proteases and acids of the intestinal tract and infections to hosts can be caused from a very low dose. One study (Popovitch, 1982) reported survival of *Sh.sonnei* and *Sh.flexneri* of up to about 50 days at 25°C in river water. By contrast another study found survival of *Sh.flexneri* for only 4-7 days at 25-35°C (Popovitch, 1982) (Guillot, 2010).

UNIVERSITY of the
WESTERN CAPE

1.1.10 Campylobacter

Campylobacter organisms are spiral-shaped bacteria that can cause disease in humans and animals. Campylobacter are slender, spirally curved, motile, gram-negative bacteria. They are strictly microaerophilic, non-saccharolytic and oxidase positive. These species are also called thermophilic campylobacter's in that they are able to grow at 43°C (Alary, 1990).

Campylobacter species, particularly *C. jejuni* and *C. coli*, are increasingly being identified as the causes of bacterial gastroenteritis in humans (Alary, 1990). The major recognized sources of infection are raw meat (particularly poultry), untreated water, raw milk and pets (Savill et al. 2001). Contaminated drinking water has been the cause of several large outbreaks of *Campylobacter* enteritis in colder countries of the northern hemisphere. Outbreaks, involving up to 3 500 individuals, have been related to the drinking of untreated or inadequately chlorinated water (Vogt, 1982).

These bacteria are fragile. They cannot tolerate drying and can be killed by oxygen (Health Canada, 2006). They grow only in places with less oxygen than occurs in the atmosphere. *Campylobacters* have frequently been isolated from surface water, such as streams, rivers and lakes, due to discharges from wastewater treatment plants, runoff from pastures after rain, and direct contamination by animals (Brennhovd, 1992) (Savill, 2001). They have occasionally been isolated from groundwater but numbers detected were low (Savill, 2001).

The species *Campylobacter jejuni* is particularly problematic. *Campylobacters* can survive for several weeks in aquatic environments (in surface water and groundwater) at temperatures around 4°C (Hazeleger, 1998). *Campylobacters* are not uncommon in surface waters (Brennhovd and Langeland, 1992). *C. jejuni and C. coli* survive in cold water at temperatures below 10°C (Hazeleger, 1998) much longer than they survive in water at temperatures higher than 18°C (Vogt, 1982).

1.1.11 Yersinia

Yersinia ssp are gram negative, facultatively anaerobic, non-spore forming bacilli. They are motile at 25°C but not at 37°C (Hunter, 1997).

UNIVERSITY of the
WESTERN CAPE

Yersiniosis causes fever, diarrhea and abdominal pain, which lasts for about one to three weeks. Other clinical infestations include greatly enlarged painful lymph nodes. The incubation period for the species is between 3 and 7 days (Hunter, 1997). A large waterborne outbreak of *Yersiniosis* was reported in Montana 1974 (Eden 1977), but many of the strains isolated for the water sources were later identified as non-pathogenic, leaving the cause of this outbreak uncertain (Hunter 1997).

Y. enterocolitica are everywhere, being isolated frequently from soil, water, animals, and a variety of foods. *Y. enterocolitica* strains detected in drinking water are more probably non-pathogenic strains of probable environmental origin (WHO 2004). Some species and strains of *Yersinia* may replicate in water environments under the conditions of minimum nutrition and low temperatures (4°C) (Buzoleva 2000) (WHO 2004).

The lengthy survival of *Y. enterocolitica* in water was noted by (Buzoleva 2000), who detected survivors in sterile spring after 64 weeks at 4°C. Liao et al. 2003 showed the long

term survival of *Y. enterocolitica* in sterile water (several years at room temperature). Other works reported the long-term survival of *Y. enterocolitica*, *Y. pseudotuberculosis* and other Yersinia species at reduced temperatures (4-6°C) (Liao et al. 2003). Chao et al. 1988 found that survival was greatly reduced with increasing temperatures. The presence and distribution in water of these pathogens together with their survival capacity should be investigated in greater depth.

Limitations of previous work done on column experiments

Transport and fate studies were reported since 1970. These studies included bench type experiments based on packed columns as well as field studies (Levy et al.'s 2007). Packed columns have since been used to investigate specific aspects of the biological, chemical and physical factors that play a role in the transport and deposition kinetics of bacteria in porous media (McCaulou and Bales, 1995).

Biological factors investigated include cell surface macromolecule length and composition, cell motility, cell size and shape, organism type, and growth phase. Chemical and physical conditions investigated include soil grain size and shape, presence of surface coatings on collector media, fluid velocity, solution ionic strength and composition, and cell concentration (Weiss et al. 1995).

A significant finding of the studies on bacteria and viruses was that attachment and deposition kinetics are mainly determined by electrostatic interactions (Knapp et al. 1998). Findings also indicated that increased temperatures are associated with a substantially higher adsorption rate to particulate matter in an aquifer and that decreasing temperatures play a role in detachment (Huysman and Verstraete, 1993). Temperature is also the only variable significantly correlated with the decay rates of viruses for instance (Knapp et al. 1998). Studies also showed that the transport and deposition behavior differ between viruses and bacteria as they respond differently to specific chemical and physical factors (Huysman and Verstraete, 1993).

Many studies have been devoted to the experimental investigation of bacterial transport through aquifer materials (Levy et al.'s 2007). Most of these previous efforts involved

column experiments. Many factors controlling transport of bacterial in the subsurface have been identified through these previous experiments. These factors include the properties of bacteria themselves (McCaulou and Bales, 1995; Weiss et al. 1995; McCaulou et al. 1994; Huysman and Verstraete, 1993), physical and chemical properties of aquifer materials (Knapp et al. 1998; Morley et al. 1998; Johnson and Logan, 1996; Fontes et. al., 1991;), groundwater chemistry (Jewett et al. 1995; Gross and Logan, 1995; Scholl and Harvey, 1992), and hydraulic conditions (Morley et al. 1998; Hendry et al. 1998).

Few studies on the transport of bacteria through intact sediments have been reported. Smith et al. (1985) studied the transport of *Escherichia coli* (*E. coli*) through intact soil columns. They used highly structured top soil rather than aquifer sediments in their experiments and the focus of their study was on the effect of channeling of *E. coli* transport due to roots and soil fauna. Fuller et al. (2000) were perhaps one of the first to study bacterial transport through intact aquifer cores under well controlled conditions.

Although recently there has been some research of bacterial transport through intact aquifer materials reported (Fuller et al. 2000), their findings highlight the need of more experimental work in this area so that the controlling factors for bacterial transport through intact aquifer sediments can be identified. Furthermore, no attempt has been made to compare the transport behavior of bacteria through intact cores with that through repacked columns (Levy et al.'s 2007). Thus, it remains unclear whether the transport of bacteria through intact sediments is significantly different from transport through repacked sediments.

UNIVERSITY of the
WESTERN CAPE

The transport medium used in Fuller et al, 2002 was horizontal cores retrieved from three distinct sedimentary facies. They found that total effluent recovery of bacteria varied with facies type and appeared to be most influenced by mean grain size and total metal hydroxide content. However, the conclusion is preliminary. First; there was not much variability in the mean grain size among the three cores used in their experiments (2.8 mm, 2.39 mm, and 1.74 mm, respectively) and all the three cores have relatively narrow ranges of grain size distributions (about 1.5 mm to 3 mm). Secondly; other potential factors such as total organic content and degree of sorting were not considered in their study. The authors pointed out the need of further examination of the role of physiological heterogeneity in determining the extent of bacterial transport through subsurface sediments (Fuller et al. 2002).

The simulation and prediction of the movement of microbes through different media under different conditions is complex as the survival and transport of the microbes are influenced by a nonlinear combination of physical, chemical and biological processes.

Experimental setup in previous studies

1.1.12 Typical laboratory setup

Levy et al.'s (2007) examined the transport of *E. coli* through intact cores of glacial-outwash sediment with the overall goal of enhancing our ability to predict the transport and fate of bacteria in similar geologic environments using easily-measured sediment characteristics.

Levy et al.'s (2007) objectives were to characterize the transport of *E. coli* and the variability of transport among many cores of intact natural sediments and to compare bacterial transport to conservative solute transport in the same media. This study focused on assessing the influence of sediment characteristics. Therefore, some other factors affecting bacterial transport were kept constant including groundwater chemistry and the type of bacteria used.

Sediments used for the laboratory experiments comprised of sand. The main advantage of using sand for the laboratory experiments is that it comes directly from the aquifer. The columns assembled were setup according to Darcy's specifications (Levy et al.'s 2007), both bromide and bacterial transport experiments were performed on 16 cores (referred to as N1 through N16). The column assembly comprised a headspace purging system to keep the system anaerobic, a peristaltic pump for influent delivery, and the sediment core with top and bottom Plexiglas caps and a fraction collector for effluent recovery (Figure 0-4) (Levy et al.'s 2007). Prior to the setup of a transport experiment, the column apparatus, including all the fittings and tubing, was sterilized with methanol and thoroughly rinsed with distilled water.

UNIVERSITY of the
WESTERN CAPE

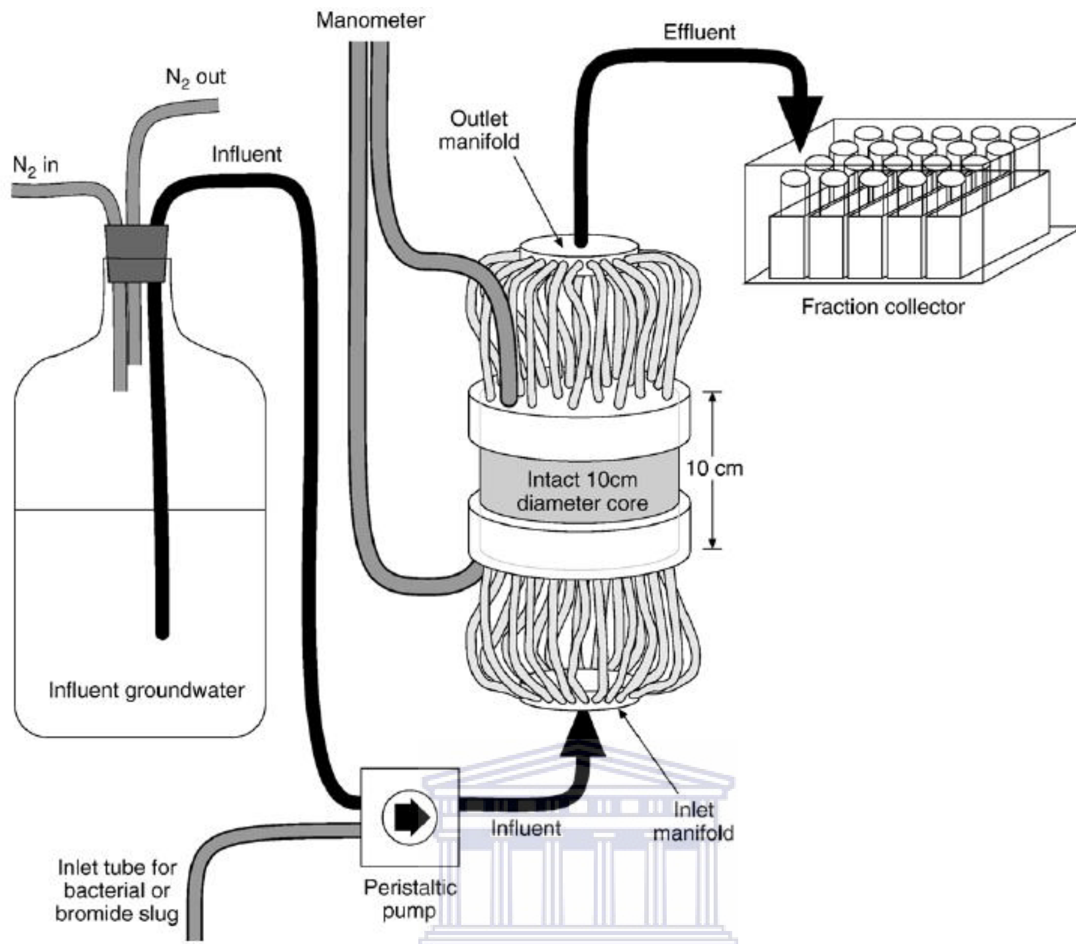


Figure 0-4: Experimental setup for laboratory column experiments (after Levy et al.'s 2007)

WESTERN CAPE

The column top and bottom caps were then attached to the polycarbonate liner containing the sediments (Levy et al.'s 2007). Two grooves were made along the wall of each cap and O-rings were recessed into the grooves to provide an airtight seal between the column caps and the core section. Influent groundwater was pumped through the column assembly with a variable-speed, digital Masterflex® L/S peristaltic pump. Water was first pumped into a PVC manifold separating the flow into 20 influent tubes (Levy et al.'s 2007). The 20 tubes were connected to the bottom cap with 20 equally spaced ports for an equal distribution of flow across the entire core. Effluent left the top of the column assembly through 20 equally-spaced ports in the top cap and was combined in a second PVC manifold before flowing to the fraction collector.

The entire flow-through apparatus was kept in a refrigerator to maintain a representative aquifer temperature of 12 °C. The ISCO 328 automatic fraction collector contained sterile sample-collection vials and was maintained at 4 °C (Levy et al.'s 2007). To closely mimic the

natural aquifer system, transport experiments were performed under anoxic conditions using the headspace purging system in which a low-flow of nitrogen gas was maintained through the air space in the bottle containing the influent groundwater (Levy et al.'s 2007). The influent groundwater used for all experiments was taken from a local aquifer and had low variability with respect to pH (range of 7.26 to 7.37), specific conductance (0.8 mS/cm) and dissolved oxygen content (range of 0.5 to 2.0 mg/L) (Levy et al.'s 2007).

Levy et al, 2007 discovered for each core, between one and three bromide transport experiments were conducted before the bacterial breakthrough experiments. For all of the cores except N9 and N16, bromide transport experiments were also conducted after the bacterial experiments. Bromide transport before and after bacterial experiments was compared to determine if running bacterial transport experiments physically altered core flow characteristics (Levy et al.'s 2007). For all breakthrough experiments, a flow rate of about 2 ml/min was used corresponding to an average flow velocity of about 0.16 cm/min or 2.3 m/d.

This velocity was chosen to correspond to velocities that are common at the field site from which the cores were taken (Levy et al.'s 2007). For the bromide experiments, immediately following flow of bromide-free groundwater, a 100 mg/L in groundwater bromide solution was introduced into the core as a 10-min, 20-ml pulse followed again immediately with resumption of bromide-free water. Effluent was collected in the fraction collector at 4-min intervals. Bromide experiments were run for 3 pore volumes. Bromide concentrations were measured using a high-performance liquid chromatograph (Levy et al.'s 2007).

UNIVERSITY of the
WESTERN CAPE

Only a few investigations have studied bacterial transport using cores of intact, natural sediments (Smith et al. 1985; Bolster et al. 1999; Fuller et al. 2000; Dong et al. 2002). Such studies are important as repacked cores may not replicate the structure and preferential flow pathways inherent in natural sediments (Dong et al. 2002). Most of the previous column studies with intact, natural cores used aquifer sediments consisting of relatively homogeneous sands (Bolster et al. 1999; Fuller et al. 2000; Dong et al. 2002).

Ryan et al (2002), conducted laboratory experiments to investigate inactivation of viruses attached to mineral surfaces.

The laboratory experiments consisted of two types of inactivation experiments — solution inactivation and surface inactivation.

The solution inactivation experiments were conducted in raw and amended groundwater from the contaminated zone at the Cape Cod site (Ryan et al. 2002). The surface inactivation experiments were conducted in raw and amended contaminated groundwater and contaminated sediment from the Cape Cod site (Ryan et al. 2002). The sediment was collected within 5 m of the field experiment site using a hollow stem auger, a piston core barrel, and a liquid nitrogen circulation device that sealed the bottom of the core barrel with a plug of ice (Murphy, 1996).

The laboratory inactivation experiments were conducted on bacteriophages PRD1 and MS2. Following the technique of Loveland et al. (1996), the two viruses were dual radio-labelled by growing the host bacteria to log phase in a methionine-deficient glucose growth medium (Ryan et al. 2002). The suspensions were incubated until lysis occurred and new viruses were released (*c*.3 h). Cell debris was removed from the suspensions by centrifugation (10 000 rpm, 20 min, Sorvall RC-5B centrifuge, GSA rotor), and the supernatants were filtered through 0.25 μm cellulose acetate filters. These suspensions were centrifuged again (19500 rpm, 6 h, SS-23 rotor) to pellet the viruses and re-suspended in phosphate buffered saline at pH 7.5 (Ryan et al. 2002).

These experiments tested the rate of inactivation of unlabelled and dual radio-labelled viruses in raw and amended contaminated groundwater from Cape Cod at 5 °C (Ryan et al. 2002).. Groundwater was amended by addition of the LAS mixture used in the field experiment (25 mg L-1) and sodium dodecylbenzene sulfonate (DBS; 25 mg L-1) (Ryan et al. 2002). A 1 mL aliquot of virus suspension was added to 19 mL of groundwater in a glass vial, mixed by hand, and incubated at 5 °C for 30 days. Samples (0.1 mL) were removed every 24 h, and virus concentration was measured by plaque assay. Each of these experiments was conducted in triplicate (Ryan et al. 2002).

As a result of the experiment the PRD1 appeared to be slightly more resistant to inactivation than MS2 in the contaminated Cape Cod groundwater but the differences in the inactivation rate coefficients were not statistically significant for comparisons between PRD1 and MS2 (Ryan et al. 2002). The half-lives of PRD1 and MS2 infectivity in the contaminated

UNIVERSITY of the
WESTERN CAPE

groundwater were 32(17 and 13(6 days, respectively. The additions of LAS, DBS, and the dual radiolabels (^{32}P, ^{35}S) resulted in small, but insignificant, increases in the inactivation rates of PRD1 and MS2 (Ryan et al. 2002).

In the laboratory experiments, 50-92% of the infective PRD1 and MS2 attached to the contaminated zone sediments in the first pore volume (Blanc and Nasser, 1996). Although MS2 and PRD1 possess similar surface charges, infective MS2 attachment during the first pore volume was more extensive than infective PRD1 attachment. In contrast, attachment of ^{35}S-PRD1 was more extensive than that of ^{35}S-MS2. Most recent laboratory and field studies have found that PRD1 attachment is more extensive than MS2 attachment to a wide range of aquifer sediments (Blanc and Nasser, 1996), including Cape Cod sediments (Kinoshita et al. 1993). The research team suspects these discrepancies may indicate that MS2 surface inactivation and release is more rapid than that of PRD1 (Blanc and Nasser, 1996).

The laboratory surface inactivation experiment did provide insight into the fate of MS2 (Ryan et al. 2002). Only small fractions (0.84 and 5.6%) of the infective MS2 were released during the 29 groundwater flushes, whereas one-fifth of the ^{35}S (protein capsid) and nearly half of the ^{32}P (nucleic acid) were released. The difference in release rates is not statistically significant (unpaired t test, $p > 0.09$ for both cases), but the result is consistent for both surface inactivation experiments and the pH 11 flushing experiment (Ryan et al. 2002). These differences between the amount and rate of ^{35}S and ^{32}P release suggest that the inactivated MS2 was disintegrated by surface inactivation; that is, it may have been released as separate protein capsids and nucleic acid. Proteins also readily adsorb to mineral surfaces, but the disruptions and distortions in their structure caused by attachment result in denaturing and spreading (Van Tassel et al. 1998, Servagent-Noinville et al. 2000) that may strengthen or weaken attachment. It is not clear from the literature if this behavior promotes or inhibits protein desorption kinetics, but the adsorption of most proteins is partially reversible and the fraction of reversible adsorption is dynamic.

