THE FATE OF MICROBIAL CONTAMINANTS IN THE SUBSURFACE WITH A SOUTH AFRICAN CASE STUDY

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A thesis submitted in fulfilment of the requirements for the degree of Magister Scientae in the Department of Earth Science,
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KEYWORDS

Pathogens

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Groundwater Quality

Groundwater Protection Zones

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Phelendaba, Kwa-Zulu Natal

ABSTRACT

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The time bound agenda of the Millennium Development Goals (MDG's) aims at reducing poverty, extending gender equality and advancing opportunities for health and education by addressing current and future water resource and sanitation needs. In many rural areas of South Africa, the cost implication of routing surface water supplies and providing water borne sewerage may far exceed the budgets of local water service authorities. This has resulted in a major thrust in service provision via localised sources, mainly boreholes and springs as well as on site sanitation options.

Whilst the National Water Act (Act 36 of 1998) mandates the South African government to provide potable water to all citizens in an equitable manner, this needs to be balanced against the preservation of the country's water resources both quantitatively and qualitatively to ensure sustainability. It is imperative that this fine balance between protection and effecting societal demands and economic development through large-scale water provision be maintained, as successful strategising will be resultant of integrated social, economic and environmental issues especially in economically developing countries. In order to fulfil the mandate of the NWA, policies and strategies for effective protection and use of groundwater resources have been drawn up and are in the process of being drawn up by the national Department of Water Affairs and Forestry (DWAF).

The major scope of research in this thesis stems from feasibility studies commissioned by the DWAF for the implementation of a groundwater protection zoning policy for the management and protection of groundwater resource quality. The research work focuses on specifically the microbiological zone of protection and attempts to determine the fate of various pathogens that emanate from on site sanitation facilities as they move through the subsurface. The research was predominantly proposed as a desktop collation and analysis of existing published data however; it was later decided to include a local case study site.

A South African case study site was set up in the unconsolidated aquifer of the Zululand coastal plains and groundwater microbial quality was monitored for a period of 11 months around a high loading school pit latrine. A series of boreholes were sited and drilled in line with the groundwater flow direction at varying distances away from the pit latrine to investigate over what distance the majority of faecal contaminants would be filtered out from the groundwater resource and thus render it safe to consume. Samples were analysed for total coliform using the plate count technique.

The analysis of samples indicated that over a distance of 3m the faecal coliform counts registered no readings within this unconsolidated aquifer (0 cfu/100ml). The total coliform counts however were higher than the standard recommended for domestic water quality as required by DWAF. The results however, did seem to vary over the drier winter months as compared to the high rainfall period of September and November. Whilst the faecal coliforms are assumed to be filtered out within the 3m distance, it would seem that the results obtained for total coliforms might be attributed to a surface source of microbes and not emanate from the pit latrine and horizontal flow. The benchmark used for comparison of results was the standards of the South African Water Quality guidelines for human consumption.

In general, the effects of such microbial contamination should be directly noticeable on the end users of the water source, with periodic reports of water borne diseases, however samples analysed from the community borehole suggests a higher total coliform count than the research boreholes, with no reported side effects on the consumers. Knowledge of issues around microbial pathogens is critically essential in managing and circumventing potential health threats that may result from the consumption or contact with groundwater that has been contaminated.

DECLARATION

I declare that **THE FATE OF MICROBIAL CONTAMINANTS IN THE SUBSURFACE WITH A SOUTH AFRICAN CASE STUDY** is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full Name: Yasmin Rajkumar **Date**: 14th May 2009

Signed: _____

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To my late dad who thought me the value of education and perseverance and my late brother, Rajesh, who always encouraged me to pursue my dreams – This thesis is dedicated in memory of you both.

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To my many colleagues at DWAF (especially the groundwater sector), friends, academics, teachers, professors who have throughout my life influenced the path I took, encouraged me to think out of the box, and most of all, who showed me how to live life with passion and contentment, I salute you all!

ABBREVIATIONS

CFU	Colony Forming Units
DWAF	Department of Water Affairs and Forestry
E.coli	*Escherichia coli*
(G)RDM	(Groundwater) Resource Directed Measures
KZN	Kwa-Zulu Natal
MAP	Mean Annual Precipitation
MAMSL	Metres Above Mean Sea Level
MBGL	Metres Below Ground Level
MDGs	Millennium Development Goals
NWA	National Water Act
NWRS	National Water Resource Strategy
PSP	Professional Service Provider
UN	United Nations
VIPs	Ventilated Improved Pits

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CHAPTER 1
INTRODUCTION

Water is life; sanitation is dignity. These are maxims that the Department of Water Affairs and Forestry (DWAF) of the South African government use in the provision of services to its people.

The promulgation of the National Water Act (NWA) (Act 36 of 1998) has seen South Africa heeding these principles of service delivery to the historically disadvantaged for the upliftment of human living conditions. It has also enforced a change in water management objectives of the South African government, to include pro-active protection of water resources.

Just over ten years ago, with a change in political leadership of South Africa, a major thrust in service provision began. There was major pressure for the rapid development of resources and this led to inadequate planning, management and protection of resources to ensure long term sustainability in many of the smaller towns. Groundwater often constituted an important part of water resources in meeting the requirements for basic needs. Following on from this thrust, South Africa as a part of the United Nations (UN) member states, resolved “to halve by the year 2015 the proportion of people who are unable to reach, or to afford, safe drinking water” and “to stop the unsustainable exploitation of water resources, by developing water management strategies at the regional, national and local levels, which promote both equitable access and adequate supplies” for the Millennium Development Goals (MDGs).

In a semi arid country like South Africa, trying to fulfil these MDG requirements equates to extensive use of groundwater resources, particularly in rural communities where this locally developed resource can be the most cost effective option. Whilst potable water supplies for these communities are sourced from groundwater resources, sanitation is provided via pit-latrines or ventilated improved pit-latrines (VIPs). It is important that the latrines do not impinge on the quality of the groundwater. The installation of many pit latrines in high density rural areas opened up a new debate on its impacts on the receiving environment and ultimately its risk potential for contamination. Jackson (1997) questioned whether the danger of groundwater pollution, from on-site sanitation systems was being over exaggerated by

environmental enthusiasts. His argument was that the natural attenuative and assimilative capacity of the environment was sufficient to prevent any major contamination of aquifers provided that proper siting and construction of these facilities were conducted.

Protection and management of groundwater resources and understanding of the interactions between surface water, groundwater and human activities are crucial for sustainable water resources development and planning. In areas where proper management and protection measures are not effected high rates of viral outbreaks can be noted as in Delmas, Mpumalanga (Nel et al., 2009; Le Roux and du Preez, 2008; Pienaar and Xu, 2007). These outbreaks, including diarrhea, typhoid and cholera amongst others, leads to poor community health and thus lowers productivity and may result in death in extreme cases.

Contaminants resulting from sewage are predominantly of microbiological or chemical origin. These subsurface contaminants may be naturally removed or modified depending on the physical, hydrogeochemical and biogeochemical processes that may occur. During transport through the sub-surface environment, processes of adsorption, ion exchange and biodegradation may result in the removal or decomposition of the pollutants. However this natural removal of the contaminant is also dependent on other factors including contaminant travel times, the geological nature of the aquifer and vadose zone (fracture density and distribution, soil moisture holding capacity, clay content, ionic properties, soil micro and macro structures), climate (including rainfall and recharge to groundwater, temperature) and predation etc (Yates et al., 1985).

South Africa as a developing country has a mix of first world technology in a third world socio-economic environment. The anthropogenically generated waste from this technology and other practices could be potentially detrimental to our natural resources if not managed properly. This scenario is not unique to South Africa, but is echoed through much of sub-Saharan Africa. Whilst it's almost impossible for absolute protection of groundwater resources without hindering socio and economic growth, we are able to implement effective strategies for the control and minimisation of the extent of pollution our resources.

Protection zoning entails the establishment of zones around specific and socially important groundwater sources and takes into consideration the travel times required for contaminants

to reach the water source. This method seeks primarily, to control land use activities close to groundwater resources, thus preventing, limiting or controlling pollution of the resource.

1. Objective of the Study

Evidence from studies indicate that control of land use activities and zonal planning around recharge areas and abstraction points of groundwater resources would decrease the microbial quality impact (Nsubunga et al., 2004; Xu and Usher, 2006 and Aleman et al., 2004).

This thesis focuses on the both the theoretical and practical fate of microbiological contaminants within the subsurface, with a literature review of published data on pathogen travel in the sub-surface and an elementary site setup in an unconsolidated aquifer in the Kwa-Zulu Natal (KZN) Zululand coastal plains.

It is envisaged that the information collected and analysed in this thesis will assist in the clarification of issues surrounding the delineation of the microbiological protection zone, which is required for the implementation of a feasibility study commissioned by the national DWAF on groundwater protection zones.

2. Current Legislative Framework for Groundwater Protection in SA

Aquifer protection can be effected by implementation of protective legislation. There are currently three pieces of governmental legislation that directly deal with the protection of groundwater resources:

- The National Water Act (*No. 36 of 1998*)
- The Environmental Conservation Act (*No. 73 of 1989*)
- National Environmental Management Act (*No. 73 of 1989*)

From these stem various policies and strategies for the protection of groundwater resource quality and quantity including the:

- Groundwater Quality Management Strategy of South Africa (DWAF, 2000)
- National Water Resource Strategy (DWAF 2004b) and National Groundwater Strategy
- Groundwater Protection Zoning Policy (in progress) (DWAF, 2008)

The NWA (Act 36 of 1998) is the main legislative framework behind the effective and sustainable management of water resources in this country. It recognises that the management principles implemented should recognise basic human needs (of both present and future generations), the need to protect water resources, the need to promote social and economic development through the use of water, the need to fulfil international obligations in terms of sharing water resources and the need to establish institutions to bring into effect the legislation stated in the NWA (DWAF, 1997a)

The old water act (Act No 54 of 1956) fell short in groundwater protection aspects. Apart from government subterranean water control areas, it offered groundwater resources virtually no protection due to its 'private use' status (DWAF, 1997a). The new NWA now takes into cognisance the different components of the hydrological cycle offering more protective legislature towards groundwater.

Section 24 of the constitution (Act No. 108 of 1996) states that any development and use of our natural resources must be environmentally sustainable. Before any licensing is granted, initial consideration will be given to the Reserve (both human and ecological), international obligations, future requirements and existing users. One of the tools developed in accordance with chapter 3 of the NWA, is the resource directed measures process (RDM), the groundwater resource directed measures (GRDM) in context of groundwater. The GRDM includes the classification of water resources, the setting of the reserve and the setting of resource quality objectives and even includes a software tool developed so that water managers may assess the potential of specific resources which they may be developing (Dennis and Wentzel, 2007).

Whilst the GRDM process works mainly at managing water use at catchment level, the National Water Resource Strategy (NWRS) strategically directs the management of water from a national perspective, offering long term planning to meet the challenges of South Africa's aridity and limited water supply (DWAF, 2004b). The NWRS also encompasses the ideals of the policy and strategy for groundwater quality management in South Africa (DWAF, 1997b & 2000), which describes the means and measures available to achieve groundwater quality management.

The mission of the Groundwater Quality Management Strategy is to ensure integrated and sustainable management of groundwater quality thus maintaining adequate levels of protection of the resource via source directed controls, resource directed measures and remediation activities. Feeding into the NWRS, it provides a regulatory and institutional framework, allowing for a detailed groundwater quality management procedure to be developed and implemented and thus entails both specific management strategies as well as broad functional strategies (DWAF, 2000). These functional strategies include source directed measures like land use planning and allocation, which is also highlighted within the Groundwater Protection Zoning Policy (DWAF 2008).

There are specific challenges related to authorisation, protection and management of groundwater resource use. The historic legislation led to a perception that groundwater is private and this perception remains entrenched among many groundwater users. The "hidden" nature of groundwater makes it difficult to detect when a person is using the water without the necessary authorisation. Groundwater monitoring is also not always effective in determining levels of protection and sustainable utilisation of aquifers, notably when all users are not registered and/or complying with their licence conditions.

3. Protection Zoning

Zoning generally offers three zones for differentiated protection. These are the wellhead operational zone, the inner protection zone and the outer protection zone (Jolly and Reynders, 1993; Adams and Forster, 1992).

Wellhead operational zone: This is the primary protection zone, which allows for protection of the immediate area around a borehole (Figure 1). The distance can be determined according to the safe minimum distance concept (Xu and Braune, 1995a). The hydraulic gradient and flow direction will determine whether the zone is circular or elliptical in shape. In South Africa this minimum distance ranges between 15-50m depending on the geological nature of the aquifer. Ideally, in this zone no other activity except abstraction should be permitted. Careful control should be exercised to prevent pollutants reaching the source (Forster et al., 2002; Xu and Braune, 1995b; Adams and Forster, 1992).

Figure 1: Rural wellhead protection measures effected in rural Africa (IAEA, 2000)

Inner protection zone: This zone is based on travel times required for the horizontal movement of microbiological contaminants in the subsurface environment. The natural attenuation processes of the unsaturated zone will filter out microbiological pathogens deposited in the subsurface environment. Flow times can vary between 10 – 400 days depending on the life span of the contaminant. However a 50 day travel time is considered

reasonable to define this zone in terms of both economical and safety reasons (Jolly and Reynders, 1993; Forster et al., 2002; Adams and Forster, 1992).

The outer protection zone: This zone works on protection of the resource on an aquifer level. The recharge area of the aquifer is protected from pollutants that can affect water quality on a long-term basis. These are pollutants that are not easily decomposed and are persistent in the subsurface environment. A 500 day travel time has been citied by Jolly and Reynders, 1993 for South African conditions. This travel time however, can be increased or decreased according to the protection needs of the aquifer as well as the vulnerability of the aquifer.

Zonation allows for specific activities that are permitted within each zone. Table 1 gives a list proposed of land use constraints according to each zone.

Table 1: Land use constraints for protection zones (Jolly and Reynders, 1993; Forster et al., 2002).

Zone	Land use constraint
Wellhead operational zone	All constraints of inner protection zone and outer protection zone Agriculture Traffic – both pedestrian and automotive
Inner protection zone	All constraints of outer protection zone Informal waste disposal Cattle kraals Sewage sludge Small settlements Pit latrines Mining Fuel storage Cemeteries Workshops Farm stables and sheds Roads and railways Parking lots
Outer protection zone	Hospitals Wastewater and sewage treatment facilities Solid waste sites Mass livestock Airports and military facilities Oil refineries Chemical plants and nuclear reactors Large informal settlements using pit latrines Storage of hazardous substances underground

Several methodologies exist for the delineation of aquifers into protection zones. These range from simplistic analytical models to elaborate numerical models. And whilst the more elaborate numerical models are preferred, constraining factors in which method is used are cost and reliability. Numerical models require large amounts of data and is time consuming, but results obtained are technically superior. These models can take into account all hydrogeological parameters to provide an accurate and realistic flow model.

Analytical models are much more simplistic and require less hydrogeological information. They are low cost and easily applied but the reliability of results may come into question (Forster et al., 2002).

Careful consideration must be taken when delineating a protection zone such that all aspects between technical and socio economic impacts are carefully weighed against the perceived benefit of protected drinking water supplies.

With the adoption of protection zoning of groundwater resources in many first world countries, the challenge of developing countries to apply the same protection strategies is related to the economics of the country. Bearing in mind the general state of poverty in third world countries, it must be realised that successful strategising for protection of natural resources will be resultant of integrated social, economic and environmental issues. This poses a potential problem in that, whilst the economics of a country can not be changed overnight, the social and environmental protection issues will have to be balanced so as to best benefit the local citizens.

4. Action Plans and Case Study

Research carried out for this thesis was conducted in a two-fold manner. Data collation and analysis was done with both existing published data as well as data collected from a local case study site.

With the wide range of contaminants to consider and each have its own attenuation capacity and travel times through the unsaturated zone, a literature survey was conducted and

published data collected regarding transport and attenuation characteristics of mainly the enteric pathogens and their indicators. This data was analysed together with the data collected at the case study site.

The case study site was set up in Northern Kwa-Zulu Natal in the primary Zululand coastal aquifer to determine the effective distance pathogens would take to filter out in the natural environment under natural conditions. This field-based project required a multi-disciplinary, multi-institutional approach. Field measurements, sample acquisition, laboratory analyses and analysis and interpretation of results required knowledge on local geohydrology, geochemistry and microbiology.

The site was setup was in Phelandaba, approximately 60km south-west from Kosi bay at a school called Khofi Primary. Phelandaba is situated approximately 24km from the sea with an altitude of approximately 80 mamsl.

Site set up included the siting and drilling of five research boreholes at increasing distances away from a high loading pit latrine servicing the school, within the direction of flow of the groundwater in the area. This included the drilling of one reference borehole hydraulically up-gradient of the point pollution source to provide data on the background groundwater microbial quality at the school.

Boreholes were sampled on a monthly basis for a period of 11 months for microbiological quality. All sampling was carried out by DWAF geotechnicians using aseptic techniques and sample analysis was conducted at the Mlathuze Water laboratory in Richards Bay, using the membrane filtration technique.

CHAPTER 2
GEOLOGICAL CLASSIFICATION

1. Simplified South African Geology

South Africa is a geologically complex country. Vegter (2001) defined 64 groundwater regions based on opening types (primary or secondary), lithostratography, physiography and climate. See Figure 2 and Appendix 1.

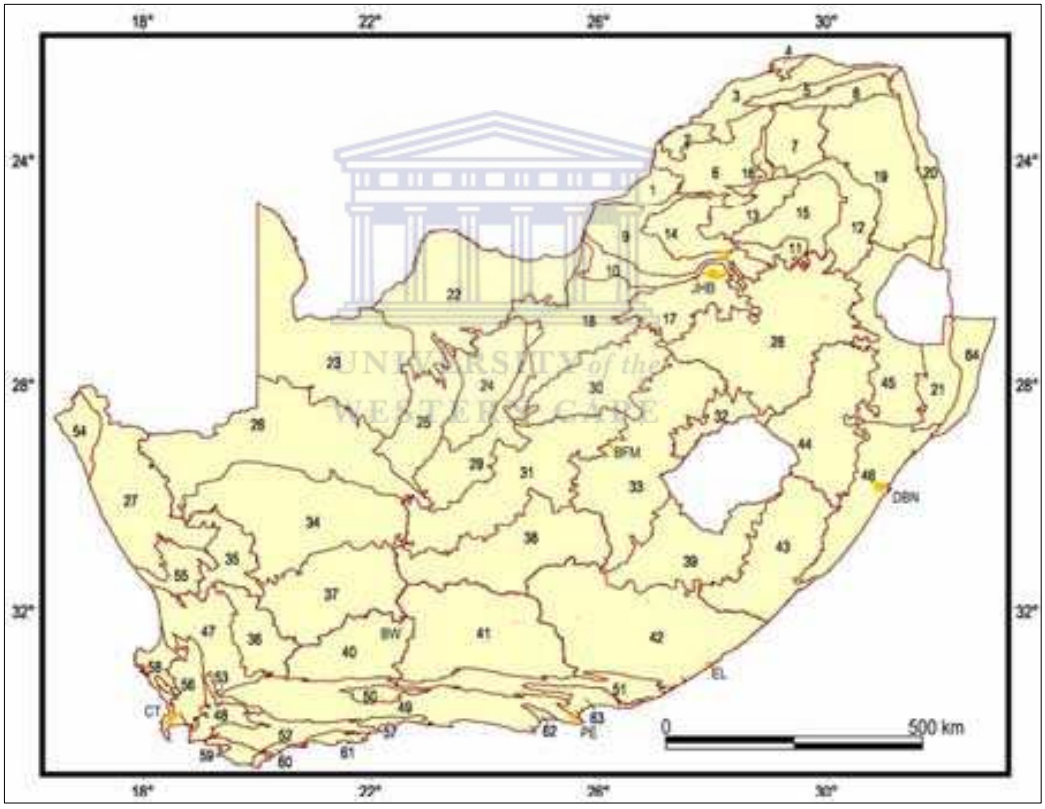

Figure 2: Groundwater regions as defined by Vegter, 2001.

South Africa's primary geological units are those that have openings or interstices that originated contemporaneously with the formation of the rock. These units are to be found mainly along the coastal belts and make up a small fraction of South Africa's aquifers.

About 90% of South African aquifers are secondary in nature with a smaller fraction of primary aquifers situated mainly along the coastal belt (Vegter, 2001) (Figure3).

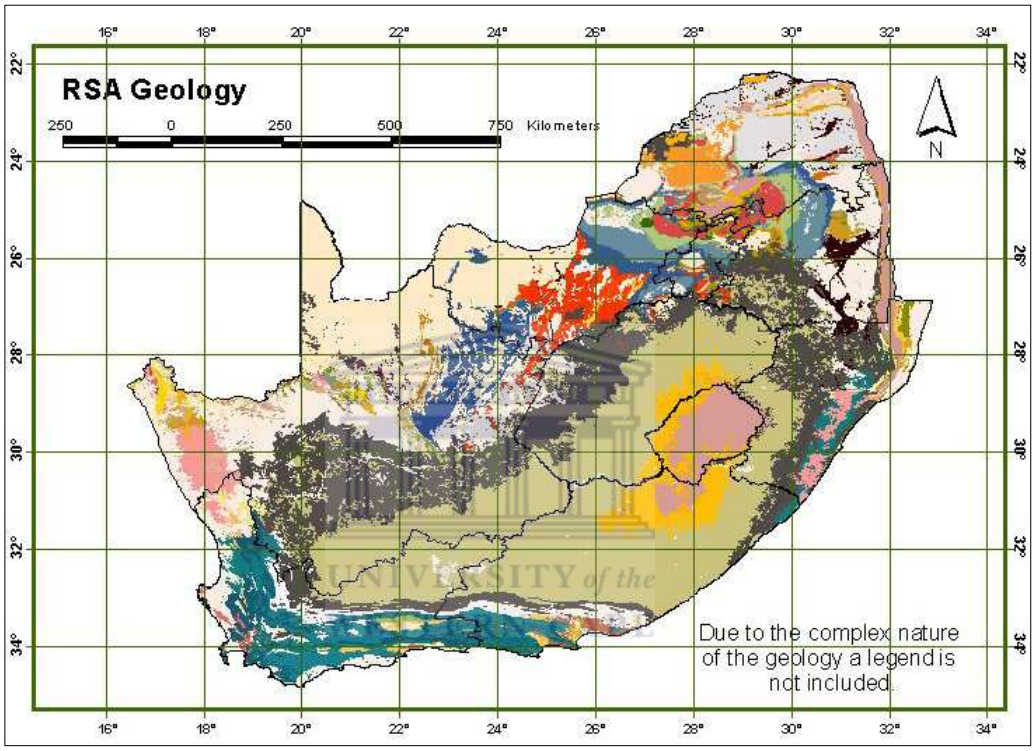

Figure 3: Geological map of South Africa, scale 1:1 million (Source: Council for Geoscience, map obtained from DWAF - GRAII, 2003)

Secondary openings develop by processes that affect rocks after they were formed (tectonic deformation, weathering processes and unloading by erosion). South Africa's and secondary porosity aquifers are subdivided into the following main rock types:

- Basement Rocks
- Consolidated Sedimentary Rocks
- Unconsolidated Sedimentary Rocks

Basement rocks: Comprising mainly of ancient crystalline and metamorphic rocks, these pre-Cambrian aquifers supply negligible quantities of water if unweathered. However, the weathered mantle and fractured bedrock, if targeted make significant aquifers (MacDonald and Davies, 2000; Titus et al., 2005). These rocks are characterised by extreme heterogeneity in their hydraulic properties. Groundwater in basement rocks tends to be shallow, and with the contributing factors of high permeability and porosity in the soil zone, the groundwater is vulnerable to pollution (MacDonald and Davies, 2000). Whilst yields of these aquifers are typically low, their widespread occurrence makes them regionally important aquifers (Morris et al., 2003).

Sedimentary rocks: Sediments of marine and continental origin form these consolidated aquifers when compacted and cemented. Comprising of sandstone, limestone, siltstone and mudstones, these rocks become consolidated with age. Primary porosity is moderate to poor but secondary porosity (fractures) formed from tectonic origin can be significant. Groundwater yields are dependent on the sediment type and nature of binding. Sandstones and karstic limestones generally yield high amounts of water because of their large pore sizes and fracturing (MacDonald and Davies, 2000). Shallower consolidated sediments are susceptible to pollution from both chemical and microbiological contaminants because of pore sizes and fracturing. Sandstone aquifers in Southern Africa are important regional aquifers. These include the Table mountain group of South Africa, Transvaal group metasediments and Ventersdorp lava of South Africa. Large areas of South Africa are covered by Permian to Triassic Karoo sedimentary basins.

Several aquifer systems, although poorly permeable are useful sources of water in the semi-arid areas. These include the Waterberg Sandstone aquifer of South Africa and Botswana (Morris et al*.,* 2003).

Unconsolidated sediments: These sediments range from coarse gravels and sands to silts and clays and are sediments that have originated from major rivers, deltas or shallow seas. Unconsolidated sediments are characterised by the good groundwater storage in pores. Shallow aquifers are vulnerable to long term contamination from chemical contamination rather than microbiological contaminants. The pores, through which groundwater flows, have a filtering effect on the microbial contaminants based on size exclusion as they are

transported through the pores. (MacDonald and Davies, 2000; Taylor et al., 2004; Morris et al., 2003). Examples of these aquifers are the unconsolidated quarternary sands of the Zululand Coastal belt in KZN (Meyer et al., 2001).

In aquifers such a basement rock and consolidated sedimentary rocks, groundwater flow occurs in preferential pathways resultant of the high degree of secondary pore structure. Fracture density and distribution, depth to bedrock and thickness of the overlying soil layer are all factors that determine the risk potential of that particular aquifer system to contamination from pathogens. If the bedrock is close to the surface and hence the presence of an overlying soil layer is minimal, the amount of residence time for the percolating recharge water would be decreased before reaching the aquifer. Hence the interactive process that occurs between the rock, soil and water interfaces, which help in the elimination of pathogens in the subsurface, is decreased (Conboy and Goss, 2000).

The presence of shale and clay layers in the saturated zone (aquitards) are important features as these form an impermeable layer which retards the transport of pathogens into the subsurface.

2. South African Soil coverage

The South African soil system is classified into approximately 73 different soil forms (Soil Classification Working Group, 1991). In attempt to further simplify these soil categories for the benefit of hydrological professionals, Fey (2005) proposed that these 73 soil forms be re-classified into 14 groups. Classification was carried out by identifying the first four groups according to topsoil or subsoil horizons, the rest follows from these in an eliminative sequence. Attached to these classifications are hydro-geochemical properties.