After *c.*15- 20 pore volumes, however, the infective virus release increased, most noticeably for MS2 following attachment by contaminated groundwater. Similar results have been reported for MS2 during solution inactivation studies testing the effects of iodine as a disinfectant and temperature (Ryan et al. 2002). In the case of iodine disinfection, this phenomenon, termed “rebound”, was attributed to the consumption of iodine by beef extract.

UNIVERSITY of the
WESTERN CAPE

In the temperature study, the rebound was attributed to reactivation of virions that had undergone rearrangement or loss of capsid components at high temperature (Ryan et al. 2002). It cannot be speculate on the rebound observed in the experiments conducted because there is not sufficient knowledge about the exact mechanism of surface inactivation of viruses (Ryan et al. 2002).

1.1.13 Typical Field experimental setup

Harvey et al (2008), examines a field study that introduces a simultaneous pulse of a non-reactive tracer (SF_6, an inert gas) and oocyst-sized microspheres into karst limestone of the Biscayne aquifer. Karst aquifers are important sources of drinking water in many parts of the United States of America (Harvey et al. 2008). However, these aquifers are highly vulnerable to contamination by pathogens, because they can include solution-enlarged conduits and fracture systems that can result in rapid transport along preferential flow paths.

The objective of this study was to assess the vulnerability of the municipal supply wells within the North West Well Field (NWWF) to contamination by *C. parvum* oocysts from existing and newly proposed borrow pit lakes (Harvey et al. 2008). The study focused on the outcome and interpretation of in situ (injection-and-recovery) colloidal transport tracer test conducted on 5 February 2004 and supporting laboratory studies (Harvey et al. 2008).

UNIVERSITY of the
WESTERN CAPE

Approximately 164 L of groundwater (formation water collected from the injection zone) containing the microspheres and approximately 172 L of groundwater containing the nonreactive tracer SF_6 (gas) were pumped into an interval of injection well isolated by a downwell packer device built specifically for this experiment (Harvey et al. 2008). A detailed description of the injection apparatus is given by Shapiro et al. (2008). The isolated interval included a 0.8 m thick, high-permeability groundwater flow zone (Renken et al. 2005). The microspheres and conservative tracer (dissolved SF_6) were pumped from separate tanks simultaneously downhole using a system of valves and pumps (Shapiro et al. 2008). Total numbers of 1.6, 2.9, and 4.9 µm diameter microspheres added to the borehole during a 19.5 min interval were 9.5×10^{13}, 1.2×10^{11}, and 4.5×10^{10}, respectively. This was followed by addition of a ‘‘chaser’’ volume (approximately 380 L of formation water containing no microspheres or conservative tracers) that helped ensure complete displacement of the injectate from the borehole into the limestone aquifer (Harvey et al. 2008).

Following the injection of microspheres and SF6 groundwater samples were collected periodically from production well S-3164, located 97 m down gradient from the injection well G-3817 and analysed for each size class of microspheres (Harvey et al. 2008). The pumping rate of the production well was initially 476 L/s and remained relatively steady during the first 50 h of the test.

Samples were collected every 15 min during the first 12 h, every 30 min during the next 12 h, and every hour thereafter. Only data obtained during the first 26 h following injection were used because subsequent microsphere concentrations were too low to enumerate accurately. Separate samples were collected for the purpose of assessing concentrations of conservative tracers (Harvey et al. 2008). Breakthrough curves were determined for each size class of microspheres. Total numbers of microspheres transported to the well were estimated for each size class using numerical integrations of the respective breakthrough curves and the pumping rates corresponding to the time the samples were taken (Harvey et al. 2008).

1.1.14 Mathematical models and assumptions used to describe chemical and microbial transport

Several mathematical models have been proposed to describe bacterial transport through groundwater (Ginn et al. 2002). All of the models are based on the traditional advection-dispersion equation of solute transport. Some of the existing mathematical models of bacterial transport are too complex to be used in practice (Dykhuizen, 1987). Other simpler phenomenological models, such as the one incorporating a kinetic sorption process, have been successfully used to reproduce experimental data. However, these phenomenological models were simply calibrated to experimental data; no attempt has been made to relate the transport parameters to sediment properties. In order to increase the predictive capability of the mathematical models, it is very important to quantify such relationships (Dykhuizen, 1987).

Flow and transport in structured porous media are frequently described using double - porosity (dual) models (Ginn et al. 2002). Central to the dual-porosity approach is the assumption that the medium can be separated into two distinct pore systems, both of which are treated as homogeneous media with separate hydraulic and solute transport properties. The dual-porosity medium is considered to be a superposition of these two systems over the same volume (Dykhuizen, 1987). The two pore systems interact by exchanging water and

UNIVERSITY of the
WESTERN CAPE

solutes Figure 2-5 in response to pressure head and concentration gradients. Hence macroscopically, the porous medium at any point in time and space is characterized by two flow velocities, two pressure heads, two water contents, and two solute concentrations (Dykhuizen, 1987).

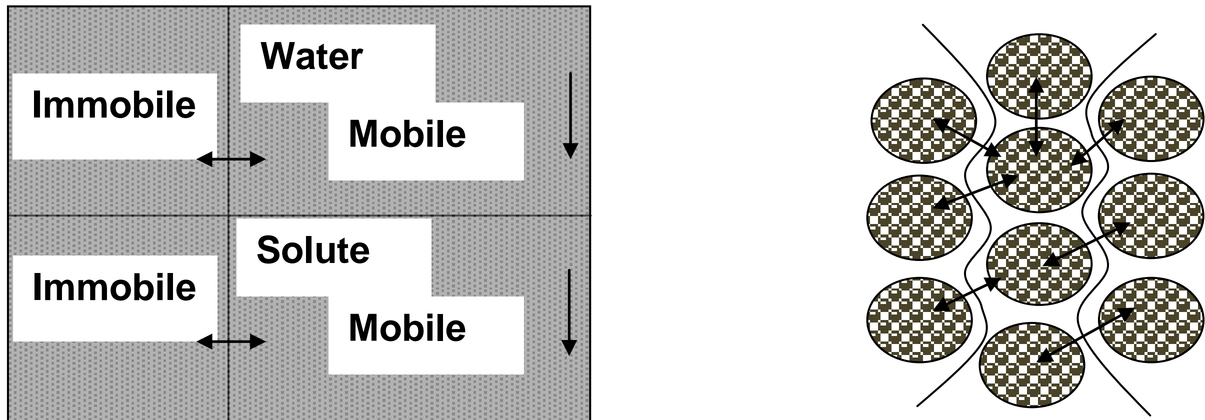


Figure 0-5: Images of Dual-Porosity under uniform water flow that only applies to Solute transport.

UNIVERSITY of the
WESTERN CAPE

Additional biological processes affecting microbial transport are expressed through the growth and decay processes and include active adhesion or detachment, survival, and chemotaxis (Ginn et al. 2002). The biological nature of these processes presents a challenge for transport modeling in that one biological mechanism is often dependent on and influenced by another biological mechanism. Thus, it may be necessary to consider the interdependency of the various biological processes (Ginn et al. 2002).

Chapter 3: Field Research Study Site

Introduction

This chapter describes the field study site and the field experiments conducted at the University of the Western Cape (sand aquifer).

2.1.1 Selection of representative microbial tracers

Bacteriophages (usually simply referred to as 'phages') are often used for tracer studies as they are non-pathogenic (other than for their target bacterial species). Their persistence in the environment is limited to a few months and they do not accumulate, which results in the background level in the environment remaining negligible (Rossi et al. 1994). A phage is typically very much smaller than a bacterium so are used to simulate viruses. A phage can only multiply once it has penetrated a host cell (Harvey and Ryan, 2004).

PRD-1 was selected as representative phage. This is an icosahedra-shaped, double stranded DNA phage of 62 nm in diameter with a relatively low isoelectric point (Bales et al. 1991). PRD-1 is an important viral tracer for studies involving sub-surface viral transport and ground water environments (Harvey and Ryan, 2004). PRD-1 is particularly useful in field applications due to its high stability in aqueous and geologic media (Yahya et al. 1993a, Straub et al. 1992). It has structural and functional similarities to mammalian adenoviruses (Belnap and Steven, 2000), some of which are human pathogens. It also has a low propensity for attachment to many surfaces compared to other phages (Yahya et al. 1993b) and imposes no health threats (Schijven et al. 1999).

UNIVERSITY of the
WESTERN CAPE

Bacteria are the most commonly used microbial tracers because they are easily cultured in large numbers and are easily detected (Cronin and Pedley, 2002).

E. coli was selected as representative bacteria. Faecal coliforms are gram-negative rod-shaped bacteria used extensively for monitoring groundwater quality as they are excreted in large quantities by humans and mammals and are useful indicators of faecal contamination. They are used to monitor the movement of sewage wastes in groundwater (Keswick et al. 1982). *E. coli* is a valuable indicator of faecal contamination due to its ease of detection (Foppen and Schijven, 2005).

Site description

The study site is situated along the western boundary of the UWC campus and covers an area of approximately 150 m^2 at an elevation of 60 m above sea level. The campus site displays different types of vegetation during the year from wild grass during winter to flowers during spring. Permanent vegetation and buildings are present within a radius of about 100 m from the boreholes. The campus site is surrounded by the Cape Flats nature reserve, the Belhar residential area and the massive Parow industrial area which is about 300 m from the production boreholes.

The test site (UWC) consists of six boreholes mainly used for research purposes. Boreholes UWC3A and UWC4 were used for the tracer experiments (Figure 0-1).

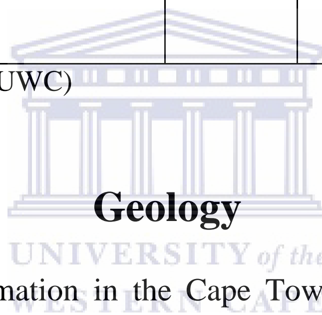
The borehole characteristics are given in Figure 0-1 and Table 0-1. Boreholes UWC1, UWC2, UWC3A and UWC4 penetrate into the upper tertiary Cape Flats Aquifer. Borehole UWC1 and UWC2 are completed with 90 mm PVC casing, while borehole 3A and 4 are completed with 160 mm PVC. Boreholes UWC1, UWC3A and UWC4 have transmissivities of between 20 and 35 m^2/d, while borehole UWC2 has a transmissivity of about 1 m^2/day. The water levels vary between 4 and 5 m below ground level. The distance between borehole UWC1 and UWC4 is *c*.40 m. Borehole UWC4 is equipped with a 3 L/s 3-phase submersible pump. Both UWC1 and UWC can be pumped using either 1 or 1.5 KW suction pumps.

UNIVERSITY of the
WESTERN CAPE

Table 0-1: Borehole characteristics of UWC campus site

ID	Aquifer type	Depth (m)	Screen top (m)	Screen bottom (m)	Diameter (cm)	Hydraulic conductivity (K – m/day)	pH
UWC1	Primary	6	1.5	6	7.5	1.5	6
UWC2	Primary	30	1.5	12	5.5	0.17	6
UWC3A	Primary	18	1.5	6	14.5		7
UWC4	Primary	22	1.5	3	14.5	1.9	6
UWC5	Secondary fractured shale aquifer	108	32	12	14.5	25	6
UWC6	Secondary fractured shale aquifer	82	64	12	7.5	15	6

(October, 2009, Pers. comm. J. Nel, UWC)


UNIVERSITY of the
WESTERN CAPE

Geology

The predominant geological formation in the Cape Town area is the Malmesbury Group (Tygerberg & Moorreesburg) is The Malmesbury Group is the oldest geological rock formation which is intruded by granite batholiths resulting in the deformation and metamorphism of the shales. The Cape Flats Granite and the Malmesbury shales are in turn uncomfortably overlain by the sandstones of the Table Mountain Group (Johnson et al. 2008). Cape Flats is an area with low lying topography, which was created by the weathering of Table Mountain Group between False Bay and Table Bay during Post-Palaeozoic erosion (Johnson et al. 2008).

The Late Precambrian Malmesbury Group underlies the Cape Flats sand deposits and forms the basement rock on the campus site. The Malmesbury Group is subdivided into three major tectono-stratigraphic domains also referred to as “Terranes” that are separated by major fault zones (Johnson et al. 2008). The most western terrane, the Tygerberg Terrane, represents the basement to the overlying unconsolidated sand deposits. The Tygerberg Terrane or Formation consists mainly of medium- to fine-grained greywacke, phylittic shale, siltstone and quartzite

(Johnson et al. 2008). At the UWC campus site, the Tygerberg Formation consists of fractured shale which becomes highly weathered at the top to form a thick clay layer.

UNIVERSITY of the
WESTERN CAPE

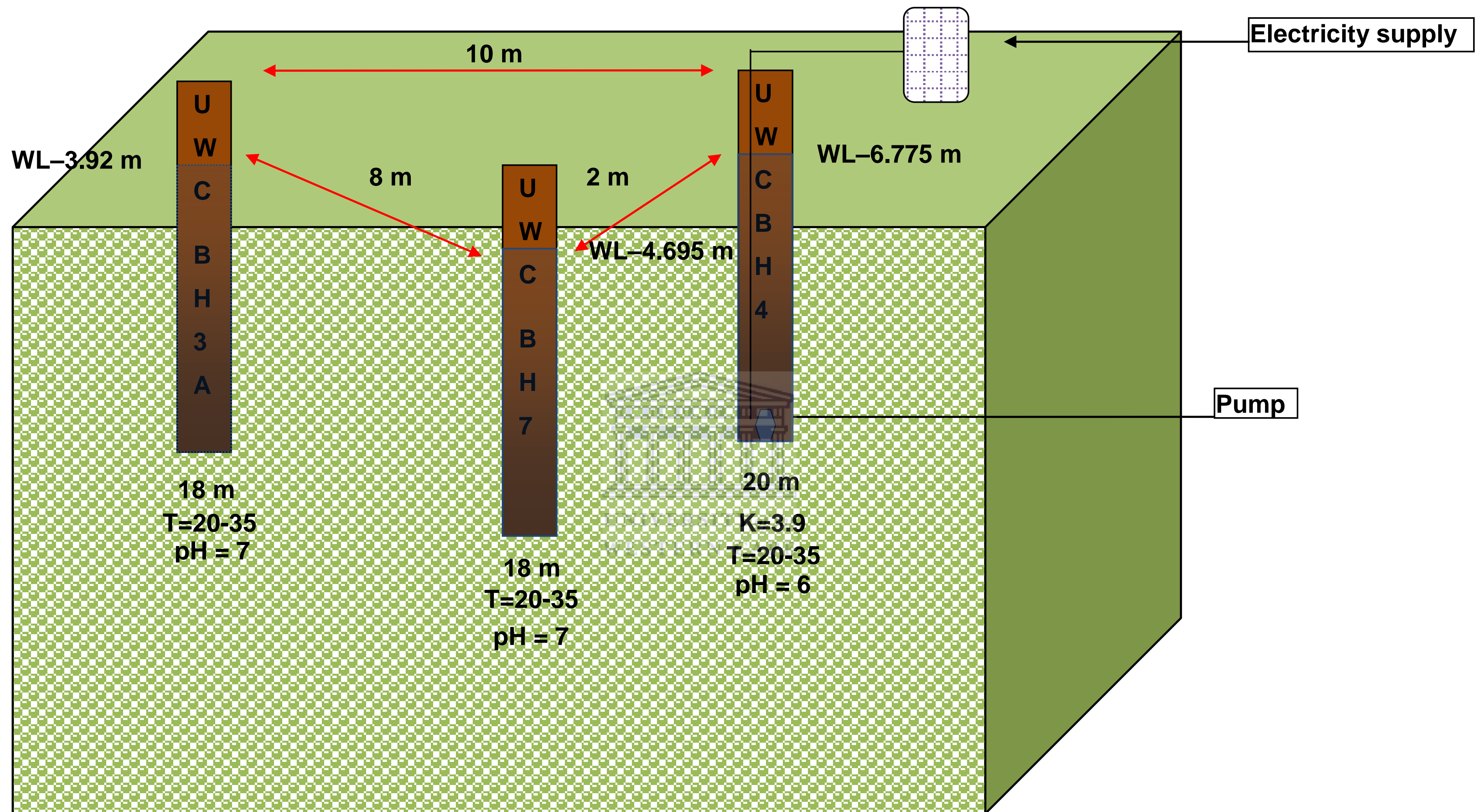


Figure 0-1: Cross section of campus site showing relevant boreholes used for experiment as well as their hydraulic characteristics.

The predominant geological formation in the Cape Town area is the Malmesbury Group (Tygerberg & Moorreesburg). This is interspersed with the Cape Granite and Klipheuwel groups, while alluvium, sand, and calcrete are found on the coastal plain (Truswell, 1970).

The Cape Flats is covered with a deep mantle of recent and Tertiary sands which in the study area calcareous in nature. Cape Flats is underlain by Malmesbury Group which consists of fractured rock formation. The Malmesbury Group is typical consist of shale and clays rock types, and the fractures are resulted due to weathering. The Malmesbury Group is uncomfortably overlain by the Neogene age (24 to 2 Ma) sandy soil and peat of the Springfontyn Formation which in turn are overlain by the younger unconsolidated Quaternary age (<2 Ma) white sand of the Witzand Formation (Geological Survey of South Africa, 1990; Theron *et al.* 1992).

Groundwater from the Cape Flats Aquifer is mainly used as a freshwater resource that supplies the industrial, domestic and agricultural industries in the Western Cape. These industries are also the largest contributors to misuse and pollution of this resource (Usher and Xu, 2006). The research site is situated close to the Parow Industrial area which could be the largest source of possible contaminants within the study area. The Malmesbury Fractured Rock Aquifers which underlies the Cape Flats Aquifers are found to be vulnerable to contamination, because of the fractures that allow for adequate transporting of contaminants and microbes. Studies indicate that the fractures might have been originated due to weathering.

UNIVERSITY of the
WESTERN CAPE

Drilling and construction

The various field hydraulic tests and tracer tests conducted by previous researchers (Mr. Adolf October and Mr. Mlamleni Kolosa) and practicals highlighted that the boreholes at the site intercepted different aquifer layers and had different levels of connection. Borehole UWC 3A is a replacement of the old collapsed borehole UWC 3 and was purpose drilled for this project. Borehole UWC3A was drilled 10m from existing production borehole UWC4 to act as tracer injection borehole. The boreholes are constructed with full length PVC screens for tracer injection purposes. UWC3A and UWC4 have the same borehole depth and same aquifer units. Experimental results (Section 4.4.2) showed that 10 m was still too far for realistic transport time frames and an additional hole was drilled 2 m from production hole.

Mud rotation drilling was the method used to construct the two new boreholes (UWC3A and UWC7) Figure 0-2. In the mud rotary drilling method, the borehole is advanced by rapid rotation of a drill bit mounted on the end of the drill rods. The bit cuts and breaks the material at the bottom of the hole into small pieces (cuttings). The cuttings are removed by pumping drilling fluid down through the drill rods and bit, and up the annulus between the borehole and the drill rods. The drilling fluid also serves to cool the drill bit and stabilize the borehole wall, to prevent the flow of fluids between the borehole and surrounding earth materials, and to reduce cross-contamination between aquifers. Among additives for increasing the density of water, salt is one of the most convenient; but one of the most widely used is a natural clay mineral known as bentonite (calcium montmorillonite), which swells enormously in water (USEPA, 1986).

Drilling mud consists of a slurry of water and polyprop or gum-gum combined in the proper proportions and has a higher viscosity than water. This drilling mud is used to exert hydrostatic pressure while the borehole is being drilled (Figure 0-2). However, a disadvantage is that gum-gum needs to be mixed and left for some 12 hours before use to allow the viscosity to build up. Polyprop can however be used immediately (USEPA, 1986).