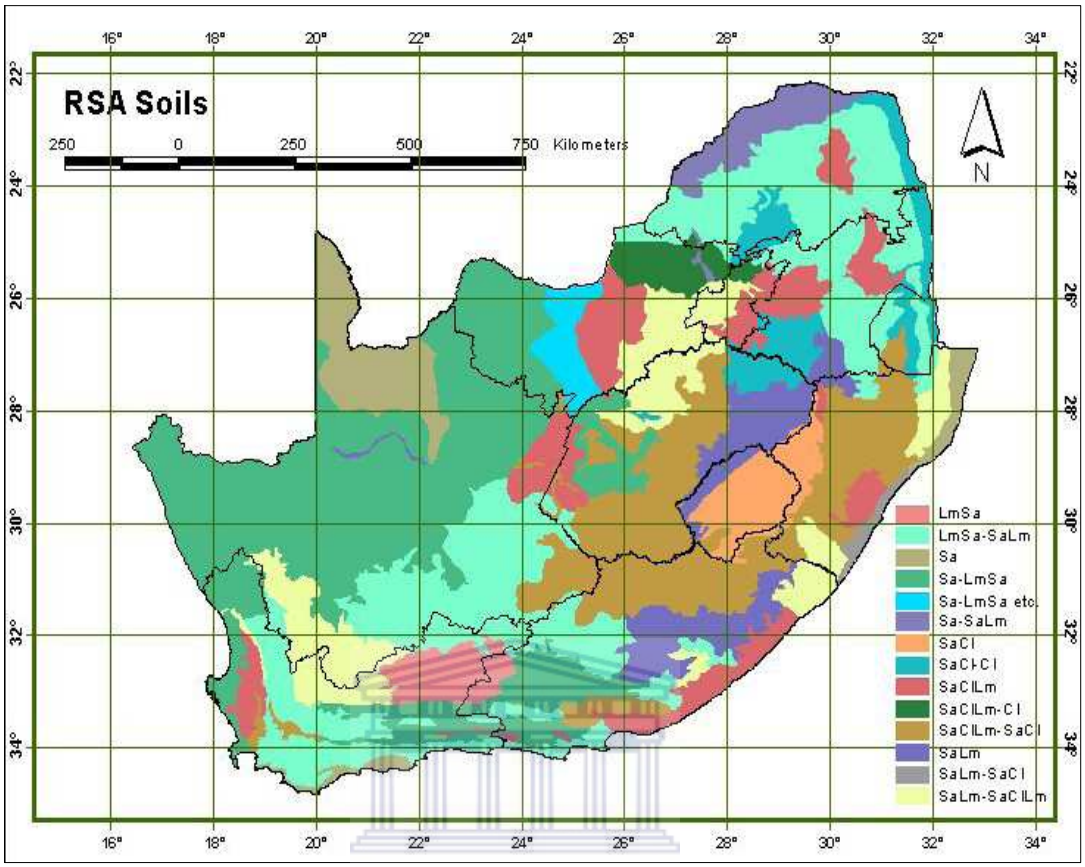

Figure 4: WR90 South African soil coverage based on the 1989 Revised Broad Homogeneous Natural Regions map produced by the Department of Agricultural Engineering, University of Natal, Pietermaritzburg (map obtained from DWAF-GRA II, 2003).

Soil bulk density and hence smaller pore sizes are directly proportional to the decrease in pathogen sub-surface transport due to the flow reduction and longer sub-surface residence time in the vadose zone (Conboy and Goss, 2000). Higher clay content effectively means a higher sorption capacity for that soil medium due to its large surface area and high cation exchange capacity (Chu et al., 2003). The soil type can be classified according to the percentage of clay and sands present in the soil, which thus affects the water retention capability. Well drained sandy, loamy soils contain less than 20% clay, whereas sandy clays and clayey soils contain more than 35% clay. These soils are regarded as strongly structured soils, with a high water retention capability (SASAES, 1999). Figure 4 shows that distribution of soil types across South Africa.

The strategic positioning of the unsaturated zone including the top soil layer, between the land surface and saturated zone renders it an important natural defense mechanism in unconfined aquifers. It forms the first defence mechanism as pollutants move through the sub-surface environment. The attenuation of pathogens in the unsaturated zone is dependent on the type of pathogen, rate of flow through the unsaturated zone, the thickness of the unsaturated zone and its capacity to adsorb contaminants (Tredoux et al., 2004; DWAF, 2003). It is estimated that 92-97% of bacteria can be removed by the top soil layer (Conboy and Goss, 2000). The soil zone's higher clay and organic matter content as well as large indigenous microbial populations renders the soil zone an effective zone for interception, attenuation and elimination of pathogenic microbes (Adams and Forster, 1992). The soil's permeability is dependent on both the soils microstructure (particle size, shape, grain arrangement) and its macrostructure (stratification, fractures and fissures, lenses).

Thus the soil medium in the unsaturated zone will effectively give an indication of the of the pathogens infiltrability (Adams and Forster, 1992). Soils with higher clay content allow for greater water retention thus allowing pathogens more time in the unsaturated zone to either adsorb onto soils particles, filter out via size exclusion or be retained long enough within this zone for natural die off of the pathogen. However, soils with high clay content in the unsaturated zone may also develop macropore structures and hence act as a preferential pathway for the pathogen. Sandy soils inhibit movement of pathogens by active filtration and adsorption (Conboy and Goss, 2000).

Soil ionic status is another important factor that helps with the reduction of the pathogen concentrations that reach the aquifer. Chemically inert soils may restrict adsorption of pathogens onto the surface of soil particles and hence allow a greater amount of pathogens to be transported to the saturated zone (Conboy and Goss, 2000; Morris, 2006).

The elimanative process will continue in the saturated zones at a slower rate due to the slower movement of groundwater in this zone as well as a reduced rate of biological activity (predation) (DWAF, 2003). However, as the depth in the soil increases there is less microbial degradation and an increased likelihood of the contaminants leaching into the groundwater.

It is hence extremely important that an unsaturated zone with sufficient thickness be maintained to minimise pathogens reaching the groundwater. In South Africa, mean depth to

the groundwater table varies mainly between 11 – 147 mbgl, with a small area ranging from 0-10 mbgl. Figure 5 depicts mean depth a groundwater levels in South Africa.

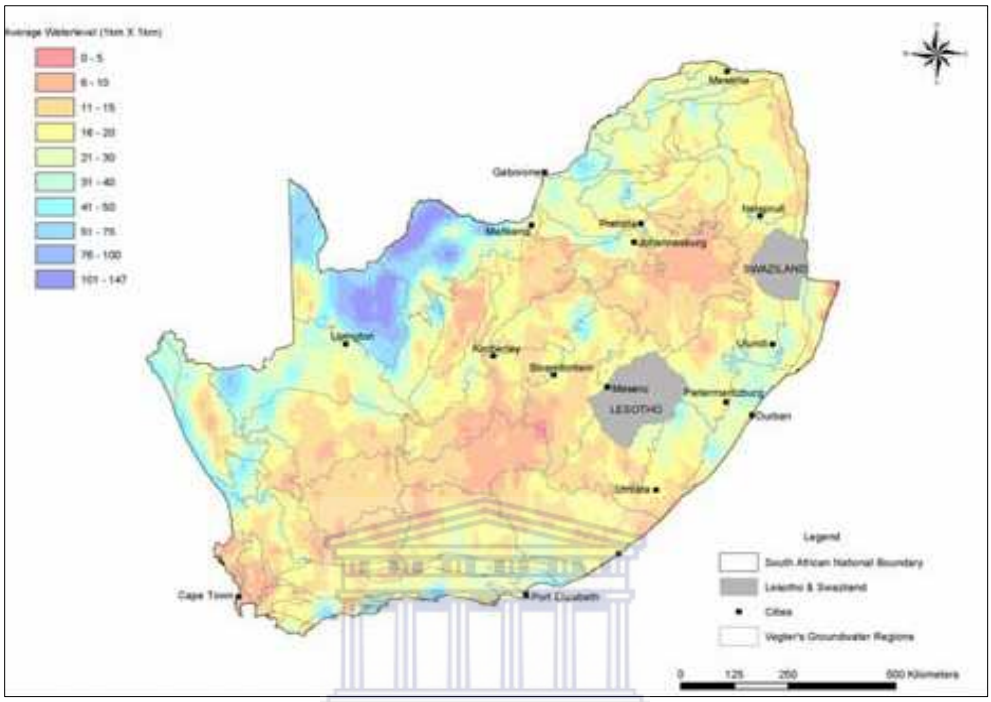

Figure 5: Mean Waterlevel Depth (DWAF – GRA II, 2003)

3. Recharge

South Africa is a semi arid country with recharge to groundwater varying according to rainfall across the country. As denoted in Figure 6, Recharge is estimated to be higher on the eastern side of the country where higher rainfall events are experienced as compared to the western side.

Rainfall events and hence recharge to the aquifer is the primary mode of pathogen transport from the surface to sub-surface. Both Celico et al. (2004) and Godfrey et al. (2005) concluded that shallow groundwater resources (both springs and boreholes) were susceptible to infiltration of surface pathogens soon after rainfall events. Changes in

microbial water quality were detected within 10-24 hours post the rainfall and recharge event in these shallow systems. Celico et al. (2004) conducted both field scale and laboratory scale experiments and were able to conclude that the pulse rainfall events resulted in high microbial counts. The intermittent flushing and migration of microbes during these events were depicted by the different break through curves obtained.

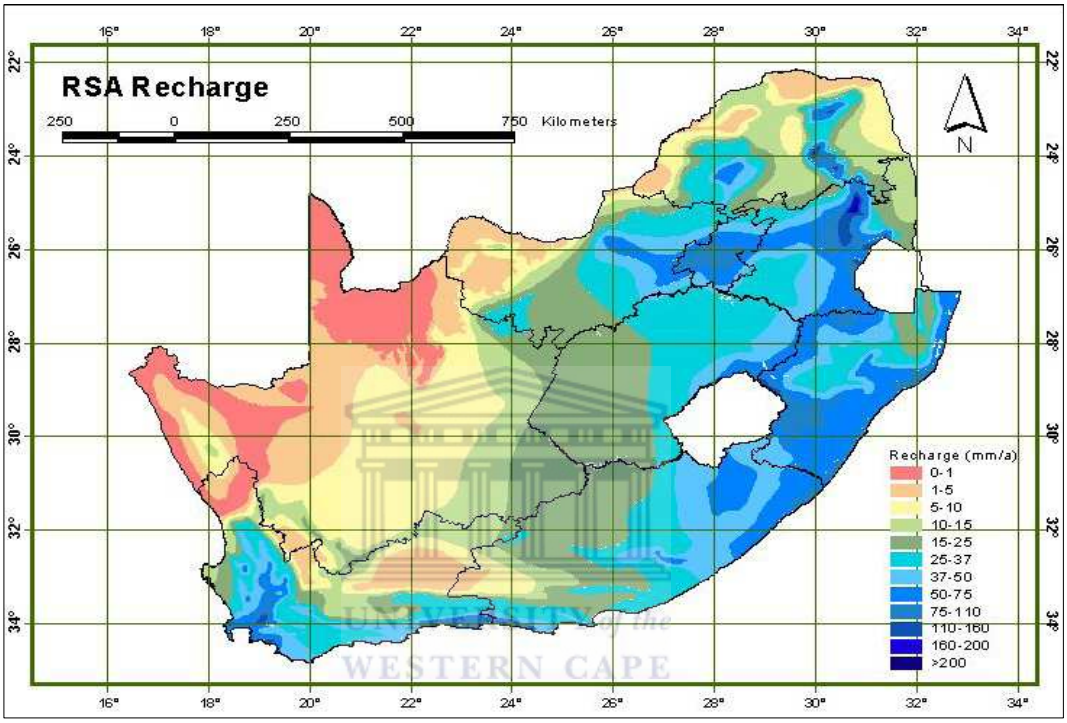

Figure 6: Groundwater Recharge (Original Source: Vegter, 1995) (map obtained from DWAF - GRAII, 2003).

4. South African Aquifer Classification

In adopting a differentiated approach to aquifer protection, where various levels of protection are given to different aquifers, a more in-depth classification system of aquifers is required. Research by Jolly and Reynders, 1993, put forth a possible classification system, which could be implemented as a part of the South African groundwater protection policy. The three primary criteria offered to assess and establish the importance of the aquifers was:

groundwater quality, yield and aquifer use. These factors were used to assess both present and potential future aquifer use. The groundwater quality and yield were seen as the main contributing factors in the assessment with the use or future potential use used to upgrade or downgrade aquifer importance (Jolly and Reynders, 1993).

Groundwater quality: The South African water quality guideline (DWAF, 1996) is the guiding document regarding water quality suitability for domestic use. This document sets out target quality levels for each constituent of water, so that water of suitable quality may be consumed, thus preventing health implications.

Borehole yield: The amount of water that can sustainably be abstracted from an aquifer should determine its suitability for domestic use. Ideally the aquifer should be able to yield enough water to cater for its specific communities needs. A borehole yielding less than 1*l/s* is considered low yielding. Moderate yielding aquifers deliver 1-5*l/s* and high yielding greater than 5*l/s*. In cases where smaller communities receive their water supplies from groundwater, even a low yielding aquifer of less than 1*l/s* is satisfactory (Jolly and Reynders, 1993).

The yield of an individual borehole does not always adequately quantify the yield of a regional aquifer which may be accessed through a well field of numerous boreholes, and may also be connected to neighbouring aquifers. Regional sustainability is an issue that must be considered when regarding the sustainability of aquifers, as well as the levels of protection that are required.

Aquifer use: This criterion is based almost exclusively on water used for domestic consumption. Depending on the amount of water used for domestic supply, the aquifers importance is rated. Bearing these criteria in mind, Jolly and Reynders (1993) proposed the following classification systems of aquifers. Refer to Table 2.

Table 2: Proposed aquifer classification (Jolly and Reynders, 1993)

Class	Aquifer Type	Description
1	Sole Source Aquifer	Aquifer supplies more than 50% of the domestic supply with no alternative source.
2	Important Aquifer	Water is of moderate quality, moderate to high yield with alternative source available
3	Minor Aquifer	Water is moderate to poor quality and yield. Predominant use is non domestic
4	Insignificant Aquifer	Water is of poor quality with low yields. No current and future use expected.

Aquifer classification maps (Parsons and Conrad, 1998) used similar, slightly modified classification categories. These maps were produced in preparation for the adoption of the groundwater quality management strategy. The modified classification system identified five different aquifer types as depicted in Table 3.

Table 3: Modified aquifer classification (Parsons and Conrad, 1998)

Aquifer Type	Description
Sole source aquifer	Aquifer supplying more than 50% of urban domestic water where there is no alternative source of water
Major aquifer	High yielding aquifer system of good quality
Minor aquifer	Moderately yielding aquifer system of moderate quality
Poor groundwater region	Low to negligible yielding aquifer system of moderate to poor quality
Special aquifer region	Aquifer system designated as such by the Minister of Water Affairs and Forestry, after due process

Understanding the processes that control movements of contaminants is necessary for developing any successful strategy to protect groundwater from contamination levels that pose significant health risks. Not all aquifers are valuable due to salinity, poor yield and other factors. Therefore monitoring and protection needs should be linked to present and potential

future use. The differentiated approach of protection zoning offers a possible solution to address this protection problem, allowing for maximum protection of important groundwater resources and less stringent protection regulations for aquifers of less value.

CHAPTER 3

LITERATURE REVIEW OF SUBSURFACE MICROBES

1. Introduction to Sub-surface Microbes

Groundwater contamination can be broadly divided into chemical or microbial. Whilst with chemical contaminants, the consequences are chronic and accumulative, microbiological contaminants of even the lowest viable concentration can result in a health risk. Water is generally the mode of transport for microbes into the saturated zone from the unsaturated zone, as it moves from the ground surface as recharge or leachate. For a very long time is has been assumed that groundwater is microbiologically safe but more and more evidence is surfacing to show that groundwater resources are indeed vulnerable to contamination from microbes, even at great depths (Abbaszadegan et al., 2003; Borchardt et al.,2007).

Microbiological pathogens can be subdivided into 3 major groups: Protozoa, Bacteria and Viruses; and can be differentiated by their physical and nuclear structures as depicted in Table 4. Structurally, microbes possess a cell wall, cell membrane and/or protein coating to increase their resistance to adverse conditions and thus ensure their survival.

Microbes generally possess a negative net charge due to the structure of the protein capsid on the surface of the microbe which has a carboxyl ($R\text{-}COO^-$) and amine ($R\text{-}NH3^+$) group. At the isoelectric pH of the microbe a net charge of zero exists as the positive and negative surface charges between the amino and carboxyl groups are balanced within an aqueous solution of specific ionic strength. Above the isoelectric pH a greater number of ionized carboxyl groups are present resulting in the net negative charge and vice versa for pH's below the isoelectric point where the ionization of a larger number amino groups takes predominance thus giving the microbe a net positive charge. (Harvey and Ryan, 2004; WHO, 2006).

Table 4: Differentiating between protozoa, bacteria and viruses using physical and nuclear information (DWAF, 2001 and Abbaszadegan, 2003)

Microorganism	Class	Size	Nuclear Membrane	Structural Description
Protozoa	Ciliata, Mastigophora, Sarcodina, Sporozoa	4-15 µm	Yes - Eukaryotic	Single cell with cell wall. Possesses cillia, pseudopodia and flagella for movement.
Bacteria	Cocci, Bacilli, Vibrio, Spirilium	0.5 – 3 µm	No - Prokaryotic	Single cell. May possess flagella for propulsion.
Viruses		23 – 80 nm	No nucleus, strand of genetic material (RNA or DNA) in a protein coat	Obligate parasites, unable to synthesize compounds on own account. Replicates at expense of host

Pathogenically induced diseases range from diarrheal infections to death in extreme cases. Those pathogens responsible for ill health in man may be infective via two routes: faecal – oral or faecal – cutaneous via infected water.

Table 5 gives an indication of waterborne diseases and their routes of infection. It also shows various diseases caused by microbiological contamination of water sources which are associated with poor hygiene and inadequate sanitation.

Table 5: Microbiological diseases related to poor water quality and sanitation (Coetzee, D. and Bourne, D.E., 1996)

Group	Disease	Route leaving host	Route of infection
Water borne diseases	Cholera	Faeces	Oral
	Typhoid Fever	Faeces / Urine	Oral
	Hepatitis	Faeces	Oral
	Giardiasis	Faeces	Oral
	Amoebiasis	Faeces	Oral
	Dracunculiasis	Cutaneous	Percutaneous
Diseases related to poor hygiene	Bacillary Dysentry	Faeces	Oral
	Enteroviral Diarrhoea	Faeces	Oral
	Amoebiasis	Faeces	Oral
	Polimyclitis	Faeces/ Secretions	Oral
	Scabies	Cutaneous	Cutaneous
	Skin Sepsis	Cutaneous	Cutaneous
	Lice and Typhus	Bite	Bite
	Trachoma	Cutaneous	Cutaneous
	Conjunctivitus	Cutaneous	Cutaneous
Diseases related to inadequate sanitation	Ascariasis	Faeces	Oral
	Trichuriasis	Faeces	Oral
	Hookworm	Faeces	Oral/ percutaneous
	Pinworm	Faeces	Oral
	Trachoma	Cutaneous	Cutaneous

It also important to note that most naturally occurring microbes in the subsurface are not harmful to man. Except for a few, those that are pathogenic to man originate from human sources i.e. Anthropogenically generated bacteria, viruses and protozoa. More specifically, resultant of human faecal sources.

Naturally occurring microbes can be of benefit to both man and the ecosystem. These microbes participate in oxidation/reduction (redox) reactions that result in the biodegradation of toxic chemical contaminants into useful materials. The redox reactions are important in biological systems as they provide a means of energy production (Bruice, 1998). Micro-organisms use the energy produced by the catalysis of the oxidative reaction. The oxidative process requires the reduction of an electron acceptor, which is O_2 in aerobic conditions. Under anaerobic conditions organic carbon (C), hydrogen (H^+), carbon dioxide ($C0_2$) or sulfate (SO_4^{2-}) may play the role of electron acceptor (Wilhelm et al., 1994). In the groundwater contamination context, in the unsaturated zone, breakdown of organic contaminants occur under aerobic conditions whilst anaerobic conditions prevail in the unsaturated zone (Engelbrecht et al., 2005). Naturally occurring microbes as well as artificially introduced microbes can hence be effectively used for *in situ* groundwater treatment of contaminated aquifers, thus improving water quality (Tredoux et al., 2004; Azadpour-Keeley et al., 1999). These microbes also act as 'in-house soldiers' helping to inactivate potential pathogens.

2. Pathogenic organisms

Many waterborne diseases are generally caused by microbes that originate in the gastrointestinal tract of man and are hence termed enteric viruses. Infections generally occur via the faecal – oral route (Table 5).

Of the different subsurface microbes viruses pose the biggest perceived threat to microbiological safety of groundwater resources, as these microbes are smaller in size and tend to travel further in the subsurface. These viruses include Noroviruses, Hepatitis A and E, Echovirus, Rotavirus A and C, Poliovirus, Coxsackievirus A and B, Enteric Adenovirus, Astrovirus and Calicivirus (Health Canada, 2004; WHO, 2006; Venter, 2003).

Some of the more common bacterial strains associated with waterborne diseases are *Shigella spp., Campylobacter jejuni, Salmonella spp., Escherichia coli, Yersinia spp., Legionella spp.* and *Vibrio cholera* (WHO, 2006; Coetzee and Bourne, 1996; Venter, 2003).

Protozoa, cysts and helminths are traditionally considered the smallest threat to groundwater quality as they most likely will be filtered out as they move through the pore spaces in the subsurface. However, microbes in this group which are considered to still produce an adverse reaction to health are: *Cryptosporidium parvum, Giardia lamblia,Ascaris lumbricoides, Trichuris trichuma, Entamoeba histolytica, Hymenolepis nana* and *Dracunculu medinensis* (WHO, 2006; Coetzee and Bourne, 1996; Grabow, 1996).

These organisms may induce health impacts ranging from diseases and illnesses like diarrhea, cholera, gastroenteritis, meningitis, respiratory diseases, fever, nausea, encephalitis, typhoid fever and in extreme cases death.

3. Sources of pathogens

Pathogens in much of rural South Africa can originate from various sources including on site sanitation, waste disposal sites, burial sites, animal kraals, run-off water and agricultural practices (DWAF, 2004a; Murray et al., 2007). This combined with a high contaminant load directly into the subsurface over a sustained period of time could lead to a high risk potential for the underlying aquifer.

Burial sites: Indiscriminate siting of mass burial sites of both human corpses and animal carcasses negatively impact on groundwater quality. Pathogens associated with mass burial sites can originate from both the natural decay process of the bodies as well as any pathogenic virus or bacteria that may have contributed to the death of that particular animal/ person. Infected corpses that are buried release these pathogenic bacteria straight back into the subsurface upon decomposition. If a burial site is not properly sited, and are in areas of high groundwater tables, permeable soils, and upslope or close to a water resource, these pathogens may enter the local groundwater supply and result in a circle of infection (DWAF 2003; Fisher and Croukamp, 1993).

Results obtained from the cemetery sites in Zimbabwe show high coliform counts in the vicinity of the graves with high total coliforms predominant around the new graves and high faecal coliforms around the more established graves (Love et al., 2006). Pathogens

emanating from a burial site are highly varied. It is not uncommon to find fecal coliform and total coliform bacteria present, as well as Clostridium and bacterial phages (Fisher and Croukamp, 1993). A large variety of pathogens originating from the infected carcasses that were primarily responsible for the demise of that animal or human will also be present. It is imperative that proper measures are taken to inactivate pathogenic microbes before the burial site is sealed e.g. use of lime on infected carcasses.

Waste disposal sites: Waste disposed of in landfill sites originate from a range of sources both within the domestic and industrial sector. These wastes can include faecal matter, pathogenic waste from hospitals and other health sectors, food and beverage industrial waste, waste from alcohol distilleries (yeast), fertilizers. Leachate generated from this toxic mix of substances will impinge on groundwater quality if the site is not properly sealed. Some microbes may act as environmental biocatalysts in the site breaking down any biodegradable waste.

Animal kraals: Faeces from animals pose a lower health risk to humans but still a risk nonetheless. Where a high number of animals congregate i.e. kraals, watering points localized concentrations of fecal matter containing microbes may occur. The contaminant loading from these 'hotspots' could be sufficient to contaminate the groundwater. Domestic animals (cattle, sheep, pigs) shed high amounts of Cryptosporidium oocyts and viruses, *E.coli*, Salmonella as well as bacteriophage PRD1 may also be found (CRC, 2004).

Agriculture: Agricultural activities affecting groundwater quality is manure management, irrigation with wastewater and tillage practices. Manure originating from animal sources can be high in microbial concentrations. Application of these wastes as manure on agricultural lands introduces these microbes into the subsurface (Jamieson et al., 2002; Conboy and Goss, 2000; Gerba and Smith, 2005). A farmer adopting a no till planting practice to prevent soil erosion and increase organic matter in the top layers leaves the soil undisturbed. This may lead to consolidation of the soil to some extent encouraging the formation of preferential pathways. Thus allowing the microbes from the applied animal manure, to move more rapidly to the subsurface (Conboy and Goss, 2000).

There are over 150 identified animal pathogens which can be transmitted to humans and land application with animal derived waste products as fertilizers and manure can form this

bridge of transmission via infection of groundwater drinking sources. Irrigation with wastewater not sufficiently treated to remove or inactivate all pathogenic microbes introduces them into the environment and these can travel into the saturated zone very quickly especially if the no till practice is adopted (Conboy and Goss, 2000; Gerba and Smith, 2005).

On site sanitation: Human faecal matter typically contains a high concentration of pathogens, biodegradable organic matter and inorganic constituents. Disposal of human waste via onsite systems (VIPs, pit latrines and septic tanks) whilst is meant to offer a safe means for disposal of faecal waste, in order to protect human health, may be a potential risk to groundwater quality. In cases where construction of these facilities has been inappropriate and maintenance over the longer periods has not been carried out (Wright, 1999; Fourie and Reyneveld, 1994; Nicosia et al., 2001; Abdel-Lad and Shamrukh, 2001) effluent will more than likely overflow or leak out of these systems directly into the groundwater table. Pathogens include the protozoal *Giardia lamblia*, bacteria such as *Escherichia coli*, *Salmonella* spp. *Shigella* spp. and *Vibrio cholerae and Vibrio typhii*, dangerous enteric viruses such as Polio, Hepatitis A and E, Adenovirus, Coxsackie, Norwalk and Rotavirus A and C; worms, both round and hook and oocysts (*Cryptosporidium*) (Coetzee and Bourne, 1996; DWAF, 2004a; Abbaszadegan et al., 2003; WHO, 2006).