UNIVERSITY of the
WESTERN CAPE

These boreholes are cased with PVC casing, the lower 14 m of casing is slotted and the top six meters is solid to prevent plant roots from entering the borehole (USEPA, 1986).

a) Preparation of the hole

b) Polyprop or gum gum used to exert hydrostatic pressure

c) Mud rotary drill rig

UNIVERSITY of the
WESTERN CAPE

d) Pumping fluid added to stabilize borehole walls

e) Removal of drilling cuttings by pumping fluid

f) Rod end which holds the drill bit

Figure 0-2: Site photographs of the drilling process

Water levels and flow gradients

There is almost no groundwater gradient for either the Cape Flats or the Malmesbury aquifer at the scale of the campus research site. All the tracer tests were therefore conducted under controlled forced flow conditions. The rest water level is approximately 4 m below the surface. No known interactions between shallow primary and deep secondary aquifer at the site (October, 2009, Pers. comm. J. Nel, UWC).

The strata present on the campus site can be divided into 4 different lithological units that of clay, peat and sand (top) shown in Figure 0-3 logs below.

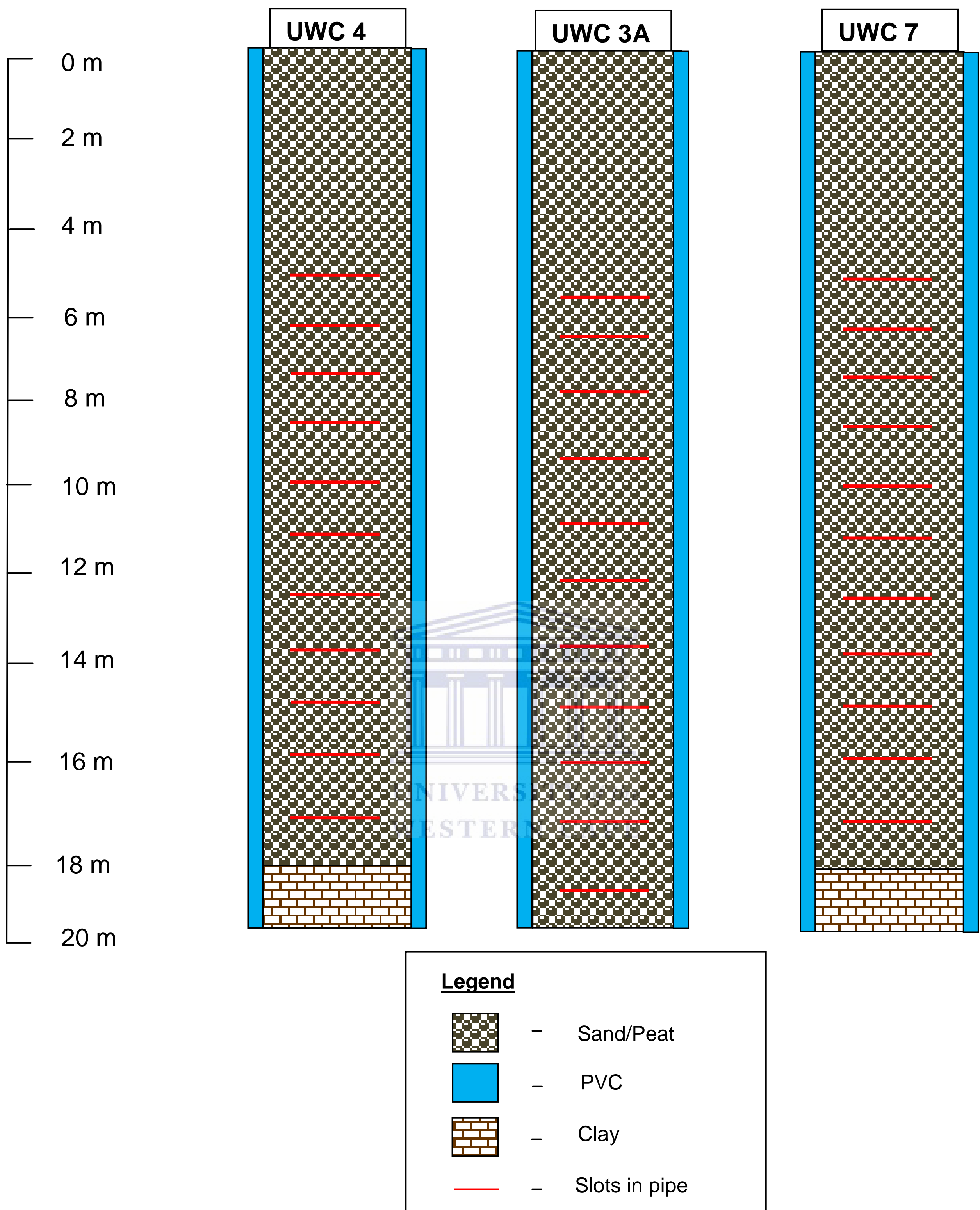


Figure 0-3: Logs of newly drilled boreholes

Chapter 4: Methods and Materials

Project team and affiliations

This project is part of a Water Research Commission (WRC) investigation to understand and model transport and fate of micro-organisms in two simulated aquifer conditions in the laboratory and the field. The project team consists of Martella du Preez who was to the project leader (affiliated WRC), Wouter le Roux the microbiologist who grew the micro-organisms for the tracer tests conducted in the laboratory and field, Marna van der Merwe the groundwater modeler, Jaco Nel the UWC project leader and mentor, Rienie Meyer and Kevin Murray who saw to the technical information and Erin Paramoer who compiled the literature review and conducted the laboratory and field experiments as part of her Master of Science degree.

Introduction

A series of controlled experiments under laboratory and field conditions was conducted. Each provides a different kind of data and information. The results from laboratory studies were used to improve the design of the field studies. In both cases, the data collected provided information on fate and transport of microbial and chemical tracers in groundwater in the Cape Flats aquifer of South Africa at UWC.

UNIVERSITY of the
WESTERN CAPE

Laboratory tests

3.1.1 Sand and water collection

Sand sediments were collected from the Cape flats aquifer at the University of the Western Cape. Hand augers were used to collect sand samples that were used in the columns. The sand was extracted from depths of between one and two meters to avoid the organic matter and disturbed top soil layers.

3.1.2 Disinfection and autoclave

The laboratory equipment was disinfected and cleaned with a mixture of household Jik and water. A 200 liter container was filled with water and 12 ml of the household Jik added. Equipment was submerged in this solution for 12 hours. This mixture was used to sterilize all

equipment and rinse the pipes and pumps. To neutralize this solution 18 g per liter of sodium thiosulphate was used.

Sand and water used for the experiments was autoclaved for some of the bacterial transport experiments. An autoclave is a device used to sterilize equipment by subjecting them to high pressure steam at 121 C typically for 15 to 20 minutes depending on the size of the load and the contents. Sterilization refers to killing of bacteria and germs on surgical instruments. Autoclaves are specially designed and made according to the heat that is demanded. Both sand and water was autoclaved in preparation for the bacterial and virus transport experiments.

3.1.3 Column setup and packing

The laboratory flow field and tracer tests adapted experiments carried out by Levy et al.'s (2007). Water is first supplied to a PVC influent manifold separating the flow into several influent tubes (Figure 4-1). The tubes are connected to the bottom cap with 9 equally-spaced ports for an equal distribution of flow across the entire inner sand column. Effluent leaves the top of the column assembly through 9 equally-spaced ports in the top cap and is combined in a second PVC effluent manifold before flowing to the fraction (sample) collector. The entire flow-through apparatus was kept in a constant temperature room (18 - 22°C) representative of the Cape Flats aquifer.

UNIVERSITY of the
WESTERN CAPE

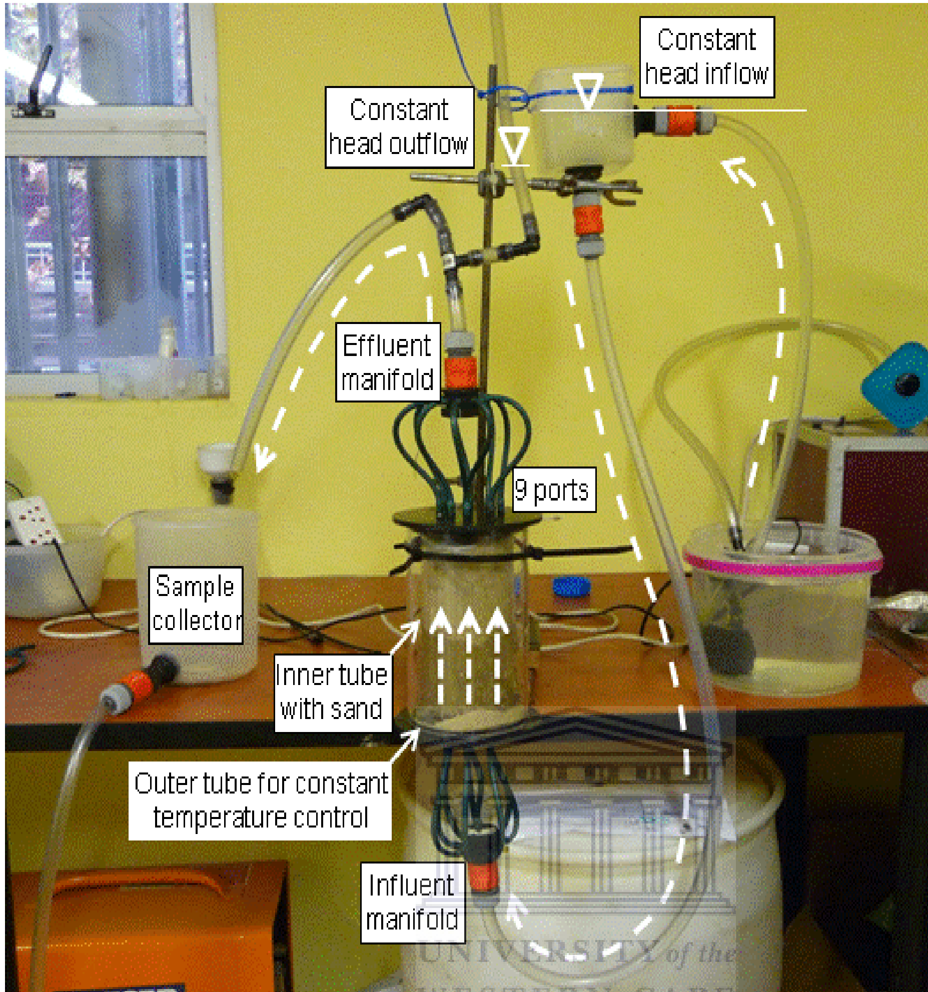


Figure 0-1: **Darcy upflow experiment setup for laboratory simulation of a sandy aquifer**

The flow of the water is provided at a constant head with water percolating upwards through the 20 cm column. The upwards percolation is primarily done to saturate the sample and to prevent air trapping. The compacted column is then immersed into a constant temperature water bath set at 20ºC. The Darcy experiment is run under a constant inflow and outflow head. The tracer is injected into the influent manifold while the Darcy experiment is running.

The sand was packed according to a standard leaching packing procedure (ASTM 4874, 2006). The objective of the standard leaching packing is to fill up the column with material in at least five consecutive layers (Figure 4-2). Each of the five layers is introduced into three sub-layers and leveled. The compaction of the sand material into the column is achieved by using a rammer with a weight of 125 g falling along a guide rod onto the compacting disk of 10 cm diameter. The rammer is applied three times onto the sample layers; each time falling from a height of 20 cm. Filter paper is used to prevent intrusion of sand into the pores/pipes

of the porous inlet and outflow pieces. The filter paper used has negligibly small hydraulic impedance.

UNIVERSITY of the
WESTERN CAPE

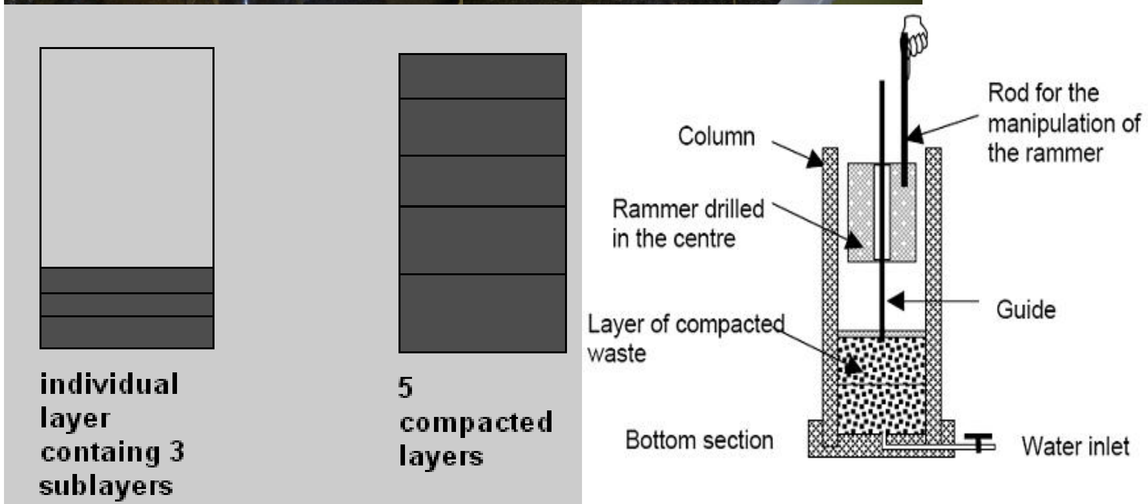


Figure 0-2: Illustration of the ASTM 4874 standard leaching packing method.

3.1.4 Tracer test

Both a salt tracer and a microbial tracer were used in all sand columns. Both tracers were injected at the distribution manifold, with the microbial tracer injected first followed by the salt tracer after the microbial test was completed.

3.1.5 Biological tracer preparation

The PRD-1 and *E. coli* tracers were propagated in the laboratories of the CSIR (Pretoria) and sent to the University of the Western Cape where the tracer experiments were carried out.

- **Bacteriophage PRD-1**.
  - **Host culture, top agar and Perti dish preparation**

    The host culture is prepared the day before the experiment. A loopful of host culture is taken from an agar slope of *Salmonella thyphimurium* and introduced into 50 ml sterile nutrient broth. Incubate for ± 18 hr.

    Petri dishes (90 mm diameter) are poured using Bacto agar 11 g; Tryptone, 13 g; NaCl 8 g Glucose 1.5 g and 1 000 ml distilled water.

    Top agar is prepared using Bacto agar 8 g; Tryptone, 10 g; NaCl 8 g Glucose 3 g and 1 000 ml distilled water.

UNIVERSITY of the
WESTERN CAPE

    All solutions and agars used for bacteriophage experiments were heated to 20ºC / 37ºC prior to use to ensure optimum conditions for bacteriophage culture.

    Add 0.2 ml of the host culture to 2.5 ml top agar. Add 1 ml of the water sample. Pour the top agar mixture onto the bottom layer of a phage agar plate. Incubate plates upside down for 18-20 hr at 35-37˚C. Count the plaques the next day. Each plaque constitutes one phage.

  - **Growth of large volumes of bacteriophage for tracer studies**

    Bacteriophage suspensions can be produced growing a confluent layer of host bacteria and bacteriophage on the agar mixture given above. Twenty plates produce approximately 10^{13} pfu/ml .The confluent layers on the Petri dishes are scraped off and re-suspend in 20 ml suitable phage buffer by gentle vortexing. Centrifuging at 4 000 g for 20 minutes separates the bacteriophage and bacteria.

The pellet of bacterial cells is discarded and the supernatant retained as it contains the bacteriophage. Centrifuging at 3 000 g for 20 minutes purifies the bacteriophage. Alternatively the suspension can be ultra-centrifuged at 25 000-30 000 g for 2 hours to pellet the bacteriophage. The supernatant is discarded and pellet re-suspended in fresh phage buffer or PBS. Store the bacteriophage suspension in the fridge 2-5ºC *c.*1 week until required for tracer tests. Run bacteriophage counts on a weekly basis to determine if storage has an adverse effect on bacteriophage. Serial dilutions are made to determine the exact concentration.

E. coli: *E. coli* (strain AMP-R) was grown overnight at 35°C in peptone water (ISO 6579, Merck) to a spectrophotometer reading of approximately 1.0 (or higher) was measured at 410 nm. This corresponds to an approximate *E. coli* cfu/ml count of 1.0×10^6. Four 50 ml centrifuge tubes were each filled with 50 ml of the *E. coli* containing broth and spun at 10 000 rpm for 2 minutes to pellet the bacterial cells. The supernatant was decanted and the *E. coli* cells re-suspended in 10 ml of sterile minimal PBS buffer. The resulting *E. coli* containing buffers were pooled, and spectrophotometer readings taken at 410 nm. Serial dilution plating was used to confirm *E. coli* cfu/ml counts. The pooled solution was diluted in minimal PBS to meet the desired final concentration for injection into the sand columns (20 ml of a 5.0E+08 cfu/ml *E. coli* solution for a sand column with 300 ml pore volume.)

UNIVERSITY of the
WESTERN CAPE

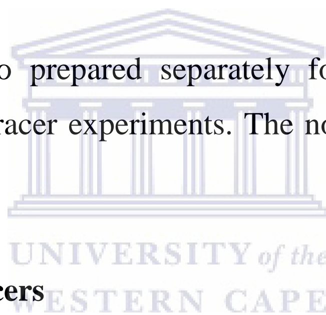
Culture method: This uses an MFC or EC agar that are selective agars for the growth of *E. coli* and other coliform bacteria. The agar was prepared according to the manufacturer' cooled to approximately 45°C and 25 mg/l Ampicillin, re-suspended in 2 ml of sterile distilled water, was added, and mixed well before pouring plates. The streak plate method was used to enumerate numbers of *E. coli* present in the water samples (0.1 ml of a suitable dilution of bacterial suspension was streaked out on a Petri dish)

Colilert® method: This method is based on the Defined Substrate Methodology (DST) for simultaneous detection of coliforms and *E. coli* with no need for confirmation. DST utilizes two indicator substrates, o-nitro-phenyl-β-D-

galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG), which are combined to simultaneously detect total coliforms and *E. coli.* Total coliforms produce the enzyme β-galactosidase that hydrolyses ONPG and thereby releases o-nitrophenol that produces a yellow color. *E. coli* produces the enzyme β-glucoronidase that hydrolyses MUG to form a fluorescent compound (Olsen et al. 1991).

The method uses a Quanti-Trays® sealer to seal Quanti-Trays that contain 51 small wells in which *E. coli* colonies grow if present. The fluorescent compound is visualized using an ultra violet lamp. The levels (Most Probable Number (MPN), counts/100 mℓ) of *E. coli* in the original sample were obtained from a table using the number of positive wells in the Quanti-Tray after 18 hours incubation at 37ºC.

Non-biological tracers were also prepared separately for these experiments and ran in conjunction with the biological tracer experiments. The non-biological tracers included Salt (NaCl); and Rhodamine.



3.1.5.1 Salt and Rhodamine tracers

The salt solution consisted of 200 ml water and 2 g NaCl with an initial specific conductance of 13 700 μS/cm. Samples were taken at a five minute intervals and with each sample taken the containers were rinsed with distilled water. A specific conductance measurement and a temperature measurement were taken for each sample. The heads remained constant to ensure the inflow and outflow was constant throughout the test. All instruments used were calibrated before the experiment.

Salt and Rhodamine WT tracer experiments were conducted after the bacteria experiments were completed to estimate the porosity and hydrodynamic dispersion in the packed columns. A salt concentration was selected to enable high enough concentrations for accurate measurement of the tracer break through curve, but also not too high to cause density driven flow conditions. The concentration of salt depended on the background value of the groundwater used and was chosen to give a peak of at least 50 per cent higher than background values.