Surface water run-off: Diffuse effluents and run off from areas of high pollution potential (e.g. Informal settlements) may also contain high levels of pathogens. This is especially true in areas where no formal sanitation services are provided and defecation may be carried out on the ground surface. The groundwater is more likely to be polluted if the runoff water collects in a low lying point where recharge will occur via seepage (Grabow et al., 2002 and Grabow et al., 1996).

4. Microbial survival in the sub-surface

Predicting the survival of microbes in the subsurface is difficult as there are various micro- and macroscopic, physical and chemical factors to consider. Survival rates are organism specific and may vary according to geology and environmental factors. Other factors that affect survival of microbes in the sub-surface are predation and microbial activity, adsorption,

nutrient availability and the ability to form cysts or spores (Murray et al., 2007; Fisher and Croukamp, 1993). The rate of degradation via predation and microbial activity decreases as depth in the soil increases. This may result in an increased likelihood of the contaminants leaching into the groundwater resource.

Most bacteria and viruses do not generally replicate outside of the host environment but there are a few known bacterial strains that do. These include Legionella, atypical mycobacteria, *Burkholderia pseudomallei* and *Naegleria fowleri* (WHO, 2006). Those organisms that do not replicate in the environment outside of their host may adopt special mechanisms to ensure their survival. These include spore/cyst formation and adsorption onto soil particles. Cyst and spore formation is a metabolically dormant protective phase that ensures longevity in the environment until the environmental conditions are suitable once again for normal microbial activity.

Microbial growth patterns for those that can proliferate generally follow the lag-exponential growth-stationary-decline phases as depicted in Figure 7. This life cycle is dependent on ideal environment conditions, nutrient availability, the presence of predator organisms and the natural life span of the microbe (Murray et al., 2007).

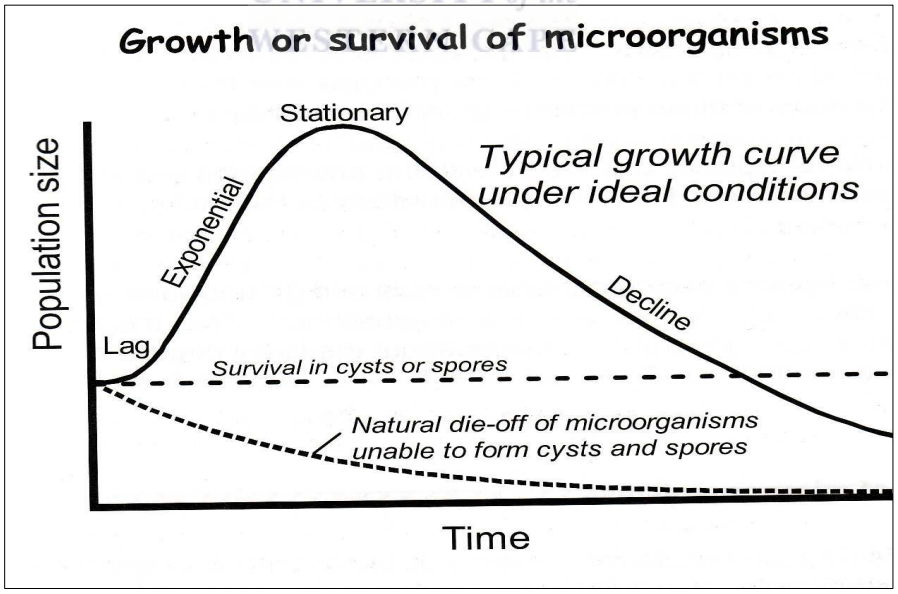

Figure 7: Life cycles of microbes outside of ideal host (Murray et al., 2007)

Organisms that are able to form spores and cysts tend to survive longer in the subsurface environment. In this state the organism is able to lie dormant until a suitable host is found in which to replicate.

Yates et al. (1985) found that environmental temperature within the subsurface (generally lower than surface water) encouraged the persistence of pathogens. Decay rates of poliovirus and echovirus 1 were analysed and persistence in the colder groundwater climate was noticed to last up to 28 days. During lab and field scale tests on the Atlantis aquifer in Cape Town South Africa, Engelbrecht, 2005 found that E.coli was able to persist for more than 40days in the laboratory.

5. Faecal indicator organisms and microbial tracers

In order to assess health risks from pollution of resources and the actions that are required for remediation, one needs to understand the origin of the pollutant. In trying to determine the safety of water microbiologically, indicator organisms are often used to check for contamination from a faecal source and microbial source tracking can be used to determine the exact physical point the pollution emanates from, using genotypic, microbiological, phenotypic and chemical characteristics (Scott et al., 2002).

Indicators circumvent the need to assay for every pathogen present (there are more than 140 enteric viruses than are known to affect human health). It would be physically and financially unreasonable to assay for every pathogen. Indicators are also used for detection when concentrations of pathogens may be below detection levels or there might not be appropriate methodologies for the detection of certain pathogens.

Indicator organisms should be non pathogenic, rapidly detectable and present in sufficient numbers within the resource. They should be easily enumerated and also have the ability to be easily cultivated in high numbers. It is important that these indicator organisms are strongly associated with the presence of pathogens and must exhibit survival characteristics in the environment similar to faecally generated pathogens. They should be durable in both the aqueous and geologically environment showing stability over a range of temperatures

with a low degree of attachment to geological sediments. Indicators also need to be structurally and functionally similar to viruses and bacteria (WHO, 2006; Health Canada, 2004; DWAF, 1996; Scott et al., 2001).

Indicator organisms mainly used in SA to detect microbiologically unsafe water are total coliforms, faecal coliforms, *Escherichia coli*, and Enterococci (DWAF, 1996) and are detected by the direct molecular method of plate counts or culturing (Cimenti et al., 2007).

Currently most indicators used are bacterial indicators and this becomes problematic when assessing viral presence in groundwater resources. Viruses tend to be more persistent and smaller in size than bacteria and have over 144 strains that may cause a pathogenic reaction in man. Only a small number of these can be detected using current methods. This problem could be eliminated by analysing for bacteriophages which are highly specific to the bacteria they infect but also morphologically similar to viruses. Bacteriophages are viruses that infect bacterial cells and can be associated with faecal pathogens (Sundram et al., 2002; Leclerc et al., 2000). However, consideration must also be given to the fact that bacteriophages, by their very natures are actually indicators of faecal indicators and hence should not be detected in isolation but should be done in congruence with the parent bacterium (Leclerc et al., 2000).

6. Case Site Indicator Organisms

The indicator of choice used at the study site was initially faecal coliform bacteria, which is an indicator specific for faecal pathogens and the presence of *Esherichia coli* gives and indication of pollution from warm blooded animals. The indicator was then changed to total coliform which gives an indication of the general hygienic quality of the water.

Although both these traditional indicators have the disadvantage of being very broad indicators, and are limited to not just faecal pathogens from humans but also warm blooded animals, which might give a false indication of pathogenic quality of the water, the limiting factor of available budget had to be taken into account. Whilst it would have been more preferable to use indicators like bacteriophages which are more specific to humans

(including somatic coliphages and F-specific RNA coliphages) or even microbial source tracking using chemical methods by analysing for pharmaceutical products excreted in human faeces, genotypic methods using ribotyping and other genetic marker and finger printing techniques (Scott et al., 2002; Cimenti et al., 2007), these analyses are all more expensive to conduct at laboratories. Appendix 2, sourced from Scott et al., 2002 gives the advantages and disadvantages of methods that can be used in microbial source tracking.

CHAPTER 4
SUBSURFACE TRANSPORT AND INACTIVATION OF MICROBES

1. Transport of Pathogens in the Subsurface

Groundwater contamination in fractured rock systems is thought to occur predominantly via defined, preferential pathways. These pathways are generally congruent with the aquifers macrostructure (fractures, fissures, stratification and the presence of geological lenses) and allow rapid transport of pathogens from the pollution source to the groundwater resources thus increasing its vulnerability to contamination (Frazier et al., 2002; Personne et al., 1998). Even deep confined aquifers show evidence of contamination with viable pathogens. This coupled with tritium aging of the recharge water suggests rapid movement of recharge water with pathogens into deep aquifers via preferential paths which may be either natural (the aquifers macrostructure) or man made (ie. Unsealed boreholes, ineffective grout seals etc.) (Borchardt et al., 2007).

The key mode of transport for pathogens in the subsurface is via the water that the pathogens are suspended in. Transport rates of pathogens through the subsurface in porous media differ greatly from that in fractured rock. Most approaches to transport rates assume that pathogens are transported at the average linear velocity of groundwater flow and hence calculate the average velocity of groundwater flow using Darcy's Law (Taylor et al., 2004). Over 90% of the aquifers in South Africa are secondary, fractured rocks. Fractured rock aquifers show extreme spatial variability in their hydraulic conductivities thus making the determination of groundwater flow rates difficult and complex. Groundwater flow in fractured rock media is not linear but highly variable and hence may predominantly follow preferential pathways with a small degree of matrix diffusion (Frazier et al., 2002; Taylor et al., 2004). Travel times calculated using Darcy's Law have drawbacks since this method is based on macroscopic flow velocities and the arrival time of the average concentration for a contaminant front or the peak arrival time for a well defined pulse.

In cases of pathogenic contamination of water, the consumption of even the lowest viable concentration of the pathogen would impact on the consumer's health. The approach that should be used for determination of microbial pathogens should hence not only concentrate on only average concentration peaks or a well defined pulse but should be able to accommodate and determine flow velocities of the first break through pathogens as they arrive in smaller concentrations.

A method for exploration of travel times for smaller concentrations of contaminants was investigated by Clarke et al., 2005. Using a modified version of the traditional advection-dispersion (ADE) model of Wheatcraft, the fractal times of arbitrary concentrations of contaminants were explored. The reasoning behind this model lies in the differential migration times of contaminants due to dispersive processes (Clarke et al., 2005).

Methods to determine the probability of a trace particle reaching a given region within a specific time, has also been explored using the backward type Kolmogorov equation. Fang et al., 2004 concluded that the backward method was much more efficient than the forward particle tracking methods normally conducted especially when the extent of protection zones is being delineated. When delineating protection zones the probability of the pathogen reaching a water resource needs to be determined together with rate of movement. Using the forward method can be tedious and time consuming since the probability for each starting point around the said area would have to be calculated resulting in numerous calculations. Using the backward method, the protection zone can be simulated in one procedure (Fang et al., 2004).

Subsurface contaminants may be naturally removed or modified depending on the physical, hydrogeochemical and biogeochemical processes that may occur. During transport through the sub-surface environment, processes of adsorption, ion exchange and biodegradation may result in the removal or decomposition of the pollutants. Analysis of groundwater flow data together with various factors regarding pathogen type, mechanisms of movement and subsurface conditions would give one a relatively good indication of the distance to be travelled by a pathogen, allowing it sufficient time for inactivation. However, in order to gain a true understanding of pathogen migration in the subsurface these factors must be all be

considered collectively within a natural environmental setting and not in isolation as many laboratory controlled experiments are setup.

2. Factors Affecting Sub-surface Transport of Pathogens

2.1 Nature of Pathogen

Pathogens vary in size, shape, survival characteristics, isoelectric points, surface coatings and appendages that assist in movement. The primary mode of pathogen removal in the sub-surface is via filtration, size exclusion, predation, natural die off and adsorption. The efficiency of the movement and removal of the pathogen by filtration is determined by its relative size. The differences in sizes and shapes of bacteria, viruses and protozoan pathogens allow them to move at different rates through the subsurface. Generally protozoan parasites (4-15 µm) move slower than bacteria (0.5-3 µm) than viruses (23-80nm) (Abbaszadegan et al., 2003).

Whilst viruses and bacteria move faster through soil as compared to helminths and protozoa they have relatively shorter life spans. Helminths and protozoa have the ability to form spores or cysts which allow them to survive for much longer periods within the subsurface environment.

Different microbes have different isoelectric points. The isoelectric point of a microbe is important as it determines the amount of microbes that will be attenuated in the subsurface. Generally most microbes and soils particles possess a negative surface charge. Hence microbes with higher isoelectric pHs will have a lower net negative charge and hence repulsion between the microbe and negatively charged soil particles are less than those microbes with a lower isoelectric pH (Chu et al., 2003).Pathogens entering the subsurface suspended in recharge water would generally have a negative charge if the pH of the water is above the isoelectric pH of the pathogen and vice versa. The recharge water possessing a different pH to the natural background pH of the groundwater may have the effect of charge reversal in either the pathogen or soil medium temporarily. This point where charge reversal occurs is called the critical pH and is determined to be 0.5 units below the highest isoelectric

pH of either the pathogen or soil media. In experiments conducted by Guan et al., 2003, a complete adsorption or viruses were observed when the critical pH was attained. Electrostatic interaction (attraction and repulsion, van der Waals forces and hydrophobic effects) is responsible for the adsorption of pathogens onto the geological medium as it is transported through the subsurface. The change in surface charge attained during the critical pH, results in a change in attachment behaviour of the pathogens and hence influences the concentrations of the pathogen in the groundwater resource as it may reach a potential user (Dowd et al., 1998).