3.1.5.2 Biological tracers

One bacteriophage, PRD-1, American Type Culture Collection (ATCC) no. 23564B, and its bacterial host *Salmonella thyphimurium*, ATCC 23564B and one bacterium *E. coli* resistant to Ampicillin were used as biological tracers for the laboratory and field experiments. The PRD-1 and *E. coli* cultures were cultured and supplied by our co-workers at CSIR.

- The principle is to mix a small volume of semi-solid nutrient medium with the water sample to be tested. A culture host strain is added and the mixture is plated on a solid nutrient medium. If any phages are present in the test sample they will enter the host strain, increase in number inside the host cell until the host cell bursts and thereby release the phage to infect more host cells.
- The *E. coli* used for the experiments is resistant to the antibiotic Ampicillin. This resistance provides an additional selective characteristic that is useful when one wants to differentiate one *E. coli* from others that may occur in one's water sample.

3.1.6 Sample injection procedure

For bacteriophage tracer injection a suspension of bacteriophage PRD1 particles with approximately 3 x 10^{14} plague forming units (PFU) was prepared as described by the International Organization for Standardization (ISO 10705-4:1995 (E) using the bacteriophage's host, *Salmonella typhimurium*. Tracer injection was carried out as follows: Water was pumped from the aquifer from a depth of 0.5 meter below the water table. It was mixed with the different tracers above surface and re-injected into borehole UWC6 at a depth of 70 meter below surface.

E. coli K12 with was used for the tracer experiments. To differentiate between possible natural or introduced *E. coli* in the groundwater *E. coli* K12, resistant to ampicillin, was additionally used as bacterial tracer.

3.1.6.1 Tracer preparation for column experiment:

Four millilitres of the stock tracer were added to 396 ml of sterilized borehole water (boiled or autoclaved). A volume of 300 ml of the above solution was used to load the column (300 ml of this tracer containing solution were let through the column) followed by another 300 ml sterilized borehole water. A control sample (100 ml of the prepared tracer solution) was sent

UNIVERSITY of the
WESTERN CAPE

back to the CSIR for analyses. The results of this sample constituted the true concentration of bacteriophage injected into the column.

3.1.6.2 PDR-1

The PRD-1 consisted of $2.4x10^6$ phage particle in 10 ml PBS. This was injected at the distribution manifold using a sterilized syringe. The 10 ml phage suspension was injected slowly into the manifold to not disturb the flow field. The injection time for this volume is less than a minute. The same injection procedure was followed in both column A and B. Experiments A and B was run on consecutive days.

A 50 ml volume sample was taken at the outlet just before injection and at 30 min intervals thereafter. These samples took about 4 min to collect. All tubes were clearly marked and stored in a fridge that was set at 10°C. The column experiments were completed and samples couriered within 3 days after receiving the phage. Courier samples was packed in a cooler with frozen dry ice blocks and delivered to CSIR with overnight delivery.

At the CSIR each sample that was received was serially diluted in sterile saline to 10^{-4} and all dilutions were plated on phage agar plates prepared as described in Section 4.3.5.

UNIVERSITY of the
WESTERN CAPE

3.1.6.3 E. coli

The *E. coli* injection solution differed between the different experimental sets. Injection took place into the distribution manifold using a syringe. The *E. coli* suspension was injected slowly into the manifold to not disturb the flow field. The injection time is about 20 ml per minute. Large volumes of tracer therefore took a bit longer to inject. Columns A and B were run at the same time where possible, alternatively they were run on consecutive days. 50 ml volumes sample was taken at the outlet immediately before injection and at 30 min intervals during the first 6 hours and hourly from 6 to 24 hours. The tube was clearly marked and stored in a cold room at 10°C until shipment to Pretoria. Courier samples was packed in a cooler with frozen dry ice blocks and delivered to CSIR with overnight delivery.

At the CSIR the sample in each tube were serially diluted to 10^{-4} and plated on MFC or EC agar or analyzed using Colilert.

3.1.6.4 Sampling frequency

During the experiment a 15 ml sample was collected every hour for twelve hours.

3.1.7 Interpretation of laboratory results

Different hydraulic gradients were used to establish the most convenient in respect of obtaining usable sample volumes at suitable time intervals. The hydraulic conductivity of the sand varies between 0.5 and 16 m/d, depending on the compaction method used. To ensure that justifiable comparisons can be made between experiments, the standard leach packing method was used at all times. The column design was improved to the point that flow remained satisfactorily constant for extended times. Tracer peaks appeared in the effluent about an hour after injection. The sharper the peak the more homogenous is the flow and the smaller the effects of dispersion, advection and adsorption. The results from laboratory testing informed the field studies.

Field studies

The field design phase of the experiment was an up-scaling of the laboratory phase of this project. The amount injected into the aquifer was increased in proportion to the size of the research site. Tracer tests using chemical and microbial tracers were carried out simultaneously.

UNIVERSITY of the
WESTERN CAPE

3.1.8 Hydraulic test / conditions

Preliminary hydraulic tests were done to ensure a stable and consistent flow rate of 1 L/s was obtained. The abstraction borehole (UWC4) was pumped two days prior to tracers being injected to allow a steady state flow field and the flow rate was determined using a bucket test. The difference in water level between the injection borehole and abstraction borehole was measured on weekly intervals to check possible changes in the flow gradient. No unexpected changes occurred during any of the tracer tests. No significant rainfall or flood events occurred during the individual tracer experiments. Previous work has been conducted using these boreholes by other student (October, 2009, Pers. comm. J.Nel, UWC).

3.1.9 Tracer tests

Two field experiments were performed on two different injection boreholes at two different occasions. Borehole UWC3A was injected using the PRD-1 bacteriophage (10 m distance to

production well) and the *E. coli* bacterium was injected at borehole UWC7 (2 m distance to production well). The biological tracer tests were combined with chemical tracer tests to determine the aquifer properties and prove connectivity between the boreholes seen in Table 4-1.

Table 0-1: Field biological tracer experiments performed

Experiment	Tracers used	Date
PRF-01	PRD-1 NaCl Rhodamine WT	16 May to 8 June 2010
ECF-01	*E. coli* Rhodamine WT	24 August to 9 September 2010

3.1.10 Biological and chemical tracer preparation

E. coli K12 and *E. coli* resistant to Ampicillin was grown overnight at 35±2°C in peptone water (ISO 6579, Merck) or nutrient broth. The cell concentration was determined spectrophotometrically. A spectrophotometer reading of approximately 1.0 measured at 411 nm wavelength corresponded to an approximate 10^6 CFU/mL. Two hundred millilitre of the broth were centrifuged at 10 000 rpm for 4 min to pellet the cells. The supernatant was decanted and the cells resuspended in 10 mL of a 1:10 dilution sterile minimal PBS buffer to give a 10^8 CFU/mL.

UNIVERSITY of the
WESTERN CAPE

The *E. coli* tracer (2 000 mL) prepared as described above was supplied for injection in 2 x 1 L Schott bottles, at a concentration of approximately 1.0E+07 CFU/mL as a stock solution. The stock (2 L) was added to 10-20 L of borehole water. After mixing very well it was added into the injection borehole. A control sample (100 mL of the prepared tracer solution) was sent back to the CSIR for analysis.

This sample constituted the time zero analysis.

E. coli enumeration was done as described in Section 4.3.6.3.

3.1.11 Tracer analyses

Tracer tests conducted between boreholes UWC5 and UWC6 showed tracer breakthrough at 1 220 min after injection using forced gradient methods. Borehole UWC6 was pumped at 0.8 l/s until steady state before injection. Borehole UWC5 was used for injection and circulated between 93 and 105 m below surface while measuring the dilution of the salt tracer. The tracer tests suggest a single fracture linking borehole UWC5 and UWC6 (October, 2009, Pers. comm. J. Nel, UWC).

Salt dilution tests on borehole UWC4 showed a natural gradient volumetric flow rate of 2.5671 m^3/day through the screened section (Peterson, 2008). No link between borehole UWC2 and borehole UWC4 could be achieved using forced gradient tracer tests (October, 2009, Pers. comm. J. Nel, UWC).

a) Salt and Rhodamine

Since there was no background concentration of Rhodamine in the groundwater the input concentration was chosen so that the breakthrough concentration was less than 150 µg. Rhodamine was used from a concentrated powder form. Aquifer tracer tests required about 5 g of Rhodamine WT mixed with 20 L of aquifer water before injection. Tracer test repeats were done after a thorough pump out and aquifer cleaning of at least one week.

UNIVERSITY of the
WESTERN CAPE

3.1.11.1 PRD-1 Phage experiment

a) Experiment PRF-01

Approximately 2.16 X 10^8 PRD-1 phage particles were injected into UWC3A injection borehole. The tracer combination of PDR-1, NaCl and Rhodamine was injected using a 25 mm pipe that is inserted into the injection borehole. The tracer mixture is injected into the pipe using a funnel. The water in the pipe is then displaced downwards by the tracer mixture. When the pipe is removed from borehole the tracer is released and the time is documented as the start time. Samples were taken over 39 days from the sampling borehole UWC4 10 m away displayed in Table 4-3. A control sample (100 ml of the prepared tracer solution) was sent back to the CSIR for analysis. They were initially taken twice daily with less frequent sampling in the latter stages of the experiment. Samples were collected at the pump from a ball valve installed for sampling. Samples are stored in a refrigerator at 10ºC until couriered to CSIR for analysis.

3.1.11.2 *E. coli* experiment

a) ECF-01

The *E. coli* tracer (2 000 ml) was supplied to UWC laboratory in 2 x 1 L Schott bottles, at a concentration of approximately 1.0E+07 cfu/ml as a stock solution. The stock (2 L) and 5 g of Rhodamine was added to 10 - 20 L of borehole water. After mixing very well it was deposited into the injection borehole. The same process as performed for PRF-01 was conducted for ECF-01 for consistency. A control sample (100 ml of the prepared tracer solution) was sent back to the CSIR for analysis. The results constituted the original concentration of bacteria that were injected into the column. Samples were collected at the pump from a ball valve installed for sampling. Samples are stored in a refrigerator at 10ºC until couriered to CSIR for analysis.

Table 0-2: Chemical and biological tracer preparation, injection and sampling

3.1.11.3 Sampling frequency

During both experiment a 100 ml sample was collected twice per day for the first three days and once per day thereafter for a total of 39 days for PRF-01 and a total of 22 days for ECF-01.

UNIVERSITY of the
WESTERN CAPE

Chapter 5: Data and Discussion

Introduction

A series of tests were conducted in the laboratory and the field to mimic what we should encounter during field experiments. Laboratory experiments were conducted using sterilized and unsterilized columns. The aim of the experiments is to evaluate the results and possibly implement it to protect our drinking water resources in the real world.

Column and field studies were conducted at the UWC to investigate the fate and transport of *E. coli* and bacteriophages. Hydraulic conductivity of the laboratory columns and the field site were of a similar magnitude, with the field site varying from 1 to 20 m/day whereas the laboratory values varied from about 0.4 to 9 m/day.

Data and discussion

4.1.1 Column experiments using bacteriophage PDR-1 and *E. coli*

Four sets of data were generated. Two columns were prepared for the experiments, A and B. The columns were either run simultaneously or on consecutive days. One experiment was undertaken using PRD1 (Experiment PRL-01, and three experiments were undertaken using *E. coli* (Experiments ECL-01, ECL-02 and ECL-03). Table 5-1 lists the experiments performed.

UNIVERSITY of the
WESTERN CAPE

Table 0-1: Laboratory biological tracer experiments performed

Experiment Name	Tracer used	Date	Columns
PRL-01	PRD-1	15 to 16 May 2010	Sterilized
ECL-01	*E. coli*	23 to 24 August 2010	Sterilized
ECL-02	*E. coli*	05 October 2010	Sterilized
ECL-03	*E. coli*	25 February 2011	Unsterilized

4.1.1.1 Experiment PRL-01

A column experiment was performed in duplicate (two columns A and B prepared in the same way, inoculated with the same tracer amounts and run on consecutive days) using bacteriophage PRD-1 as a tracer in sterilized columns (autoclaved and flushed with ethanol).

Approximately $2.4x10^6$ PRD-1 phage particles were injected into each column (suspended in 10 ml phosphate buffer). The phage tracer was injected into the manifold of the flowing column experiment and allowed to run for 12 hours. Some column parameters are presented in Table 5-2.

Table 0-2: Column parameter values for experiment PRL-01

Parameter	Value (Col A)	Value (Col B)	Unit
Flow	12.5	16.7	ml/min
Length of sand column	20	20	cm
Area of sand column	70.9	70.9	cm^2
Phage particles added	$2.4x10^6$	$2.4x10^6$	pfu
Phage particles added	10	10	ml
Specific conductance of salt tracer	13 700	13 700	µS/cm
Volume of salt tracer injected	5	5	ml
Temperature range	19.5 to 20.4	19.2 to 19.4	ºC

UNIVERSITY of the
WESTERN CAPE

The results are shown in Figure 5-1.

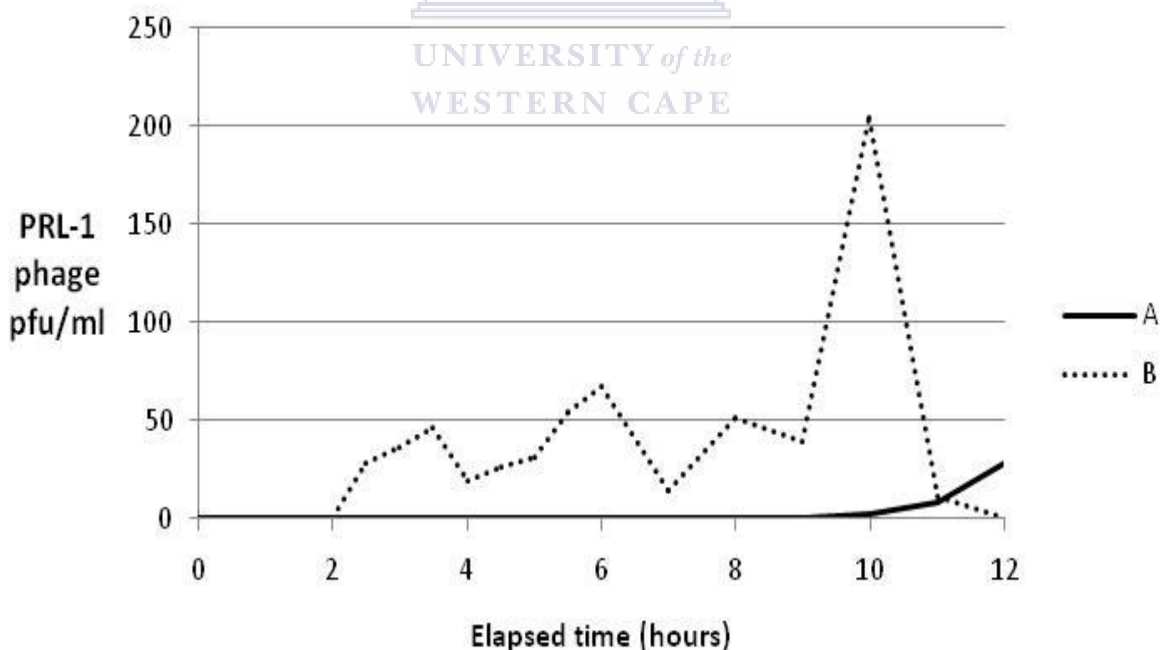
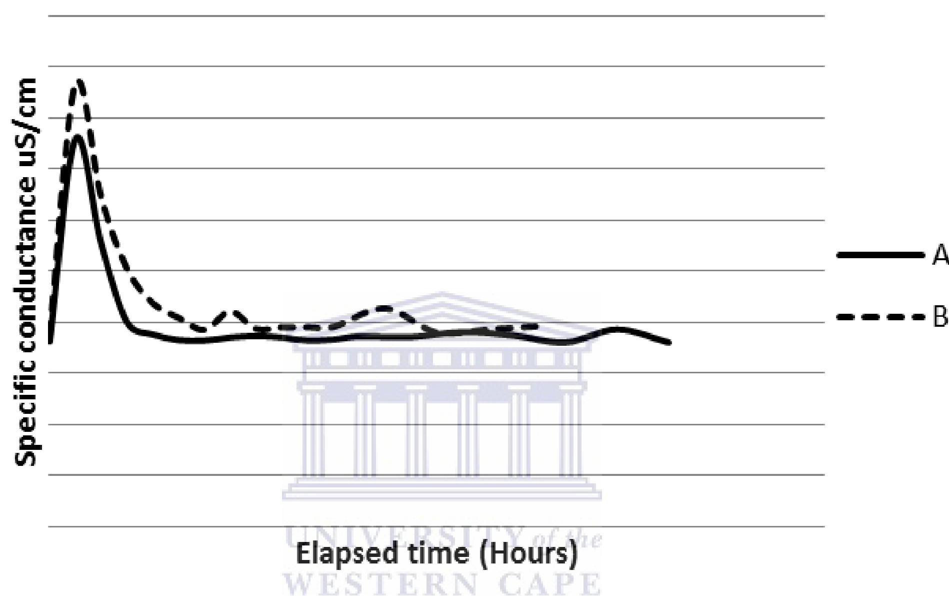


Figure 0-1: Laboratory biological tracer PRD-1 phage (pfu/ml) results – sterilized (Expt. PRL-01)

A tracer arrival is apparent at 2 hours in experiment B with the counts peaking at 10 hours. However, the phage was only detected in experiment A after 10 hours with the peak probably

occurring after 12 hours. The faster breakthrough time for experiment B may have been due to the faster flow rate (Figure 5-1). Both columns removed more bacteriophage tracer than initially anticipated. It is unclear why Experiment A reduced tracer concentrations to such low levels compared to column B.

The salt buffer of the bacteriophage buffer acted as a salt tracer and was therefore introduced to the columns at the same time as the microbial tracer. Figure 5-2 shows the results.



UNIVERSITY of the
WESTERN CAPE

Figure 0-2: **Laboratory salt tracer (µS/cm) results (Expt. PRL-01)**

The salt tracer breakthroughs for both columns are similar and occurred between 1 and 2 hours. The fact that the salt trace results indicate that the two columns are behaving similarly does not give any insight was why the microbial tracers apparently behaved so differently in the two columns. For future experiments it is recommended that higher loads of bacteriophage tracers should be injected and the test should also run for a longer time period. Samples should also be taken more frequently than in these experiments.

4.1.2 *E. coli* experiments with sterilized columns

These were again conducted with two duplicate columns as above. The packed columns were sterilized by flushing with ethanol. The sand used for packing as well as water used for flow experiment was autoclaved prior to the experiment.

4.1.2.1 Data for experiment ECL-01

4 ml of a tracer containing solution ($6x10^6$ cfu/ml) was added to 396 ml of sterilized borehole water, giving in effect a 100 times dilution. 300 ml of this tracer mixture was used to load the column reservoir. The tracer mixture was injected into the manifold of the flowing column experiment.

The volume injected provided a tracer injection period of approximately 14 minutes. 100 ml of the tracer mixture was kept as a control to account for tracer die-off/inactivation rates. 15 ml samples were taken half-hourly for the first six hours of each column experiment. Thereafter, samples were collected hourly up till 12 hours and 4 hourly up till 24 hours. This column was allowed to run for 24 hours, during which 24 samples were taken. Some column parameters are presented in Table 5-3.

Table 0-3: Column parameter values for experiment ECL-01

Parameter	Value	Unit
Flow	21.7	ml/min
Length of sand column	20	cm
Area of sand column	70.9	cm^2
Bacterial cells added	$1.8x10^7$	cfu
Volume of bacteria suspension tracer	300	ml
Specific conductance of salt tracer	19 800	µS/cm
Volume of salt tracer	5	ml

UNIVERSITY of the
WESTERN CAPE

The counts obtained are shown in Figure 5-3.

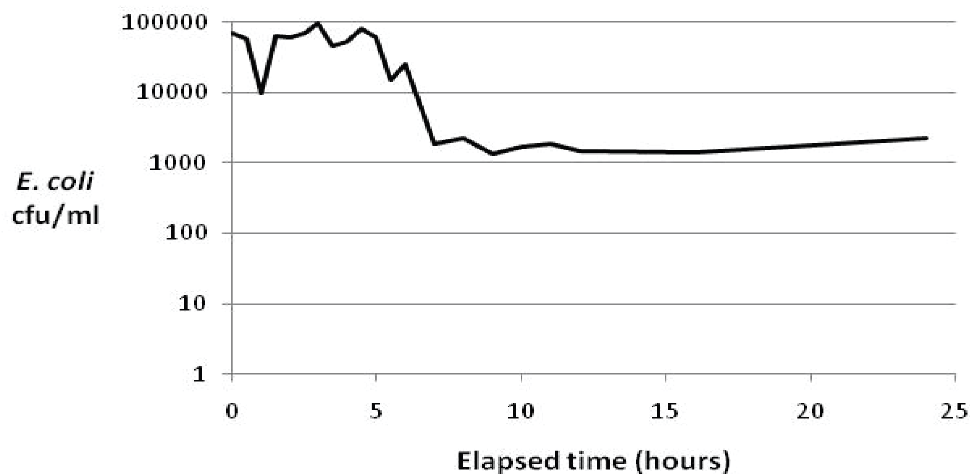
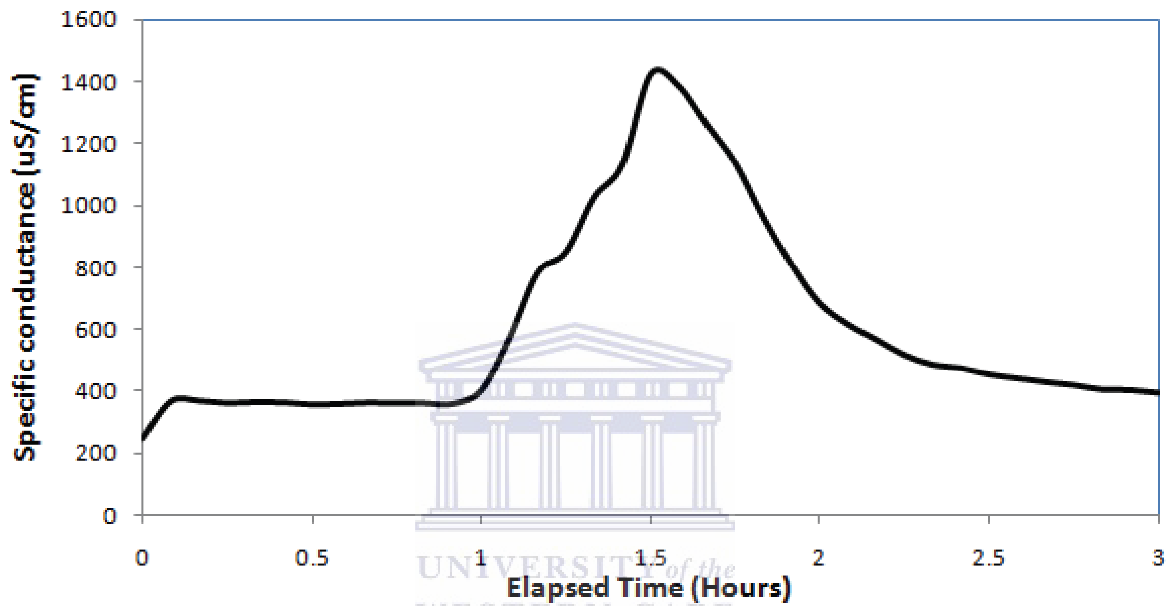


Figure 5-3: Laboratory biological tracer *E. coli* (cfu/ml results (Expt. ECL-01)

The first sample was taken at time zero (along with tracer inoculation). This sample had a very high colony count on MFC agar. The counts of the presumptive *E. coli* remained high for some hours. Unfortunately, the *E. coli* and non-*E. coli* bacteria could not be distinguished based on color and morphology.

The salt tracer was again injected as part of the *E. coli* suspension. Samples for the salt tracer were collected at 5 min intervals. The salt tracer results are shown in Figure 5-4.



UNIVERSITY of the
WESTERN CAPE

Figure 0-3: **Laboratory salt tracer (µS/cm) results (Expt. ECL-01)**

The salt tracer showed the tracer breakthrough occurring between 1 and 2 hours, similar to the PRL-01 experiment. The more frequent sampling interval provided better resolution on the shape of the tracer breakthrough graph, with good control on the tracer arrival and tail. These results also suggest that the column (or water used in the experiment) was in fact contaminated with bacteria that grow well on MFC plates, indicating that the sterilization was not completely effective. These non-*E coli* background (contaminant) bacteria masked the actual levels of *E. coli* tracer.

4.1.2.2 Data for experiment ECL-02

In the above experiment (ECL-01) the samples contained contaminant bacteria that could not be distinguished from the *E. coli* tracer used using an MFC agar plate method. In an attempt to counter this problem, an *E. coli* strain obtained from the Colorado State University (USA) was used. This strain has been engineered with resistance to Ampicillin (an antibiotic). Ampicillin was added to the MFC agar plates to inhibit the growth of bacteria that do not have ampicillin resistance, like the background contaminant bacteria. Some column parameters are presented in Table 5-4.

Table 0-4: Column parameter values for experiment ECL-02 (*E. coli* measured using MFC agar)

Parameter	Value (Col. A)	Value (Col. B)	Unit
Flow	16.13	12.25	ml/min
Length of sand column	20	20	cm
Area of sand column	70.9	70.9	cm^2
Bacterial cells added	$8.4x10^6$	$8.4x10^6$	cfu
Volume of bacteria suspension injected	45	45	ml
Specific conductance of salt tracer	19 760	19 760	µS/cm
Volume of salt tracer	45	45	ml
Temperature range	20.8-22.3	20.3-21.6	ºC

UNIVERSITY of the
WESTERN CAPE

Figure 5-4 shows the results of the *E. coli* (i.e. those with resistance to ampicillin) counts for the experiments which ran for 49 hours. For column B analyses were only performed for the first 8 hours.

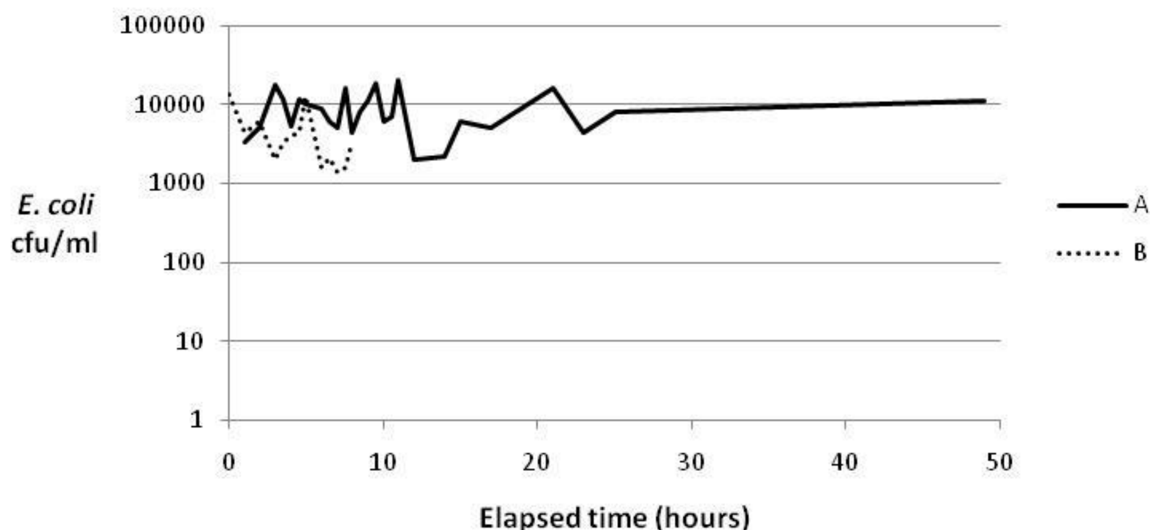
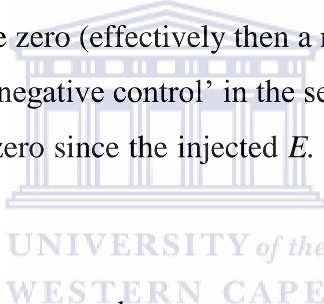


Figure 0-4: **Laboratory biological tracer *E. coli* (cfu/ml) results - with ampicillin (Expt. ECL-02)**

The same pattern was observed in this experiment as in ECL-01. High counts of bacterial colonies were measured from time zero (effectively then a negative control) to 49 hours. (The measured count at time zero is a ‘negative control’ in the sense that had sterilization been 100 per cent effective, this would be zero since the injected *E. coli* could not have had time to be eluted).


UNIVERSITY of the
WESTERN CAPE

It is apparent that the sterilization procedures were not effective. Antibiotic resistance in bacteria is widespread and it is evident from these results that the contaminant bacteria were also, to some extent, resistant to the inhibitory effect of the ampicillin, creating difficulties in detecting *E. coli*.

Colony counts on MFC agar were chosen as the analytical method because of the relatively low cost of this method for the detection and enumeration of *E. coli*. However, the presence of bacterial contaminants in the columns that resemble *E. coli* on MFC agar was not foreseen. The main disadvantage of using this detection method for *E. coli* is that it relies on a color reaction (forming blue colonies) that is not exclusive to *E. coli*. Interpretation is also subjective and brand-to-brand differences in performance of MFC agar are well known.

To address these shortcomings the detection method was changed to the Colilert method. Although much more expensive, this method better discriminates between *E. coli* and closely

related organisms. The samples obtained during experiment ECL-02 were re-analyzed using the Colilert method. The results can be seen in Figure 5-5.

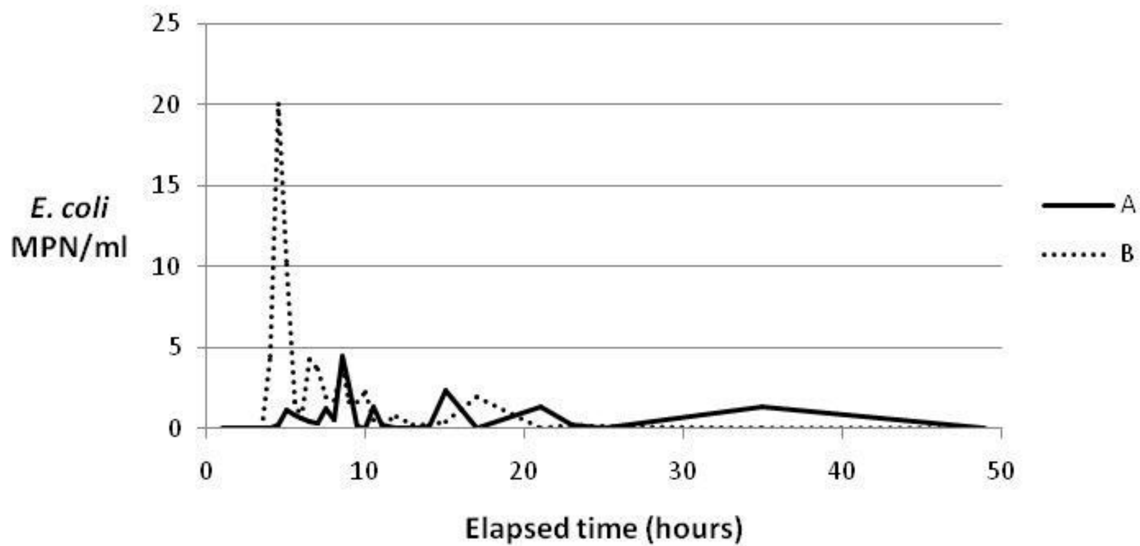


Figure 0-5: Laboratory biological tracer *E. coli* (MPN/ml) results - with ampicillin (Expt. ECL-02)

The Colilert method provided useable results. For the first time, a breakthrough of the *E. coli* tracer could be observed at 4 hours in column B. However, the *E. coli* counts were fairly low indicating that the column effectively removed most of the *E. coli* tracer originally injected. Column A in particular required higher loads of tracer to provide accurate data. In column B the *E. coli* counts apparently peaked at 4.5 hours. However, future experiments will need to confirm whether or not this is a real effect because the two relatively high values responsible for this (20 and 10 MPN/ml) may not be representative.

The results of the salt tracer are shown in Figure 5-6**.**

UNIVERSITY of the
WESTERN CAPE

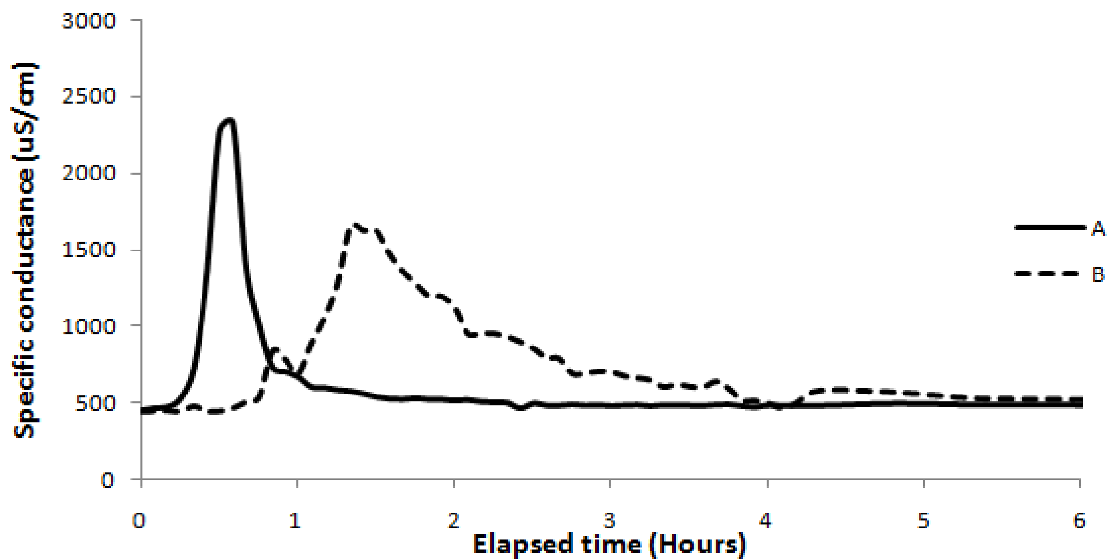


Figure 0-6: **Laboratory salt tracer (µS/cm) results (Expt. ECL-02)**

UNIVERSITY of the
WESTERN CAPE

Breakthrough of the salt tracer in column A occurred much earlier than found in previous experiments. The previous column tests, (PRL-01 and ECL-01) all showed tracer breakthrough between 1 and 2 hours after injection. Column B on the other hand shows a breakthrough just before 1 hour with a tracer tail extending between 3 and even 4 hours. This is also different than the results obtained from the previous studies. While the flow rate through these columns is comparable, the tracer tests show large differences in porosity. These differences can possibly be attributed to differences in sand structure or moisture content resulting in different packing density.

The salt tracer in column A is occurring in under an hour while the *E. coli* is only breaking through after 4 hours. The respective breakthrough and peak salt elution times are some three times longer in column B than in column A.

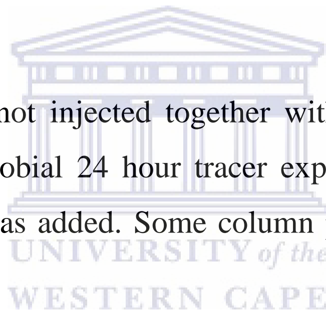
The results conclude that the Colilert method is much more effective for distinguishing *E. coli* from contaminant non-*E. coli*. The sand in the column is also apparently very effective at immobilizing *E. coli*. The *E. coli* are apparently moving about four times slower through the columns than the salt.

4.1.2.3 Experiments with uunsterilized columns and water

a) Data for *E. coli* experiments ECL-03

The aim with experiments ECL-01 and ECL-02 was to provide *E. coli* inactivation data in columns that were devoid of other bacteria and living components. However, those experiments indicated that the sterilization procedures were not completely effective, resulting in only partial removal of natural biota present in the source material. Experiments were necessary that could show the effect of this biota of the Cape Flats sand and water on biological tracer removal.

Accordingly, experiment ECL-03 was performed (again in duplicate columns A and B) without sterilizing the columns and water. The columns included the full natural component of the biota present in the Cape Flats aquifer. As in previous experiments the ampicillin resistant strain of *E. coli* was utilized as the biological tracer, and the counts obtained by means of the Colilert method.


UNIVERSITY of the
WESTERN CAPE

The salt tracer in ECL-03 was not injected together with the microbial tracer, but rather injected one day later. The microbial 24 hour tracer experiment was therefore allowed to complete before the salt tracer was added. Some column parameters are presented in Table 0-5.

Table 0-5: Column parameter values for experiment ECL-03 (*E. coli* measured using Colilert)

Parameter	Value (Col. A)	Value (Col. B)	Unit
Flow	5	10	ml/min
Length of sand column	20	20	cm
Area of sand column	70.9	70.9	cm^2
Bacterial cells added	$8.6x10^9$	$8.6x10^9$	cfu
Volume of bacteria suspension injected	45	45	ml
Specific conductance of salt tracer	19 760	19 760	µS/cm
Volume of salt tracer	45	45	ml
Temperature range	20.7-22.3	20.8-22.3	°C

Results are shown in Figure 5-7.

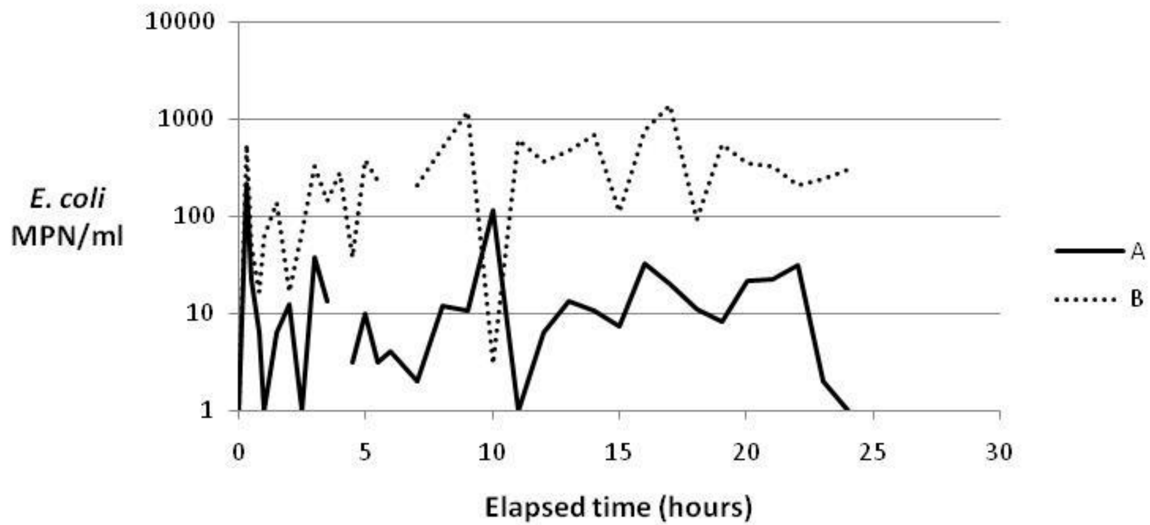


Figure 0-7: Laboratory biological tracer *E. coli* (MPN/ml) results – unsterilized (Expt. ECL-03)

The generally higher values in Figure 5-7 compared with Figure 5-8 are likely due to the higher number of cells added initially.