Pathogens may also possess appendages that aid their movement in the subsurface. Cillia, pseudopodia and flagella may all be used for movement and propulsion.

2.2 Subsurface Conditions

Various factors including geology type, soil type and pH, pore connectivity, fracture density and size, surface coatings and ionic strength may all influence the rate at which pathogens are transported to the groundwater resource (Azadpour-Keeley et al., 2003).

The mobility of bacteria and viruses depend on the size and connectivity of the water filled pores and the velocity of the water in the unsaturated zone. Smaller pore sizes will filter more efficiently and it also allows for a greater residence time of the microbiological contaminant for adsorption and deactivation. However in the saturated zone where the predominant mode of transport would be the macrostructures of the aquifer, there would be less interaction between the media and pathogen due to the higher velocity experienced in these preferential pathways that act as conduits connecting the ground surface to the aquifer. Another factor to consider is the variability in groundwater flow rates within individual fractures. If fracture walls are irregular and rough, groundwater flow is greater at the centre of the fracture as frictional forces operating along the fracture wall slows down water flow in that region (Cook, 2003; Freeze and Cherry, 1979; Taylor et al., 2004).

Both surface coating and ionic strength can affect the bacterial retention capacity of sands. Bolster et al., 2001 and Chu et al., 2003 reported that metal oxide coated soil particles attenuate higher concentrations of viruses. They concluded that the metal oxide coating increased deposition rates in two ways. Firstly the metal oxide coating may have the effect of charge reversal on the soil particle thus enabling adsorption of the negatively charged pathogen onto a positively charged soil particle via electrostatic interaction. The metal oxide may also act as a multivalent cation which increases the specific surface area of the soil particle thus allowing for a greater number of adsorption sites. Thus unique property of metal oxide coated soil particles allows them to be used as hydrological barriers for microbial transport in the subsurface (Bolster et al., 2001; Chu et al., 2003; Harvey and Ryan, 2004).

Bolster et al., 2001 also noted that a decrease in ionic strength one fold resulted in a notable decrease in microbial sticking efficiency and hence more bacterial particles were eluted rather than being adsorbed.

2.3 Mechanisms of Movement

The advective movement of flowing groundwater transports the microbes. When a small volume of water containing the microbe is released into an aquifer, it will spread out from the expected advective flow path to form a contamination plume, which broadens both along and perpendicularly to the direction of groundwater flow. Processes contributing to the formation and shape of the plume are molecular diffusion and mechanical dispersion. Molecular diffusion occurs mainly due to the thermal- kinetic energy of the contaminant and moves in the direction of the concentration gradient. Mechanical dispersion is dependent on the rate of flow of groundwater through the channels or fractures of different widths within the aquifer (Hornsby, 1999; Cook, 2003; Geophysics Study Committee et al., 1984).

Solute transport in the porous media of the unsaturated zone is relatively linear with preferential pathways sometimes being chosen as compared to dispersive transport of solutes within fractured rock (saturated zone). In fracture flow dispersion may act as an accelerator to transport of pathogens even when the overall flow velocities of the groundwater are slow. However diffusive behaviour of the pathogens into the matrix acts as

a retarding factor in the transport of the pathogen and allows for greater retention time in the subsurface before reaching the end point user (Geophysics Study Committee et al., 1984).

3. Unsaturated vs. Saturated Transport

The unsaturated zone due to its structure and content provides the opportunity for the greatest eliminative potential for microbes introduced into the subsurface. There is a high rate of biological activities in this zone that enables this process. Water in the unsaturated zone is restricted to slower velocities, smaller pores and experience larger surface areas where adsorption can occur (Adams and Forster, 1992).

As pathogens move through the subsurface they undergo a process of filtration in the unsaturated zone. If attenuated in this zone the pathogen can be inactivated by physical factors such as pH changes, temperature, light and changes in oxygen concentration.

Filtering within the unsaturated zone occurs primarily by size exclusion and adsorption. The decrease often noted in pathogenic levels in the subsurface is more often a direct result of adsorption onto soil particles and filtering rather than immediate inactivation. This was confirmed by tests done by both Anders et al., 2004 and Pang et al., 2003, where recycled water was used for artificial recharge of a porous aquifer and effluent from a septic tank on the shorelines of a lake were monitored for pathogenic behaviour and movement in the sub-surface. Pang et al., 2003 also found that the majority of bacterial species were removed by filtration (87-88%) compared to 45% of viruses removed by this same process. Viruses are generally smaller particles than bacteria and hence can be transported with greater ease in the subsurface.

Figure 8 shows the relative sizes of viral, bacterial and protozoa species as compared to properties of the underlying geology.

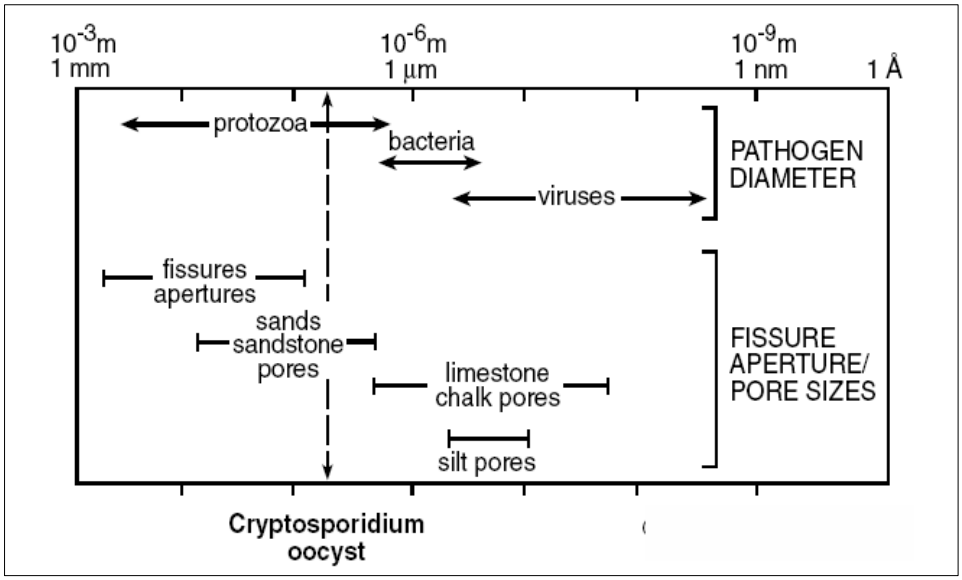

Figure 8: The size of microbial species (logarithmic scale) in relation to the properties of geological media (From WHO, 2006).

Those that are not filtered out will enter the saturated zone where their mobility will be determined by the size of the water filled pores and fractures as well as the actual velocity of the water in the pores and fractures, their surface coatings, ionic interactions (Bolster et al, 2001). Becker et al, 2003 was able to show as well that differences in size and shape of bacteria also played a role in differences in transport behavior in the subsurface. In the saturated zone there is less opportunity for pathogen elimination via adsorption and predation due to higher flow velocities as well as fewer numbers of predatory micro-organisms.

Pathogens have a specific life span and hence inactivation and natural die off plays a more significant role in minimization of viable pathogen populations in the saturated zone. It is important that the pathogen undergoes sufficient residence time in the soil before reaching a water source so that it may be rendered inactive. If this residence time is insufficient before reaching an abstraction point the results on the health of those consuming the infected water could be detrimental. Dispersion and dilution may also aid in reducing pathogen concentrations in the saturated zones (Morris et al., 2003).

In order to allow for sufficient residence times for pathogens in the subsurface minimum setback distances should be implemented. However, even a minimum setback back distance could be insufficient to completely protect a water resource if pathogens are allowed to rapidly transit to the groundwater level via preferential pathways.

4. Inactivation Rates of Pathogens

Inactivation of pathogens results when there is damage to the genetic material that allows it to replicate in the host environment. Structurally microbes have a nucleic acid encased in a protein coat, which if disrupted results in the degradation of nucleic acid and hence renders the microbe inactive (Figure 9). Exposure to adverse conditions also results in protein deformation and hence changes in the pathogenic host receptor site thus not allowing the pathogen to find and reproduce in a suitable host. (Harvey and Ryan, 2004, Anders and Chrysikopoulous, 2005, Azadpour-Keeley, 2003).

Pathogens may display different inactivation rates when adsorbed or attached to the surface of geological media. The attachment may either reduce the degradation process by protecting the pathogen from the adverse conditions in the subsurface and allow it to go into a dormant phase and hence prolong its survival; or the attachment if between a highly negatively charged soil particle will result in very strong electrostatic forces between the pathogen and soil particle. These strong binding forces can disrupt the pathogen structure and result in inactivation (Harvey and Ryan, 2004).

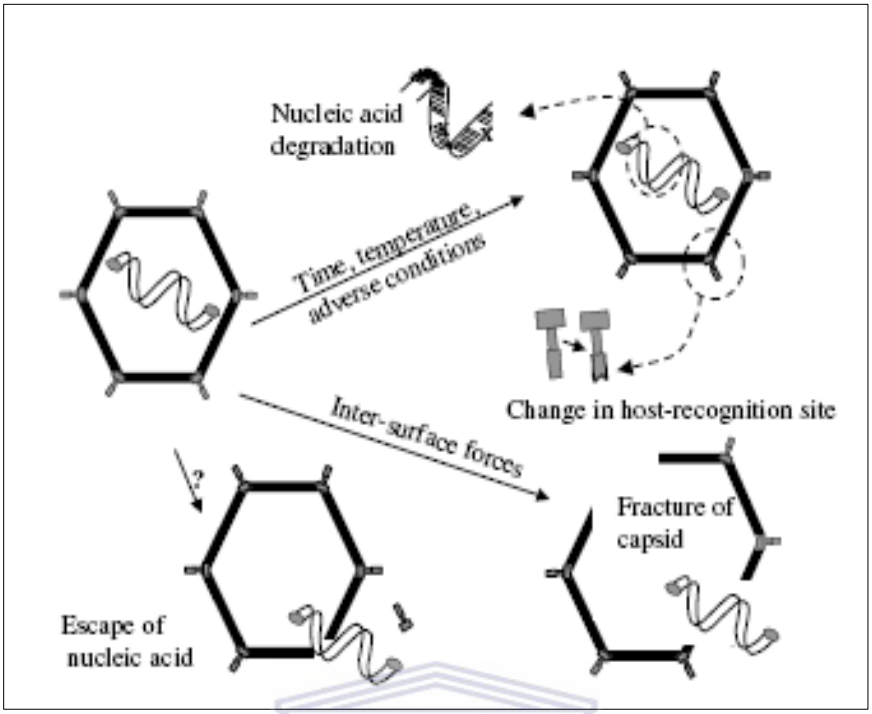

Figure 9: Potential mechanisms by which pathogens may become inactive due to loss of genetic material (Harvey and Ryan, 2004).

Generally this inactivation is described as a time dependent first order decay with a constant rate coefficient (Harvey and Ryan, 2004) and can be written mathematically as the exponential decay equation below:

$$C_t = C_0 e^{-\mu t} \quad \text{or} \quad \ln\left(\frac{C_t}{C_0}\right) = -\mu t \quad \text{or} \quad \log_{10}\left(\frac{C_t}{C_0}\right) = -\frac{\mu}{2.3} t$$

Where C_t is the final concentration of microbes present after time t and C_o is the initial starting concentration of the microbe. μ is the inactivation rate coefficient (WHO, 2006).

Inactivation rates for various microbes vary under different subsurface environmental conditions, different strains of the same microbe even display varying inactivation rates under the same experimental conditions (WHO, 2006). Chrysikopoulous and Vogler, 2001 reported that the temporal variability in microbial inactivation rates could be attributed to the

sequential inactivation of sub-populations. Fitting this data of sequential inactivation rates to a first order decay model could result in incorrect conclusions. They hence proposed a methodology using slope estimation, based on kriging, of the normalized microbe inactivation data to determine the inactivation rate coefficient. They propose that using this methodology produces more accurate time dependent inactivation coefficients than fitting data to the exponential decay model (Chrysikopoulous and Vogler, 2001).

However, most literature data available is still based on the numeric modeling of pathogens to first order decay rates and hence when being used to estimate the size of a protection zone, the inactivation of the more stable pathogens should be utilised in order to provide the most adequate and effective retention time for the removal and inactivation of pathogens (Anders and Chrysikopoulous, 2005).

Observed (literature data) (Taylor et al., 2004; WHO, 2006; Azadpour-Keeley et al., 2003) inactivation rates for bacteria in groundwater range from 0.044 – 1.7 per day:

E.coli	= 0.044 - 0.980 per day
Faecal Streptococci	= 0.066 - 0.850 per day
Salmonella spp.	= 0.190 - 0.500 per day
Shigella spp	= 0.620 - 1.700 per day
Klebsiella spp	= 0.030 - 0.072 per day

Observed inactivation rates for viruses in groundwater range from 0.010 – 1.6 per day:

Poliovirus	= 0.010 - 1.600 per day
Echovirus	= 0.019 - 1.400 per day
Coxsackievirus A and B	= 0.012 - 0.490 per day
Hepatitis virus A	= 0.038 - 0.410 per day
Rotavirus	= 0.360 - 0.830 per day

The above mentioned inactivation rates are broad classifications assigned from various literature to viruses and bacteria. It is important to note that temperature plays an important role in pathogen inactivation in the subsurface and closer inspection of the collated literature data from WHO, 2006 and Azadpour-Keeley et al., 2003 (Appendix 8) shows that at the

average groundwater temperature of 10 – 15^{o}C the observed inactivation rates for viruses are lower than bacteria suggesting a longer residence time for viable pathogenic viruses.