UNIVERSITY of the
WESTERN CAPE

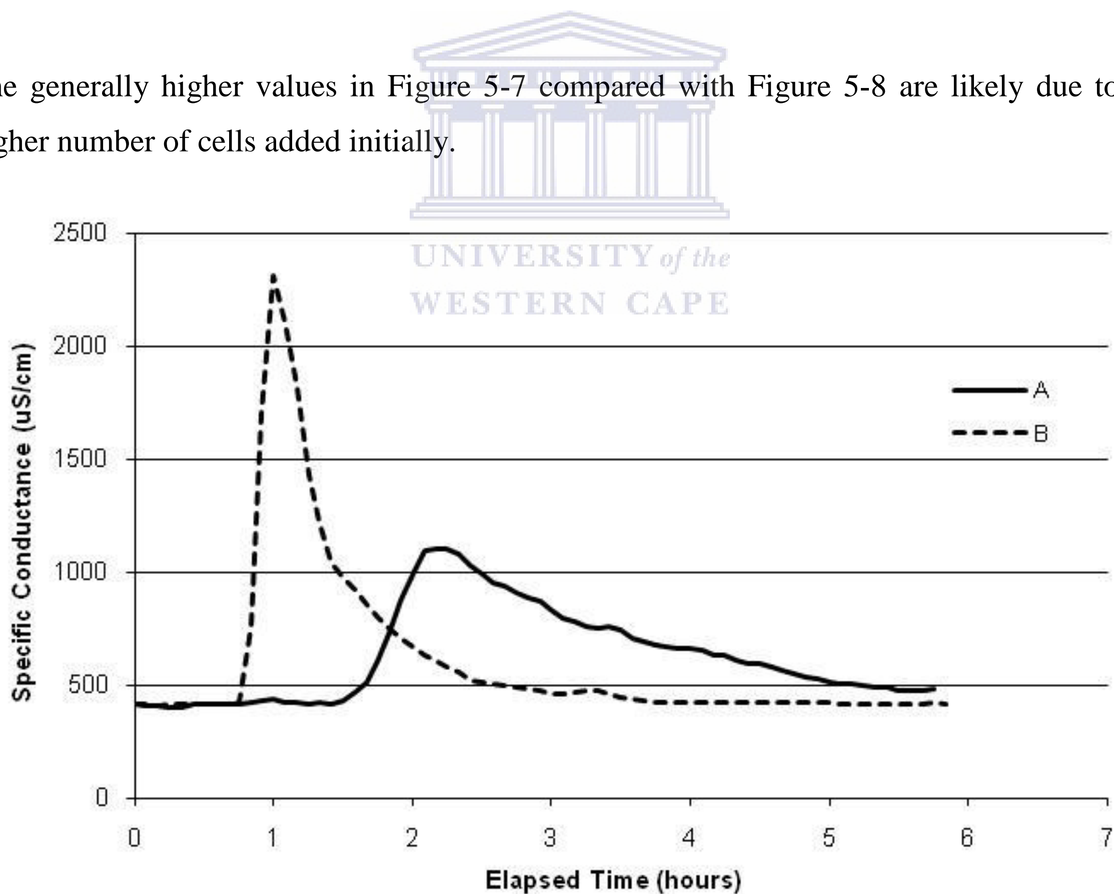


Figure 0-8: Laboratory salt tracer (µS/cm) results (Expt. ECL-03)

As in the previous experiment (ECL-02), the respective breakthrough and peak salt elution times are somewhat different in the two columns (by a factor of about two in this case). Colum B shows the salt tracer breakthrough between 1 and 2 hours, as expected. Colum A however shows a delay in tracer breakthrough at about 2 hours. The flow rate of column A was half the flow rate of column B and can explain the slower breakthrough. The difference in flow rate can only be explained by the heterogeneity of the sand with possible differences in packing density in the column.

Figure 5-7 shows that *E. coli* was detected almost immediately (even sooner than the breakthrough of salt). This suggests that the natural biota contain *E. coli*. It is not clear why this might be, particularly as this was apparent in the previous experiment (ECL-02) (Figure 5-5).

4.1.3 Interpretation of laboratory experiments

Seven different column experiments were conducted. The flow rates varied between 5 and 20 ml/min, providing hydraulic conductivity of between 0.4 and 9 m/day for the sand columns as seen in Table 5-6. This hydraulic conductivity range is similar to the observed hydraulic conductivities for the field site which varies between 1 and 20 m/d for the different boreholes.

UNIVERSITY of the
WESTERN CAPE

Table 0-6: Laboratory experiment flow parameters and hydraulic conductivity

Column Name	Flow (ml/min)	Length (cm)	Area (cm^2)	Head difference (cm)	Hydraulic Conductivity (m/day)
PRL-01 Col A	12.5	20	70.9	10	5.1
PRL-02 Col B	16.7	20	70.9	10	6.8
ECL-01	21.7	20	70.9	10	8.8
ECL-02 Col A	16.13	20	70.9	30	2.2
ECL-02 Col B	12.25	20	70.9	10	5.0
ECL-03 Col A	5	20	70.9	47	0.4
ECL-03 Col B	10	20	70.9	47	0.9

Salt tracer breakthrough mostly varied between 1 and 2 hours, indicating similar porosity for the packed columns. Some showed slightly faster velocity, while others showed a slightly slower velocity. Most experiments showed a slight tail indicating different length or different velocity flow paths.

Table 0-7: Laboratory porosity estimates for column tracer tests

Column Name	Darcy velocity (cm/min)	Salt Tracer Peak (min)	Microbe Tracer Peak (min)	Microbe Tracer Peak (min)	Velocity from salt peak (cm/min)	Porosity Estimate
PRL-01 Col A	0.176	30	>12h	>720	0.667	0.264
PRL-02 Col B	0.236	30	450	450	0.667	0.353
ECL-01	0.306	90	240	240	0.222	1.377
ECL-02 Col A	0.228	35			0.575	0.396
ECL-02 Col B	0.173	85	300	300	0.236	0.731
ECL-03 Col A	0.071	130			0.154	0.458
ECL-03 Col B	0.141	60			0.333	0.423

The results from microbial tracers on the columns suggest microbial breakthrough times of between 3 and 10 times slower than the salt tracers. Also very high removal efficiency is observed for the microbial tracers.

Several mathematical models have been proposed to describe bacterial transport through groundwater. All of the models are based on the traditional advection-dispersion equation of solute transport. Some of the existing mathematical models of bacterial transport are too complex to be used in practice. Other simpler phenomenological models, such as the one incorporating a kinetic sorption process, have been successfully used to reproduce experimental data. However, these phenomenological models were simply calibrated to experimental data; no attempt has been made to relate the transport parameters to sediment properties. In order to increase the predictive capability of the mathematical models, it is very important to quantify such relationships.

4.1.4 Field experiment

The field experiments were intended to present up-scaling of the laboratory experiments. The amount of tracer injected into the aquifer was increased in proportion to the size of the research site.

4.1.4.1 PDR-1 experiments (PRFF-01)

The results for field experiment (PRF-01) conducted are shown in Figure 5-9.

UNIVERSITY of the
WESTERN CAPE

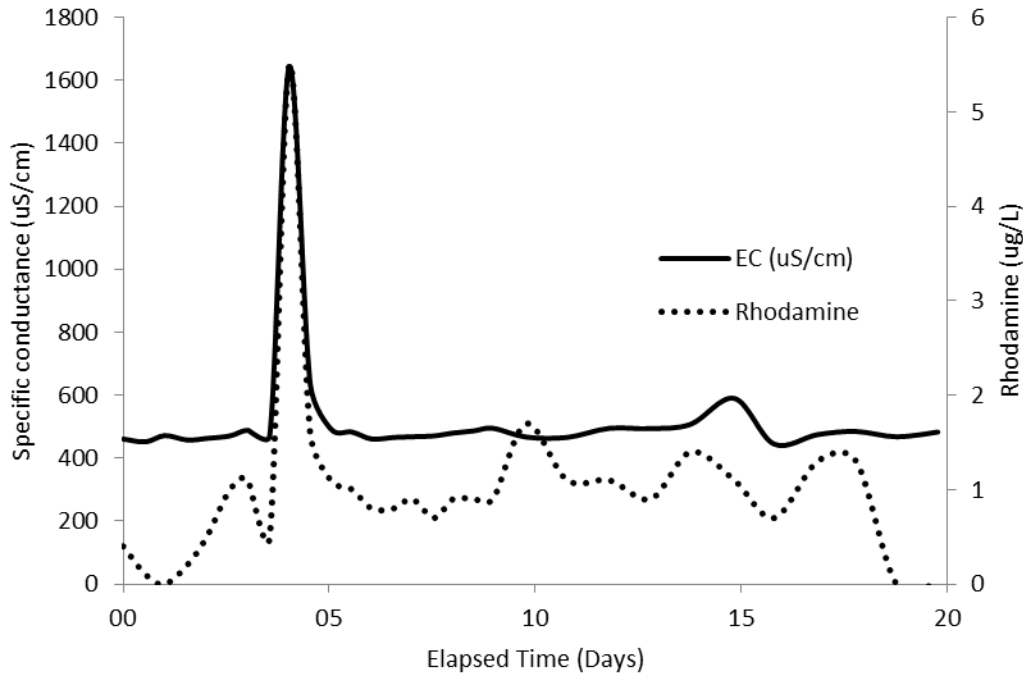


Figure 0-9: Field salt tracer EC (µS/cm) and Rhodamine (µg/L) results – (Expt. PRF-01)

UNIVERSITY of the
WESTERN CAPE

Approximately $2.6x10^8$ PDR 1 phage particles were injected into the UWC3A injection borehole. Figure 5-9 shows that Rhodamine and salt tracers broke through after about 3 days, with tails extending just past 5 days. With a limit of detection for Rhodamine of around 1 µg/L, and noise in the reading caused by interference such as air bubbles, the only result of any significance is this peak between 3 and 5 days.

With the breakthrough curves of the salt and Rhodamine around 5 days – the phage could have taken up to 20 days to move through the aquifer. The second field experiment was therefore conducted over a shorter distance and similar time period.

The Rhodamine and salt tracer results show that the injection and sampling boreholes are indeed connected. More frequent sampling intervals and higher injection concentrations would have been useful to better define the curves. However, even a microorganism as small as a bacteriophage seems to be effectively immobilised within 10 m in a sandy aquifer.

Samples were taken over 39 days from the sampling borehole UWC4 10 m away. They were initially taken twice daily with less frequent sampling in the latter stages of the experiment.

4.1.4.2 *E. coli* experiments (ECF-01)

A new injection borehole was drilled only two meters from the sampling borehole. This was injected with approximately $1x10^{10}$ cfu of *E. coli* tracer.

Samples were taken over a period of 45 days, with a higher sampling frequency in the early stages of this field experiment. Figure 5-10 shows the measured electrical conductivity (EC) and *E. coli* counts using the MFC agar plate method.

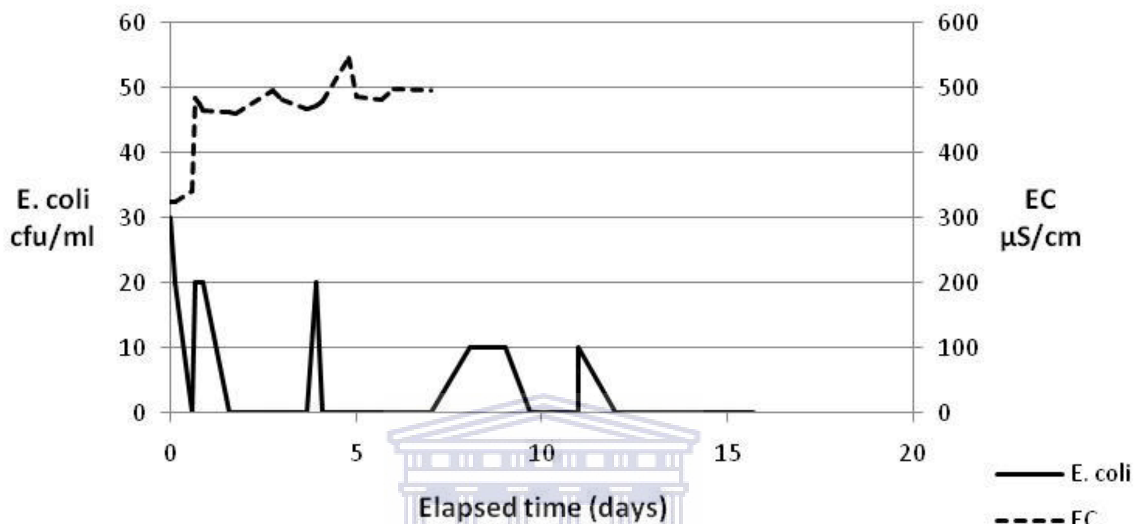


Figure 0-10: Field biological *E. coli* (cfu/ml) results – (Expt. ECF-01).

UNIVERSITY of the
WESTERN CAPE

Low CFU/mL *E. coli* values were measured from time zero (at which time no bacteria should show break through) and sporadically throughout the duration of the experiment. These are likely to be attributable to background biota (non-*E. coli* bacteria) that produced colonies of similar morphology and colour to *E. coli* on MFC agar plates (a similar occurrence was noted in the laboratory experiment ECL-01). No distinctive breakthrough was evident. The two-meter sand column effectively removed the large numbers of *E. coli* tracer injected into the aquifer.

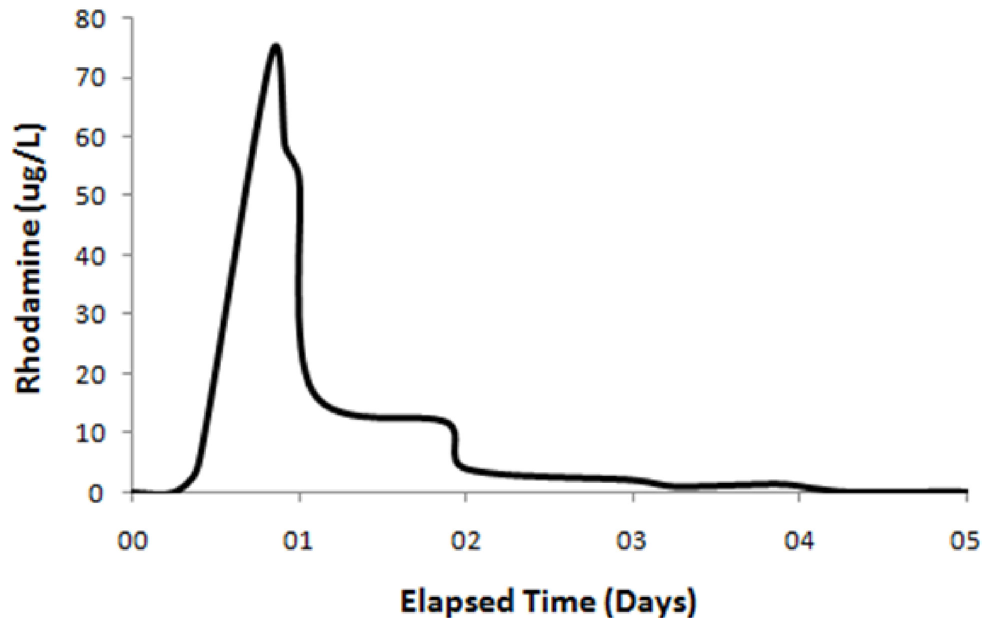


Figure 0-11: Field salt tracer Rhodamine (µg/L) results – (Expt. ECF-01)

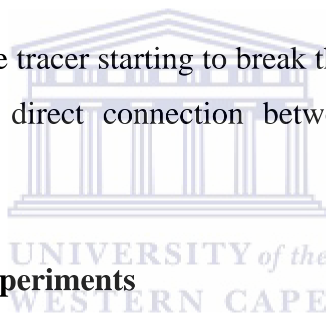

UNIVERSITY of the
WESTERN CAPE

Figure 5-11 shows the Rhodamine tracer starting to break through in less than half a day after injection, again confirming the direct connection between the injection and extraction borehole.

4.1.5 Interpretation of field experiments

A successful field chemical tracer test was conducted for both the 10m (BH3A) and 2 m (BH7) tracer tests. The salt and Rhodamine tracers showed flow rates of between 2 and 3 meters per day. Considering the slower microbial transport rates obtained in the laboratory the first microbial field experiment could possibly have been ended to soon, stopping at 20 days after injection (10 m distance). The second field test was run over 45 days with a shorter distance of 2 meters. No microbial breakthrough was observed in the either of the field experiments.

E. coli counts were obtained for the "time zero" sample when no bacteria were expected at all. Breakthrough of *E. coli* at the extraction borehole was not expected very soon after injection. These bacteria could not have been the injected bacteria but could be similar to the bacteria detected at early time for laboratory tests.

The sand aquifer at UWC effectively removed or strained/filtered most of the injected *E. coli* with irregular spikes of small numbers of *E. coli* of uncertain origin. The bacteriophages were removed totally.

Chapter 6: Conclusions and Recommendations

Insights from the study

Microbiological contaminants pose risk to human health. One conclusion is that bacteria can under some circumstances move through groundwater at rates equivalent to inorganic tracers. This is counter-intuitive but some literature evidence exists of other studies observing the same phenomenon. Upflow was used to conduct experiments in order to get the maximum saturation and prevent air from getting trapped in the columns. This raises questions about whether or not the perceived risks of micro-organisms to human health (through the water being used for drinking) might be underestimated in some circumstances. Notwithstanding this apparent movement, it is also observed that only a small fraction of the original micro-organisms move that far and that fast. This would reduce the risk.

Fate and transport of microbes are controlled and influenced by complex interaction of physical, chemical and biological factors. A brief summary indicates that the simulation and prediction of the movement of microbes through different media under different conditions is complex. A nonlinear combination of physical, biological and chemical processes affects the survival and amount of time it takes for microbes to move through an aquifer system. Importantly, microbes do not necessarily move at the same speed as the water and in the general flow direction. Due to the heterogeneous characteristics of different aquifer types and differences between similar aquifer types over short distances it is necessary to collect sufficient data to capture the heterogeneity of the aquifer systems at different scales. Field data collected at the local scale and used to parameterise a simulation model may not be representative of the larger area.

UNIVERSITY of the
WESTERN CAPE

The intention of this project was to conduct laboratory studies that would provide information for the up-scaling to field experiments. In the interim we have learnt, that column studies are intricate, lengthy and may not reproduce the field results envisioned due to column experiments being homogenous and controlled whereas field experiments are uncontrolled and heterogeneous. The column studies highlighted the fact that they might be helpful to explain single selected interactions but such findings can seldom explain the interactions of microbes and groundwater in the natural environment.

The sandy aquifers natural properties such as permeability, porosity, storativity and conductivity could not be mimicked and properties such as packing in the sand columns brought about inconsistent flow rates and variable conductivities. Natural conditions also introduce additional microbial populations which are present in any aquifer. Therefore, caution is necessary when insights from laboratory studies are up-scaled to explain what might happen in an aquifer. The field studies conducted in the sandy aquifer showed that microbes are completely retained or removed and for this particular aquifer no breakthrough of either the bacteria or the bacteriophage was recorded.

Findings and cconclusions

In the natural environment the biological tracer is exposed to different pathways, different soil composition and structures. It also interacts with the natural bacteria in its pathway. In a controlled environment biological tracers and inorganic tracers produce different results due to homogenous conditions and restrict exposure to different pathways and soil conditions and structures. The research team did not eliminate the dispersion and adsorption factors that occur beneath the surfaces. Degradation is an important element to keep in mind because no observations can be made beneath the surface, so assumptions are that the tracer degrades in a natural environment.

UNIVERSITY of the
WESTERN CAPE

5.1.1 Factors affecting transport and fate of micro-organisms in groundwater systems

Micro-organisms will be the dominant forms of life and, in most cases; they will be the only forms of life present in aquifers (Schijven, 2001). According to existing literature various studies (Harvey et al. 2002) have been conducted on the fate and transport of microbes in groundwater systems, some experiments investigated specific properties such as adsorption, inactivation, temperature, straining and dispersion. Groundwater is often at risk of faecal contamination (Ryan et al. 2002). These studies were targeted at understanding the aquifer system to prevent human health issues in existing groundwater resources (Schijven, 2001).

5.1.2 Microbial contaminants of concern to identify suitable microbial tracers

Bacteria and viruses were identified in experiments to identify contaminants of concern. Microbes have been introduced into groundwater systems to better understand hydrologic properties of aquifers (Levy et al.'s 2007). Bacteriophages commonly used as tracers in

groundwater applications are coliphages which has *E. coli* as their host. *E. coli* K12 was used due to its ability to change morphologically by decreasing in cell volume and releasing of proteins once it encounters water and the fact that this strain does not pose a risk to human's health (Schijven, 2001). *Salmonella typhimurium* is the primary host for the strain of PDR1 which also does not pose a human health risk and is well understood as a widely used conservative tracer across the world (Harvey et al. 2002). These coliphages are easily detected and usually produce good survival rates in groundwater resources (Schijven, 2001).

5.1.3 Testing the transport and fate of selected chemicals and micro-organisms

Through conducting microbial tracer tests, valuable information has been collected and the information could then be used as a useful tool for delineating the likelihood of microbial transport pathways from known sources of contamination and can provide a better delineation of microbe movement in different types of media. Microbial fate and transport through laboratory columns tests were investigated with the goal of better understanding sediment characteristics from the primary sandy aquifer. However, simulated aquifer conditions did not give the desired outcome for the natural Cape Flats aquifer. Microbes will most likely follow a preferential pathway under natural conditions whereas in the simulated environment, the pathway was forced.

UNIVERSITY of the
WESTERN CAPE

5.1.4 Research results to protect drinking water under real world conditions

Gerba, 1975 study was pinnacle in understanding; *the outbreaks of typhoid fever at the turn of the century from eating raw vegetables grown on soil fertilized with raw sewage resulted in extensive studies of the survival of enteric bacteria in soil.* For this reason, experiments were conducted using viruses and bacteria, to understand the management and prevention of outbreaks of illness. The results of this thesis could serve as a guideline for future scientists investigating waterborne pathogens. This research also has uncovered the need to pin point survival rates and transport mechanisms to combat and understand pathogens.

Recommendations

In testing, discrepancies emerged which required amendments and improvements of the approaches. A key finding from initial tests was that field experiments were too short in

duration. Lengthening the tests resulted in more stable chemical tracer breakthrough curves but did not make a difference in the biological tracer.

When results were interpreted discrepancies emerged in the mass balance of the biological tracers. In some cases, more tracers came out of the column than was injected. It is recommended that the tests be run over longer time periods — even doubling the time of the experiments in this research project. The tests worked well under controlled environments whereas the in the natural environment it was less predictable.

Recommendations for laboratory studies would be to run either duplicate or triplicate columns over longer periods of time. This could not be implemented for this project as funds and time was limited. Another option would be to achieve better control of laboratory flow rates by possibly due to a design flaw. Instead of anticipating similar flow rates from similar input head conditions, the columns should be run at different flow rates to compare the results with existing results.

UNIVERSITY of the
WESTERN CAPE

Chapter 7: References

Abbaszadegan M, Stewart P and Lechevallier M (1999). A Strategy for Detection of Viruses in Groundwater by PCR. *Applied and Environmental Microbiology* **65**(2): 444-449.

Abudalo RA, Bogatsu YG, Ryan JN, Harvey RW, Metge DW and Elimelech M (2005). Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. *Environmental Science and Technology* **39** (17): 6412-6419.

Adamson RH (1993). Waterborne Pathogens: Assessing Risk. *Health and Environment Digest* **7**: 1-8.

Alary M and Nadeau D (1990). An outbreak of Campylobacter enteritis associated with a community water supply. *Canadian Journal of Public Health* **81**(4): 268-271.

Anders R and CV Chrysikopoulos (2006), Evaluation of the factors controlling the time-dependent inactivation rate coefficients of bacteriophage MS2 and PRD1, *Environ. Sci. and Tech.*, **40**(10), 3237-3242.

ASTM D5084 (2003). *Standard test methods for measurement of hydraulic conductivity of saturated porous materials using a flexible wall permeameter*. ASTM International. West Conshohocken, PA. www.astm.org.

ASTM D4874-95 (2006). *Standard test method for leaching solid material in a column apparatus.* ASTM International. West Conshohocken, PA. www.astm.org.

AWWA Committee Report (1999). Emerging pathogens - bacteria. *Journal of the American Water Works Association* **91**(9): 101-109.

Azeredo J, Visser J and Oliveira R (1999). Exopolymers in bacterial adhesion: interpretation in terms of DLVO and XDLVO theories. *Colloids and Surfaces B: Biointerfaces* **14**: 141-8.

Baer JT, Vugia DJ, Reingold AL, Aragon T, Angulo FJ and Bradford WZ (1999). HIV Infection as a risk factor for shigellosis. *Emerging Infectious Diseases* **5** (6): 820-23.

Bales R, Gerba CP, Grindin GH and Jensen SL (1989). Bacteriophage transport in sandy soil and fractured tuff. *Applied Environmental Microbiology* **55**: 2061-2067.

Bales RC, Hinkle SR, Kroeger TW, Stocking K and Gerba CP (1991). Bacteriophage adsorption during transport through porous media: Chemical perturbations and reversibility. *Environmental Science and Technology* **25**: 2088-2095.

Bales RC, Li S, Maguire K, Yahya M, and Gerba CP (1993). MS-2 phage and poliovirus transport through porous media: sorbent hydrophobicity and chemical perturbations, *Water Resource Research*, ***29***, 957–963.

Barron E and Dziembowski ZM (2003). *An explanation of the 1:500 000 general hydrogeological map*. Department of Water Affairs and Forestry, Republic of South Africa. Pretoria.

Baygents JC, Glynn JR, Albinger O, Biesemeyer BK, Ogden KL and Arnold RG (1998). Variation of surface charge density in monoclonal bacterial populations: implications for transport through porous media. *Environmental Science and Technology* **32**: 1596-1603.

Beller M, Ellis A, Lee SH, Drebot MA, Jenkerson SA and Funk E (1997). Outbreak of viral gastroenteritis due to a contaminated well. International consequences. *Journal of the American Medical Association* **278:** 563-568.

Bitton G, Farrah SR, Ruskin RH, Butner J and Chou YJ (1983). Survival of pathogenic and indicator organisms in groundwater. *Ground Water* **21**, 405–410.

Blanc R; Nasser A (1996). Water Science Technology, Pages 33, 237-242.

Bolster CH, Herman JS, Hornberger GM, Mills AL (1999). Spatial distribution of deposited bacteria following miscible displacement experiments in intact cores. Water Resource Research. **35** (6), 1797–1807.

UNIVERSITY of the
WESTERN CAPE

Bourne DE and Coetzee N (1990). *An atlas of potentially water related diseases in South Africa, Volume 1, Mortality 1990*. WRC Report No 584/1/96. Water Research Commission, Pretoria, South Africa.

Bradbury KR, Muldoon MA, Zaporozec A, and Levy J (1991). *Delineation of wellhead protection areas in fractured rocks*. Wisconsin Geological and Natural History Survey and US EPA, Washington, DC.

Bradbury KR, Gotkowitz MB, Cherry JA, Hart DJ, Eaton TT, Parker BL and Borchardt MA (2006). *Contaminant Transport Through Aquitards: Technical Guidance for Aquitard Assessment.* American Water Works Association Research Foundation, Denver, CO.

Bradford SA, Yates SR, Bettahar M and Simunek J (2002). Physical factors affecting the transport and fate of colloids in saturated porous media. *Water Resources Research* **38**(12)**:** 1029-1340, 2002.

Brennhovd O, Kapperud G and Langeland G (1992). Survey of thermotolerant *Campylobacter* spp. and *Yersinia* spp. in three surface water sources in Norway. *International Journal of Food Microbiology* **15**(3-4): 327–338.

Buchan GD and Flury M (2004). Pathogen transport by water. In: *Encylopedia of Water Science*, Stewart BA and Howell TA (Eds.), Marcel Dekker, New York, DOI:10.1081/E-EWS 120021169.

Burge WD and Enkiri NKJ (1978). Environmental Quality. Page 7, 73.

Caldwell EL and Parr LW (1937). Ground water pollution and the bored-hole latrine. Journal of Infectious Diseases **61**: 148-183. Cited in Harvey RW (1997). Micro-organisms as tracers in groundwater injection and recovery experiments: a review. *FEMS Microbiology Reviews* **20**(3-4): 461-472.

Camesano TA and Logan BE (2000). Probing bacterial electrosteric interactions using atomic force microscopy. *Environmental Science of Technology* **34**(16): 3354-3362.

UNIVERSITY of the
WESTERN CAPE

Chao CC, Bird P, Gething MJ and Sambrook J (1987). Pasttranslational translocation of influenza virus hemagglutinin across microsomal membranes. *Molecular and Cellular Biology* **7**(10): 3842-3845.

Chapelle FH (2001). *Ground-water microbiology and geochemistry*. 2nd Ed. John Wiley and Sons. New York.

Cherry WB, Hanks JB, Thomason BM, Murlin AM, Biddle JW and Croom JM (1972). Salmonellae as an index of pollution of surface waters. *Applied and Environmental Microbiology* **24**(3): 334-340.

Colvin C (1999). *Handbook of groundwater quality protection for farmers*. Water Research Commission Report No: TT 116/99. Pretoria, South Africa.

Corapcioglu MY and Haridas A (1984). Transport and fate of micro-organisms in porous media: a theoretical investigation. *Journal of Hydrology* **72**:149–69.

Crane SR and Moore J (1984). Bacterial pollution of groundwater: a review. *Water, Air, and Soil Pollution* **22**(1): 67–83.

DeBorde DC, Woessner WW, Kiley QT and Ball P (1999). Rapid transport of viruses in a floodplain aquifer. *Water Research* **33**(10): 2229-2238.

De Marsily G (1986). *Quantitative hydrogeology* (translated by G. de Marsily). San Diego (CA): Academic; 440 pp.

DiRita VJ, Hendrixson DR and Akerley BJ (2001). Transposon mutagenesis of Campylobacter jejuni identifies a bipartite energy taxis system required for motility. *Molecular Biology* **40**(1): 214-224.

Ditthorn F and Luerssen A (1909). Experiments on the passage of microbes through soil. *Engineering Record* **60:** 642.

Dodds J (1982). La chromatographie hydrodynamique. *Analusis* **10**: 109-19.

UNIVERSITY of the
WESTERN CAPE

Dong H, Onstott TC, DeFlaun MF, Fuller ME, Scheibe TD, Streger SH, Rothmel RK, Mailloux BJ, (2002). Relative dominance of physical versus chemical effects on the transport of adhesion-deficient bacteria in intact cores from South Oyster, Virginia. Environmental Science Technology **36** (5), 891–900.

Dowd SE, Pillai SD, Wang S and Corapcioglu MY (1998). Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Applied and Environmental Microbiology* **64**: 405-410.

Duah AA and Gyau-Boakye P (2002). *Groundwater Assessment and Exploitation in Ghana*. Conference paper at the International Conference on Hydrology and Watershed Management, 18-20th December, 2002 at Hyderabad, India.

DWAF (2005). 1*:2 000 000 compilation of 1:500 000 hydrogeological mapping series*. Department of Water Affairs and Forestry.

Dzeda B, Kaiser M and Mach S (1997). Bacteria and contamination. In: *Groundwater Primer Types of Contaminants*. CE 4594: Soil and Groundwater Pollution. Civil Engineering Department, Virginia Technology

Dykhuizen RC (1987). Transport of solutes through unsaturated fractured media, *Water Research, 21, 1531-1539.*

Dyson et al. (2009). Department of Water Affairs, Strategy and Guideline Development for National Groundwater Planning Requirements. Potential Artificial Recharge Areas in South Africa. PRSA 000/00/11609/1 - Activity 14 (AR04).

Eden Kenneth V, (Center for Disease Control), Rosenberg Mark L, Stoopler, Mark, Wood, Bruce T, Highsmith, Anita K, Skaliy, Peter, Wells, Joy G, and Feeley, James C (1977): Waterborne gastrointestinal illness at a ski resort. Isolation of Yersinia enterocolitica from drinking water. Public Health Reports Volume 92, May-June, pp. 245-250.

UNIVERSITY of the
WESTERN CAPE

Elliot ET, Anderson RV, Coleman DC and Cole CV (1980). Habitable pore space and microbial trophic interactions. *Oikos* **31**(3): 327-335.

Ferguson C, de Roda Husman AM, Altavilla N, Deere D, Ashbolt N (2003). Fate and transport of surface water pathogens in watersheds. Critical Review in *Environmental Science and Technology* 33:299–361.

Fetter CW (2001). *Applied Hydrogeology*. Fourth ed. Prentice Hall, Upper Saddle River, NJ.

Flynn RM, Rossi P and Hunkeler D (2004). Investigation of virus attenuation mechanisms in a fluvioglacial sand using column experiments. *FEMS Microbiology Ecology* **49**: 83-95.

Foppen JWA and Schijven JF (2005). Transport of *E. coli* in columns of geochemically heterogeneous sediment. *Water Research* **39**: 3082-3088

Ford TE (1999). Microbiological safety of drinking water: United States and global perspectives. *Environmental Health Perspectives* **107**: 191-206.

Fry NK, Frederickson JK, Fishbain S, Wagner M and Stahl DA (1997). Population structure of microbial communities associated with two deep, anaerobic alkaline aquifers. *Applied and Environmental Microbiology* **63:** 1498-1504.

Fuller ME, Dong H, Mailloux BJ, Onstott TC, (2000). Examining bacterial transport in intact cores from Oyster Virginia: effect of sedimentary facies type on bacterial breakthrough and retention. Water Resource Research **36** (9), 2417–2431.

Gannon JM, and Shinall EA (2000). Why Watersheds are Important: A Lesson from the Rock Valley Project. *Iowa Geological and Water survey.*

Gantzer C, Gillerman L, Kuznetsov M and Oron G (2001). Adsorption and survival of faecal coliforms, somatic coliphages and F specific RNA phages in soil irrigated with wastewater. *Water Science and Technology* **43**: 117-124.

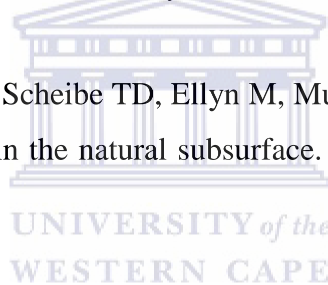
UNIVERSITY of the
WESTERN CAPE

Geldreich EE, Fox KR, Goodrich JA, Rice EW, Clark RM and Swerdlow DL (1992). Searching for a water supply connection in the Cabool, Missouri disease outbreak of *Escherichia coli* O 157: H 7. *Water Research* **8**: 1127-1137.

Gerba CP, Melnick JL and Wallis C (1975). Fate of wastewater bacteria and viruses in soil. *Journal of the Irrigation and Drainage Division* **101**(3): 157-174.

Gerba CP, Yates MV, and Yates SR (1991). Quantitation of factors controlling viral and bacterial transport in the subsurface, in Modelling the Environmental Fate of Micro-organisms, edited by C. J. Hurst, pp. 77–88, American Society for Microbiology, Washington D C.

Ginn TR (2000). Comment on ''stochastic analysis of virus transport in aquifers'' by L.L.C. Rehmann, Claire Welty, and Ronald W. Harvey. *Water Resource Reserve* **36**(7): 1981-2.



Ginn TR, Wood BD, Nelson KE, Scheibe TD, Ellyn M, Murphy EM and Clement TP (2002). Processes in microbial transport in the natural subsurface. *Advances in Water Resources* **25**: 1017-1042.

UNIVERSITY of the
WESTERN CAPE

Goldscheider N, Hunkeler D and Rossi P (2006). Review: Microbial processes in pristine aquifers and an assessment of investigative methods. *Hydrogeology Journal* **14**: 926-941.

Gordon C and Toze S (2003). Influence of groundwater characteristics on the survival of enteric viruses. *Journal of Applied Microbiology* **95**: 536-544.

Görres JH, Savin MC, Neher DH, Weicht TR and Amador JA (1999). Grazing in a porous environment: I. The effect of pore structure on C and N mineralization. *Plant Soil* **212**: 75-83.

Goyal SM and Gerba CP (1979). Comparative Adsorption of Human Enteroviruses, Simian Rotavirus, and Selected Bacteriophages to Soils. *Applied and Environmental Microbiology* **38**: 241-247.

Grabow WOK (1996). Waterborne diseases: Update on water quality assessment and control. *Water SA* **22**: 193-202.

Guillot E and Loret JF (2010). Waterborne Pathogens: Review for the Drinking Water Industry. Published by IWA publishing, London, UK.

Harris T, Johns J, Merriman J and Merricks J (1997). Viral contamination. In: *Groundwater Primer Types of contaminants*. CE 4594. Soil and Groundwater Pollution. Civil Engineering Department, Virginia Tech. (URL:http://www.cee.vt.edu/program_areas/environmental/teach/gwprimer/virus/virus.htm)

Harter T, Wagner S and Atwill ER (2000). Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. *Environmental Science of Technology* **34**: 62-70.

Harvey RW (1991). Parameters involved in modeling movement of bacteria in groundwater. In: *Modeling the Environmental Fate of Micro-organisms*. Hurst CJ (Ed.). Washington, DC: American Society for Microbiology. 89-114.

Harvey RW (1997). Micro-organisms as tracers in groundwater injection and recovery experiments: a review. *FEMS Microbiology Reviews* **20**: 461-472.

UNIVERSITY of the
WESTERN CAPE

Harvey RW and Garabedian SP (1991). Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer. *Environmental Science of Technology* **25**(1): 178–85.

Harvey RW, Kinner NE, Bunn A, MacDonald D and Metge D (1995). Transport behavior of groundwater protozoa and protozoansized microspheres in sandy aquifer sediments. *Applied and Environmental Microbiology* **61**(1): 209-17.

Hazeleger WC, Wouters JA, Rombouts FM and Abee T (1998). Physiological activity of Campylobacter jejuni far below the minimal growth temperature. *Applied and Environmental Microbiology* **64**(10): 3917-3922.

Health Canada (2006). *Guidelines for Canadian drinking water quality: Guideline technical document - bacterial waterborne pathogens - current and emerging organisms of concern*.

Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Herzig JP, Leclerc DM and Le Goff P (1970). Flow of suspensions through porous media. In: *Flow through porous media*. Washington, DC: American Chemical Society. 129–57.

Hunter PR (1997). *Waterborne disease: Epidemiology and Ecology.* John Wiley and Sons Ltd. 154-230.

Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD and Walter MV (Eds.). ASM Press, Washington, DC.

Inoue M, Nakashima H, Ishida T, and Tsubokura M (1988).Three outbreaks of Yersina pseudotuberculosis infection. Zentralb Bakteriol Mikribiol Hyg (B) 186:504-511.

Jemison J (2002). *Bacteria in water supplies Part 1: Problem bacteria and how to test for them*. http://www.umext.maine.edu/waterquality/publications/7114.htm.