Spores and cysts are estimated to survive in the environment for longer than 70 weeks in the environment in their dormant states. Observed literature inactivation co-efficient values for *Bacillus subtilis* spores and *Clostridium perfringens* spores range from 0.0714 – 0.1382 per day in a 70 week period (WHO, 2006)

CHAPTER 5
CASE STUDY IN NORTHERN KZN

1. Case study location

The Zululand coastal aquifer is situated on the east coast of South Africa and covers and area of approximately 7000 km^2 between the Mozambican border in the north and the town of Mtunzini in the south. It is the largest primary coastal aquifer in South Africa and is a significant water resource for the rural population of the previous homeland area referred to as Kwa-Zulu (Meyer et al., 2001; Still and Nash, 2004). The aquifer houses several ecologically sensitive features including the Greater St Lucia Wetland Park. Because of the unconsolidated nature of the aquifer and shallow groundwater tables, water is often collected via shallow boreholes and wells (Still and Nash, 2004).

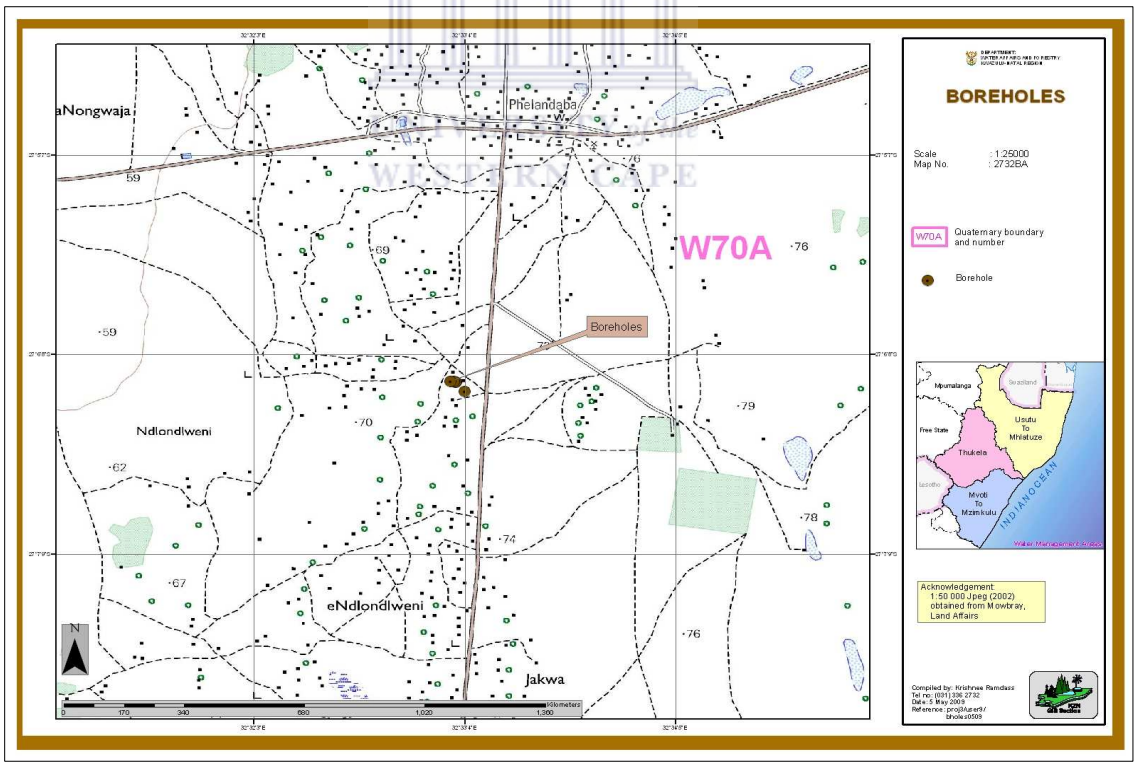

Figure 10: Locality of case study site, Khofi School, Phelandaba, Kwa Zulu Natal

The site was setup in Phelandaba, approximately 60km south-west from Kosi bay at a school called Khofi primary (Figure 10). The site was ideal in that the pit latrines being used at the school were established (> 5yrs) and unlined (Pers comm Mthembu, 2006). Five boreholes were drilled at increasing distances away from the pit latrine. The altitude is approximately 80 mamsl and drainage is inland into the Muzi channel on the western side of the site.

2. Hydrogeology

Geologically, the Phelandaba area is underlain by cretaceous age basement rocks as the secondary aquifer, with a layer of unconsolidated quaternary alluvium forming the primary aquifer. The groundwater table is shallow and ranges between 2-5 mbgl. Aquifer permeability is estimated at 15.6 m/d. Recharge to the aquifer was determined using the chloride method and is estimated to be 9.5% mean annual precipitation (MAP) (Meyer et al., 2001).

Pedologically, the soils of the Phelendaba area can be described as fine grained sands of the Maputa soil series, with parent material of Grey Recend Sand (Recent alluvium). These sands are typically highly erodible and have excessive drainage patterns. Clay and silt content is less than 5% (SASAES, 1999).

The unconsolidated nature of the aquifer lends itself to a high risk potential in terms of pollution. However, the rural nature and low population density per km^2, equates to pollution sources which are localised instead of regional.

3. Methodology

3.1 Site selection

In order to determine the impact and fate of pathogens emanating from a faecal point source, a site in an unconsolidated aquifer was chosen in the northern areas of the coastal Zululand aquifer. Site selection was based on certain criteria:

a) The site had to be an established pit latrine (> 5yrs).
b) The pit preferably should be unlined.
c) Water levels at the site should be relatively shallow.
d) The site should be located within an area / property where safety was not at too much of a risk during site visits and sampling sessions.

In consultation with the professional service provider (PSP) Partners in Development, who have worked extensively in the northern Natal coastal areas, an interview with the principal of the school (Mthembu, 2006) and a preliminary site visit was conducted in December 2006 to verify suitability of the site at Khofi School. The principal at the school agreed to allow the research to be conducted at the school in the Phelandaba area provided that the reference borehole was handed over to the school at the end of the research period as the school had no source of water except for a community borehole some distance away from the school.

Whilst the abovementioned criteria were used as the formal requirements for selecting a site, budgetary constraints also had to be taken into consideration.

3.2. Funding

Funding for the site set up and laboratory analysis was provided by DWAF: KZN regional office. It was agreed that a sum of R50 000 would be made available for all site work (siting, drilling and equipping) of the five research boreholes. Additionally, the expense of laboratory analyses over a period of six months and provision of a DWAF geotechnician and pump for sampling as well as travel expenditure would also be covered by DWAF within this period. The six month period was later extended to 11 months during which sampling were done.

3.3. Preliminary assessments and field work

Subsequent to the finalisation of the site selection, the PSP was appointed to carry out all geohydrological assessments and drilling of the five research boreholes (Appendix 3).

Owing to the nature of the aquifer (primary porosity) and shallow groundwater tables, it was decided that geophysical work was not necessary to conduct. Hence the work consisted of a desktop study of the area (drainage patterns, altitude, vegetation and other water users within a 1km radius around the site) and determination of the groundwater flow directions on site prior to the drilling of the boreholes.

3.4. Groundwater flow direction

Groundwater flow direction was determined by hand auguring six piezometric holes at the site. Three holes were augured in a triangular pattern and three in a linear fashion cutting through the school property in an east-west direction. Figure 11 shows the schematic representation of the piezometric hole set-up at the site.

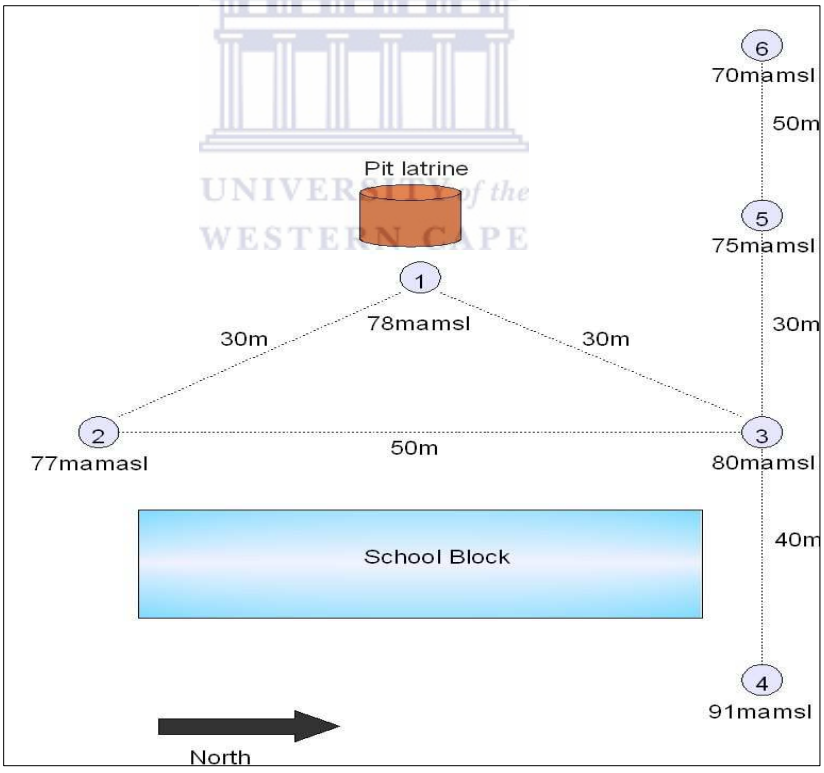

Figure 11: Schematic representation of piezometric holes at site used to determine groundwater flow direction.

The groundwater levels were then taken, using a dip meter, in relation to a fixed arbitrary marker to compensate for differences in altitude between the piezometric holes. The distances between the water level reading in each hole in relation to the arbitrary marker was recorded (See results section, Table 6).

3.5. Drilling

Following the determination of the groundwater flow direction, four research holes were sited from the pit latrine, as a starting point, at increasing distances (3m – borehole identifier KZN070007, 10m – borehole identifier KZN070006, 25m – borehole identifier KZN 070005, 50m – borehole identifier KZN070004) away from the pit latrine in the direction of the groundwater flow. The fifth borehole was sited upstream of the latrine and was utilised as a reference borehole (borehole identifier KZN070003). See site plan, Figure 12.

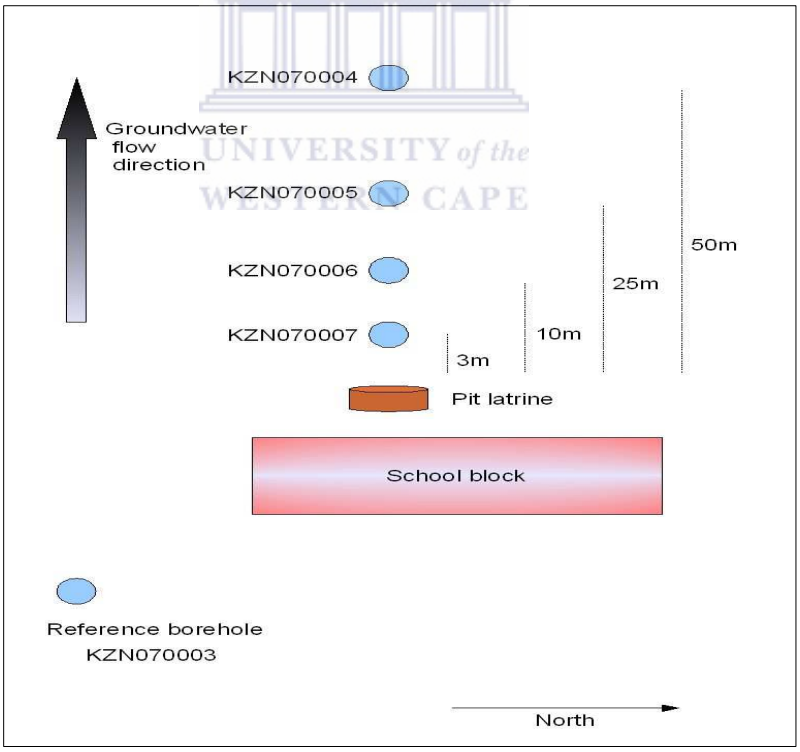

Figure 12: Site plan of research boreholes drilled at Khofi School.

Drilling commenced in mid January 2007, using the mud rotary method. Owing to the unconsolidated sediments and shallow groundwater, a non-mechanical rig was used to drill the holes (see Figure 13). The rig used was a modified Vonder rig and principally works similarly to a mud rotary rig. The wall stabilising agent used was Polyflip which can also act as a biodegradable medium for microbial growth.

Figure 13: Mud rotary drilling using a hand rig to drill the research boreholes at the site in Khofi School

Geological samples were also taken at 1m intervals for each hole drilled (Figure 14).

Figure 14: Geological samples taken at 1m intervals from ground surface (R-L) to bottom of hole.