Jiwan JS and Gates G (1998). Microbiological contamination of groundwater in New South Wales, Australia. In: Weaver TR and Lawrence CR (Eds.) *Groundwater: Sustainable Solutions*. Proceedings of the International Association of Hydrogeologists International Groundwater Conference; 1998 Feb. 8-13; Melbourne, Australia. Indooroopilly, Australia: International Association of Hydrogeologists, Australian Chapter, 473-478.

Johnson MR, Anhaeusser CR, Thomas RJ (2008). The Geology of South Africa, South Africa: Council for Geosciences.

Jonck F and Meyer S (2002). *Hydrogeological map series of the Republic of South Africa*. Department of Water Affairs and Forestry. Pretoria.

Kinoshita T; Bales RC; Maguire KM; Gerba CPJ (1993). Contamination Hydrology, 14, 55-70.

UNIVERSITY of the
WESTERN CAPE

Kinner NE, Harvey RW, Blakeslee K, Novarino G and Meeker LD (1998). Size selective predation on groundwater bacteria by nanoflagelates in an organic contaminated aquifer. *Applied and Environmental Microbiology* **64**: 618-625.

Kinoshita T; Bales RC; Maguire KM; Gerba CP (1993). Journal of Contamination Hydrology. Pages 14, 55.

Kramer MH, Herwaldt BL, Craun GF, Calderon RL and Juranek DD (1996). Surveillance for waterborne disease outbreaks - United States, 1993-1994. *Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report* **45**(SS 1): 1 33.

Kudryavtseva EA (1972). Stable conjugation invariants of Hamiltonian systems on two-dimensional surfaces. Dokl. Ross. Akad. Nauk **361**(3): 314-317 English translation in *Soviet mathematics - Doklady* **58**(1): 67-70.

Lawrence JR and Hendry MJ (1996). Transport of bacteria through geologic media. *Canada Journal of Microbiology* **42**:410–22.

Lehman RM, Colwell FS and Bala GA (2001). Attached and unattached microbial communities in a simulated basalt aquifer under fracture- and porous-flow conditions. *Applied and Environmental Microbiology* **67**(6): 2799–2809.

Levy J, Sun K, Findlay RH, Farruggia FT, Porter J, Mumy KL, Tomaras J and Tomaras A (2007). Transport of *Escherichia coli* bacteria through laboratory columns of glacial-outwash sediments: Estimating model parameter values based on sediment characteristics. *Journal of Contaminant Hydrology* **89**: 71-106.

Lewus P and Ford RM (2001). Quantification of random motility and chemotaxis bacterial transport coefficients using individual-cell and population-scale assays. *Biotechnology Bioengineering* **75**(3): 292-304.

Li X and Johnson WP (2005). Nonmonotonic variations in deposition rate coefficients of microspheres in porous media under unfavorable deposition conditions. *Environmental Science and Technology* **39**: 1658–1665.

UNIVERSITY of the
WESTERN CAPE

Li X, Scheibe TD and Johnson WP (2004). Apparent decreases in colloid deposition rate coefficients with distance of transport under unfavorable deposition conditions: a general phenomenon. *Environmental Science and Technology* **38**: 5616–5625.

Linn DM and Doran JW (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal* **48**(6): 1267-1272.

Loveland JP, Ryan JN, Amy GL and Harvey RW (1996). The reversibility of virus attachment to mineral surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **107**: 205-221.

MacDonald AM, Darling WG, Ball DF and Oster H (2002). Identifying trends in groundwater quality using residence time indicators: an example from the Permian aquifer of Dumfries, Scotland. *Hydrogeology Journal* **11**(4): 504-517.

MacLeod FA, Lappin-Scott HM, and Costerton JW (1988). Plugging of a model rock system by using starved bacteria. *Applied and Environmental Microbiology* **54**(6): 1365-1372.

UNIVERSITY of the
WESTERN CAPE

Magnan S, Lynfield R, Danila RN and Kizzee B (2008). *Annual summary of communicable diseases reported to the Minnesota Department of Health,* **36**(1).

Mallén G, Maloszewski P, Flynn R, Rossi P, Engel M and Seiler K-P (2005). Determination of bacterial and viral transport parameters in a gravel aquifer assuming linear kinetic sorption and desorption. *Journal of Hydrology* **306**: 21–36.

Martin MJ, Logan BE, Johnson WP, Jewett DG and Arnold RG (1996). Scaling bacterial filtration rates in different sized porous media. *Journal of Environmental Engineering* **122**: 407-415.

Mayo Clinic (2007). *Shigella*. Retrieved August 22, 2007 from Mayo Clinic Web site, http://www.mayoclinic.com/health/shigella/DS00719.

McCarthy T and Rubidge B (2005). *The story of earth & life: A southern African perspective on a 4.6-billion-year journey*. Kumba Resources. Struik Publishers, Cape Town.

McCaulou DR, Bales RC and Arnold RG (1995). Effect of temperature-controlled motility on transport of bacteria and microspheres through saturated sediment. *Water Resources Research* **31**(2): 271-280.

McDowell-Boyer LM, Hunt JR and Sitar N (1986). Particle transport through porous media. *Water Resource Reserve* **22**: 1901-1921.

McFeters GA, Bissonnette GK, Jezeski JJ, Thomson CA, and Stuart DG (1974). Comparative survival of indicator bacteria and enteric pathogens in well water. *Applied Microbiology* **27**: 823-829.

Mercer JR, Ford RM, Stitz JL and Bradbeer C (1993). Growth rate effects on fundamental transport properties of bacterial populations. *Biotechnology Bioengineering* **42**(11): 1277-1286.

Meschke JS and Sobsey MD (2003). Comparative reduction of Norwalk virus, poliovirus type 1, F+ RNA coliphage MS2 and Escherichia coli in miniature soil columns. *Water Science and Technology* **47**(3): 85-90.

Metge D (2002). *Fate and transport of bacterial, viral, and protozoan pathogens during asr operations - what micro-organisms do we need to worry about and why?* U.S. Geological Survey, Boulder, Colorado 80303.

Mitchell DO and Starzyk MJ (1975). Survival of Salmonella and other indicator micro-organisms. *Canadian Journal of Microbiology* **21**: 1420-1421.

Moe CL (1997). Waterborne transmission of infectious agents. In: *Manual of environmental microbiology*.

Murphy F, Herkelrath WN (1996). Ground Water Monitoring Remediation. Page 16, 86-90.

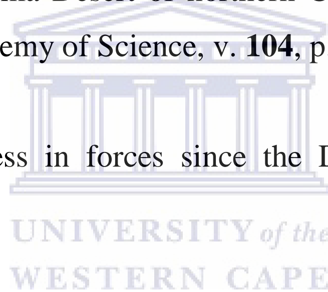
UNIVERSITY of the
WESTERN CAPE

Murphy EM, Ginn TR, Chilakapati A, Resch CT, Phillips JL, Wietsma TW and Spadoni CM (1997). The influence of physical heterogeneity on microbial degradation and distribution in porous media. *Water Resource Reserve* **33**: 1087-1103.

Murray JP and Parks GA (1980). Particulates in water: Characterization, fate, effects, and removal. In: Kavanaugh MC, Leckie, JO, (Eds.). *Advances in Chemistry Series 189*; American Chemical Society, Washington, DC. 97-103.

Murray K, du Preez M, Taylor MB, Meyer R, Parsons R, van Wyk E, Kühn A, van Niekerk H and Ehlers MM (2004). *National microbial monitoring programme for groundwater: Research report*. Water Research Commission report No. 1277/1/04. Pretoria, South Africa.

Nester PL, Gayo EM, Latorre C, Jordan TE, and Blanco N (2007). Perennial stream discharge in the hyperarid Atacama Desert of northern Chile during the latest Pleistocene: Proceedings of the National Academy of Science, v. **104**, p. 19724-19729


UNIVERSITY of the
WESTERN CAPE

Ninham BW (1999). On progress in forces since the DLVO theory. Advanced Colloid Interface Science. **83**:1–17.

Novarino G, Warren A, Butler H, Lambourne G, Boxshall A, Bateman J, Kinner NE, Harvey RW, Mosse RA and Teltsch B (1997). Protistan communities in aquifers: a review. FEMS Microbiology Reviews **20** (3-4)**:** 261-275.

October AG (2009). Personal oral communication. MSc Student. Bellville. 14 August 2009.

Odai SN and Dugbantey DD (2003). *Towards pollution reduction in peri-urban water supply: A case study of Ashanti region in Ghana*. Diffuse Pollution Conference, Dublin, 2003. Poster paper. Dublin, South Africa.

Park NS, Blandford TN, Wu YS and Huyakorn PS (1980). *CANVAS: a composite analytical-numerical model for viral and solute transport simulation. Documentation and User's Guide, Version 1.0*, HydroGeoLogic, Inc., Herndon, VA.

Pedley S and Howard G (1997). The public health implications of microbiological contamination of groundwater. *Quarterly Journal of Engineering Geology* **30**(2): 179-188.

Peterson R (2008). *A report on the characterisation of the UWC campus borehole research site*. Unpublished BSc Hons mini thesis. University of the Western Cape, Bellville.

Pietersen KC (2004). *A decision making framework for groundwater management in arid zones (with a case study in Namaqualand).* Unpublished PhD thesis, University of the Western Cape.

Popovitch G and Bondarenko V (1982). Shigella and Salmonella viability in river water at varying temperatures. Biology Abstraction, **74**.

Porter J, Mobbs K, Hart CA, Saunders JR, Pickup RW, and Edwards C (1997). Detection, distribution and probable fate of Escherichia coli O157 from asymptomatic cattle on a dairy farm. Journal Applied Microbiology, **83** (3), 297 – 306.

Powell SC and Attwell R (2000). Appendix B guidelines for confirmation of foodborne-disease outbreaks. *Morbidity and Mortality Weekly Report* **49**(SS01): 54-62.

Powelson DK; Simpson JR and Gerba CP (1991). Applied Environmental Microbiology. Page 57, 2192.

Powelson DK, Gerba CP and Yahya MT (1993). Virus transport and removal in wastewater during aquifer recharge. *Water Research* **27**: 583-590.

Price J (1997). Soil moisture, water tension, and water table relationships in a managed cutover bog. *Journal of Hydrology* **202**: 21-32.

Rajagopalan R and Tien C (1976). Trajectory analysis of deep-bed filtration with the sphere-in-cell porous media model. *American Institute of Chemical Engineers Journal* **22**(3): 523-533.

UNIVERSITY of the
WESTERN CAPE

Rao VC and Melnick JL (1986). *Environmental virology.* Berkshire England: Van Nostrand Reinhold.

Redman JA, Grant SB, Olson TM, Estes MK (2001). Pathogen filtration, heterogeneity, the potable reuse of wastewater. *Environmental Science and Technology* **35** (9):1798–805.

Rehmann LLC, Welty C and Harvey RW (2000). Reply. *Water Resource Reserve* **36** (7): 1983–1984.

Rice EW (1999). Escherichia coli. In: *AWWA manual M48: Waterborne pathogens*. American Water Works Association, Denver, CO. 75-78.

Rice EW, Rodgers MR, Wesley IV, Johnson CH and Tanner SA (1999). Isolation of *Arcobacter butzleri* from ground water. *Letters in Applied Micobiology* **28**(1): 31 35.

Rijnaarts HHM, Norde W, Lyklema J and Zehnder AJB (1995). The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. *Colloids Surface Benefits: Biointerfaces* **4**(4): 191–197.

UNIVERSITY of the
WESTERN CAPE

Rossi P, Dörfliger N, Kennedy K, Müller I and Aragno M (1998). Bacteriophages as surface and ground water tracers. *Hydrology and Earth System Sciences* **2** (1): 101-110.

Ryan J (2000). *Transport of viruses, bacteria and protozoa in groundwater*. Environmental Engineering Seminar (PowerPoint presentation).

Ryan JN, Harvey RW, Metge D, Elimelech M, Navigato T and Pieper AP (2002). Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand. *Environment Science and Technology* **36**: 2403-2413.

Sakthivadivel R (1966). *Theory and mechanism of filtration of noncolloidal fines through a porous medium.* Report HEL 15-5. Hydraulic Engineering Laboratory, University of California, Berkeley.

Sakthivadivel R (1969). *Clogging of a granular porous medium by sediment*. Report HEL 15-7. Hydraulic Engineering Laboratory, University of California, Berkeley.

Savill MG, Hudson JA, Ball A, Klena JD, Scholes P, Whyte RJ, McCormick RE and Jankovic D (2001). Enumeration of Campylobacter in New Zealand recreational and drinking waters. *Journal of Applied Microbiology* **91** (1): 38-46.

Savin MC, Görres JH, Neher DA and Amador JA (2001). Biogeophysical factors influencing soil respiration and mineral nitrogen content in an old field soil. *Soil Biology and Biochemistry* **33**: 429-438.

Schieman DA (1990). Yersina enterocolitica in drinking water. In drinking water Microbiology. Pp. 322-339. McFeters, GA., edition. New York: Springer-Verlag.

Schijven JF (2001). *Virus removal from groundwater by soil passage, modeling, field and laboratory experiments (thesis)*. Delft, Netherlands: Technische University Delft.

Schijven JF and Hassanizadeh SM (2000). Removal of viruses by soil passage: overview of modeling, processes, and parameters. *Critical Reviews in Environmental Science and Technology* **30**(1): 49-127.

Schijven JF, Hassanizadeh SM and de Bruin RHAM (2002). Two-site kinetic modeling of bacteriophages through columns of dune sand. *Journal of Contaminant Hydrology* **57**: 259-279.

Servagent-Noinville S; Revault M; Quiquampoix H; Baron MH (2000). J. Colloid Interface Science, 221, 273-283.

Sinton LW (2001). Microbial contamination of New Zealand's aquifers. In: *Groundwaters of New Zealand*. Rosen MR and White PA (Eds.). Wellington, New Zealand: New Zealand Hydrological Society Incoporated 221-251.

UNIVERSITY of the
WESTERN CAPE

Smets BF, Grasso D, Engwall MA and Machinist BJ (1999). Surface physicochemical properties of Pseudomonas fluorescens and impact on adhesion and transport through porous media. *Colloids Surface B: Biointerfaces* **14**: 121–39.

Smith MS, Thomas GW, White RE, Ritonga C, (1985). Transport of Escherichia coli through intact and disturbed soil columns. Journal of Environmental Quality **14** (1), 87–91.

Stanley K, Cunningham R and Jones K (1998). Isolation of Campylobacter jejuni from groundwater. *Journal of Applied Microbiology* **85** (11), 187-191.

Stephens A and Bredenkamp DB (2002). *Institutional arrangements for groundwater management in dolomitic terrains*. Water Research Commision Report KV140/02. Water Reasearch Commision. Pretoria.

Stevik TK, Aa K, Ausland G and Hanssen JF (2004) Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. *Water Resource* **38** (6): 1355–1367.

Theron J, Gresse P, Siegfried H, and Rogers J (1992). The Geology of the Cape Town area. Explanation: sheet 3318 (1:250 000), 140pp.

Tickner J and Gouveia-Vigeant T (2005). The 1991 cholera epidemic in Peru: Not a case of precaution gone awry. *Risk Analysis* **25** (3): 495-502.

Thomason BM, Biddle JW and Cherry WB (1975). Detection of Salmonellae in the environment. *Applied and Environmental Microbiology* **30** (5): 764-767.

Thompson JS and Gravel MJ (1986) Family breakout of gastroenteritis due to Yersina enterolcolitica serotype 0:3 from well water. Can J Microbial **32**:700-701.

Truswell JF (1970). An Introduction to the Historical Geology of South Africa: Cape Town, (Purnell & Sons).

UNIVERSITY of the
WESTERN CAPE

Tufenkji N (2007). Modeling microbial transport in porous media: Traditional approaches and recent developments. *Advances in Water Resources* **30**: 1455-1469.

Tufenkji N and Elimelech M (2005). Breakdown of colloid filtration theory: role of the secondary energy minimum and surface charge heterogeneities. *Langmuir* **21**: 841-852.

Tufenkji N, Redman J and Elimelech M (2003). Interpreting deposition patterns of microbial particles in laboratory-scale column experiments. *Environmental Science and Technology* **37**: 616-623.

Tufenkji N and Elimelech M (2004). Deviation from the classical colloid filtration theory in the presence of repulsive DLVO interactions. *Langmuir* **20**: 10818-10828.

Ukhahlamba District Municipality (2008). Investigations on child deaths in Senqu sub-district.

Vance DB (2002). *2 The 4 Technology Solutions: Anisotropic Hydrology - Part I Fracture Flow Systems*. Document URL: http://2the4.net/anisofrac.htm.

UNIVERSITY of the
WESTERN CAPE

Van Tassel PR; Guemouri L; Ramsden JJ; Tarjus G, Viot P; Talbot J (1998). J. Colloid Interface Science, *207*, 317-323.

Vaughn JM, Landry EF, Beckwith CA and Thomas MZ (1981). Virus removal during groundwater recharge: effects of infiltration rate on adsorption of poliovirus to soil. *Applied and Environmental Microbiology* **41**(1): 139-147

Vegter JR (2001). *Groundwater development in South Africa and an introduction to the hydrogeology of groundwater regions*. WRC report TT134/00. Water Research Commission. Pretoria.

Vogt RL, Sours HE, Barrett T, Feldman RA, Dickinson BS and Witherell L (1982). Campylobacter enteritis associated with contaminated water. *Annals of Internal Medicine* **96**(3): 792–796.

Wait DA and Sobsey MD (1983). Method for recovery of enteric viruses from estuarine sediments with chaotropic agents. Applied Environmental Microbiology. 46: 379-385.

WHO (1996). *Guidelines for drinking water quality. 2nd Edition. Volume 2: Microbiological criteria*. Geneva: World Health Organization.

WHO (2002). *Toxic cyanobacteria in water - a guide to their public health consequences, monitoring and management*. World Health Organisation, South Africa.

Webb EK, Anderson MP (1996). Simulation of preferential flow in three-dimensional heterogeneous conductivity fields with realistic internal structure. Water Resource Reserve. **32** (3), 533–545.

Wilson JT, Leach LE, Henson M, and Jones JN (1986). In situ biorestoration as a ground water remediation technique. *Ground Water Monitoring and Remediation* **6**(4): 56-64.

Winfield MD and Groisman EA (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* **69**(7): 3687-3694.

UNIVERSITY of the
WESTERN CAPE

Woodford AC and Chevallier L (2002). *Regional characterization and mapping of Karoo fractured aquifer systems - An integrated approach using a geographical information system and digital image processing*. WRC Report 653/1/02. Water Research Commission. Pretoria.

Xu Y and Usher B (2006). Issues of groundwater pollution in Africa. In: *Groundwater Pollution in Africa*. Xu Y and Usher B (Eds.). London: Taylor & Francis/Balkema, 3-9.

Yates MV, Gerba CP and Kelley LM (1985). Virus persistence in groundwater. *Applied and Environmental Microbiology* **49**(4): 778-781.

Yates MV, Yates SR, Warrick AW and Gerba CP (1986). Use of geostatistics to predict virus decay rates for determination of septic tank setback distances. *Applied and Environmental Microbiology* **52**(3): 479-483.

Yates MV, Yates SR and Gerba CP (1988). Modeling microbial fate in the subsurface environment. *CRC Critical Reviews in Environmental Control* **17**(4): 307-344.

Zhang Y (2007). Characteristics of aquifer system deformation in the Southern Yangste Delta, China. Engineering Geology **90:** 160 – 173.

Zhang P, Johnson WP, Piana MJ, Fuller CC and Naftz DL (2001). Potential artifacts in interpretation of differential breakthrough of colloids and dissolved tracers in the context of transport in a zero-valent iron permeable reactive barrier. *Ground Water* **39**(6): 831-40.