All boreholes were drilled to a depth of 9 mbgl and fully cased using uPVC slotted casing of 125mm diameter. The full length of the casing was wrapped in Biddim geotextile to prevent the ingress of find sand particle into the borehole. The boreholes also had a cement sill constructed around it with a lockable cover (See Figure 15)

Figure 15: Lockable cover and cement seal around the borehole.

Due to the use of Polyflip, which may promote microbial growth in the boreholes, all boreholes were sterilised post drilling with chlorine. The sterilisation process was followed as stipulated in DWAF's “Minimum Standards and Guidelines for Groundwater Resource Development for the Community Water Supply and Sanitation Programme” (DWAF, 1997c).

A final concentration of 1000mg/l was required in the borehole to allow for the sterilisation process to be successful. Commercial pool chlorine HTH containing 680g Cl /1000g total product (w/w) was utilised, taking into consideration the volume of water in the borehole already as well as the volume of water in which the HTH granules would be dissolved in. The chlorine solution was injected into the boreholes using a perforated plastic tube, which was agitated around the borehole as the chlorine was being pumped to ensure that the chlorine solution was well distributed throughout the borehole.

Once disinfection of the boreholes were completed, the boreholes were purged in order to get rid of the chlorine. Purging was also conducted before every sampling session to get rid of stagnant water.

3.6. Sampling

Boreholes were sampled once a month over a period of 11 months. The holes were initially purged before every sample run by pumping four volumes of the borehole using a mechanical pump a 0.4l/s to dispose of any stagnant water in the borehole. A sample was collected at the end of the purge period into a disinfected bottle provided by the laboratory. The boreholes were then allowed to recover to original levels and samples were taken again using plastic bailers. Each borehole had a specific bailer assigned to it to prevent cross contamination. All samplers were required to use surgical latex gloves during sampling and all equipment used was wiped down with methylated spirits and then rinsed with distilled water. All collected samples were stored in cooler boxes with ice-packs on site and were handed in at the laboratory within 24hrs for analyses. The laboratory advised that the maximum time allowable between samples taken and analysis was 48hours.

3.7. Sample analysis

Samples were analysed at the Mhlatuze Water Laboratory in Richards Bay. Analyses were carried out for faecal coliforms initially and then changed to total coliforms. The method of analysis used at the laboratory was SANAS accredited method MWIM 181 (membrane filtration) and results in colony forming units (cfu) were reported against the SANS 241-2001 specifications for drinking water (See Appendix 4 for details on enumeration methodology). A full chemical analysis was also conducted on borehole KZN070004 during the first sampling session (February 2007) in order to determine the potability of the water in regards to the chemical constituents.

4. Results

The preliminary analysis yielded the following information: From the 1:50 000 topographic map of the area (2732DD Kosibay), it could be seen that owing to the altitude in the area drainage was towards the westerly direction into the Muzi channel and not into the sea as originally thought, which lies approximately 24km to the east. Groundwater users in the area were minimal with only the borehole at the school principals house adjacent to the school and a community borehole located over 500m west of the school. Most of the area is undeveloped and rural in nature with a small forestry plantation opposite the school about a kilometer away.

Based on the premise that groundwater flows from a region of higher levels to a region of lower levels and tends to mimic surface topology, it was concluded experimentally that the groundwater levels at the site flows in a westerly direction. Table 6 shows the results of the data collected from the six piezometric holes drilled at the site to determine groundwater flow directions. This data together with the topographic data, and altitude reading taken on site, all gave the indication that groundwater flow was occurring in a westerly direction. This conclusion was confirmed by flow simulation diagrams in Meyer et al., 2001, who had who had modeled groundwater flow simulations in the Zululand coastal aquifer (Appendix 5).

Table 6: Data from piezometric holes used to determine groundwater flow directions at Khofi School.

	Piezometric hole	Height difference between holes (mm)	Water Level reading in comparison to arbitrary point (m)
	1	Water level 40mm below holes 2 and 3	1.28
*	2	Level same as hole 3	1.24
*	3	Level same as hole 2	1.24
	3	Water level 100mm below hole 4	1.47
*	4		1.57
	5	Water level 90mm below hole 4	1.66
	6	170mm below hole 5	1.83

*Shallowest water levels in holes in relation to altitude

All the boreholes were logged every metre as they were drilled. The geology, as seen from the drill samples (Figure 14) confirms the desktop analysis that the underlying geology is indeed fine, light brown-grey unconsolidated sediments of recent age. The boreholes were drilled to a depth of 9 mbgl, into the primary aquifer only and did not penetrate the basement rocks. Physical details of the borehole construction are given in Table 7:

Table 7: Details of boreholes drilled at Khofi School

Borehole Identifier	Co-ordinates (DMS)		Dist from pit latrine (m)	Depth (mbgl)	Static Water Level (mbgl)
	South	East			
KZN070003	27° 06' 19.2"	32° 33' 04.0"	Ref borehole	9	2.9
KZN070004	27° 06' 16.2"	32° 32' 59.9"	50	9	2.6
KZN070005	27° 06' 16.2"	32° 33' 00.9"	25	9	2.7
KZN070006	27° 06' 16.3"	32° 33' 01.4"	10	9	2.7
KZN070007	27° 06' 16.4"	32° 33' 01.6"	3	9	2.6

Samples were initially analysed for faecal coliforms only. However, after a period of 4 months the data showed that zero counts for faecal coliform was detected in most of the holes. The analysis was then changed to a broader scope of checking for total coliforms (See Chapter 3, section 5 for further explanation for choice of faecal indicator). The results of the microbiological analyses are depicted in Table 8. The results from the initial sample show extremely high faecal counts. This sampling run was conducted a few days after completion of drilling and initial sterilisation carried out on the boreholes was not sufficient. The biodegradable medium used in sediment stabilisation during the drilling process acts as an ideal medium for growth of microbes. The boreholes were then sterilised a second time using a concentration 1000mg/l of chlorine.

Table 8: Microbiological results obtained from the 11 month sampling period at Khofi School.

Month	KZN070007 (cfu/100ml)	KZN070006 (cfu/100ml)	KZN070005 (cfu/100ml)	KZN070004 (cfu/100ml)	KZN070003 (cfu/100ml)	Indicator analysed
Feb 07	31 000	58 000	4 100	600	1 080	*E.coli* coliforms
Mar 07	0	0	0	0	0	*E.coli* coliforms
April 07	0	0	1	24	0	*E.coli* coliforms
May 07	0	0	0	0	0	*E.coli* coliforms
Jun 07	0	0	0	0	0	*E.coli* coliforms
Jul 07	21	19	18	17	17	Total coliforms
Aug 07	18	25	28	29	16	Total coliforms
Sept 07	8	0	0	480	30	Total coliforms
Nov 07	58	38	19	190	41	Total coliforms
Jan 08	0	0	0	0	0	Total coliforms

*samples could not be taken in October and December 07 due to work commitments and lack of availability of the geotechnician

** In January 08 a sample from the borehole at the school principal's house, adjacent to the school, was taken and analysed. The total coliform count was detected at 105 cfu/100 ml.

From February 2007 – June 2007, faecal coliforms were analysed. Apart from the extremely high number of counts detected in February 2007 when the boreholes were drilled and a spike at borehole KZN070004 (50m away from the pit latrine) in April 2007, all other faecal coliform counts were within DWAF ideal microbiological drinking water standards (DWAF,

1996). This would suggest that effectively all faecal coliforms, in this aquifer type would be filtered out in the first 3m around the borehole.

From July 2007 till January 2008, the analysis was changed to total coliform to check the microbiological safety of the water on a broader scale. With the total coliform analysis results varied and generally exceeded the drinking water quality acceptable limits. There was also no correlation between total coliform counts and increasing distances away from the pit latrine to suggest that the total coliforms would be filtered out completely or the concentrations minimised as one moves further away from the pollution source. During September and November, generally the higher rainfall periods, higher concentrations of total coliforms were detected in the samples.

A full chemical analysis was also conducted on borehole KZN070004. Results are depicted in Appendix 6. The results show that all chemical constituents except for iron fall within the acceptable class 1 range for drinking water standards.

Rainfall data for the area was obtained from Weather SA (Appendix 7). However the data set for this area is filled with gaps and no proper conclusion can be drawn from the rainfall data for 2007. The data does, however show that during November and December 2007, higher rainfall values were noted and this coincides with the higher rainfall patterns experienced in this area over summer.

The higher rainfall patterns experienced during November can also be confirmed in a raise in the groundwater levels at the boreholes from November due to direct infiltration of recharge water from rainfall (Figure 16). During the winter period (May – September) an average drop of around 20-30cm in groundwater levels can be noted.

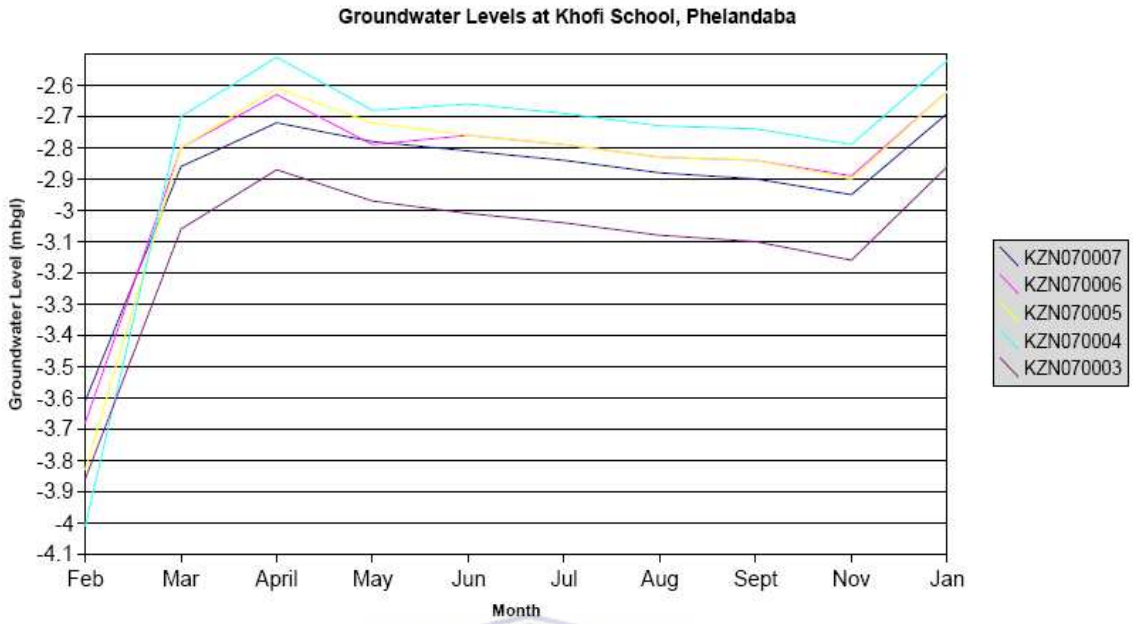

Figure 16: Groundwater level trends during sampling period (February 07 – January 08) at Khofi School, Phelandaba.

5. Discussion of case study results

The results from the case study have shown that almost no faecal coliforms can be detected, from the pit latrine, at the 3m research borehole. Faecal coliform indicators and *Escherichia coli* are used to specifically indicate the presence enteric microbes from warm-blooded animals. In most samples analysed, the closest borehole (3m away) from the latrine showed no indication of coliform presence at all (0 cfu/100ml).

Whilst it would have been ideal to drill a borehole at the distance of 1m away from the latrine and monitor faecal coliforms, this could not be done. The latrines are located on the western border of the school property (groundwater flows in a westerly direction and research boreholes had to be drilled in the direction of the groundwater flow) and the school fence posed a physical barrier, thus hindering accessibility for the drill rig to be set up at the 1m distance.

The total coliform counts in most cases exceeded the domestic drinking water quality guidelines (DWAF, 1996). It can be assumed that since faecal coliform counts were 0 cfu/100ml for most of the samples taken, that the total coliform counts were from sources other than the pit latrines. It must be borne in mind that total coliforms are general, broad-based indicators of water microbiological quality of water and does not necessarily indicate microbial pollution from a faecal source only.

The Phelandaba area is mainly rural, with livestock often being grazed on open areas. On the property outside the schools fence, where the research boreholes were drilled, remnants of cattle faeces could often visually be detected on each monthly visit. This could possibly serve as the source of microbial contaminants resulting in the high total coliform counts. This can be further supported by the lack of correlation between the increasing distance away from the pit latrine and total coliform counts, to indicate a filtering out process and hence decrease in microbial counts. Furthermore, during periods of higher rainfall, an increase in total coliform counts can be noted (September - November 2007) in all boreholes. In order to confirm that total coliform counts are from animal sources, the faecal enterococci should be analysed in future together with the faecal coliforms and the ratio between the two if below 0.7 would indicate an animal source (Celico et al., 2004).

In a primary aquifer such as the Zululand coastal aquifer, recharge is a result of direct infiltration of rain into the aquifer. With a shallow groundwater level (<3mbgl), microbial contaminants from the surface would directly infiltrate the aquifer during a rainfall event. Taking this into consideration, it seems that the higher total coliform counts, in the higher rainfall months would indicate that the origin of these microbes would be from the surface, entering the aquifer via direct infiltration. This effectively means that any surface source of microbial contaminant (whether animal or human) would pose a high risk, in terms of groundwater microbial quality in this type of aquifer.

Another plausible explanation can be directly related to the chemical nature of the soil and thus adsorption of existing microbial populations to soil particles and the subsequent elution during a rainfall event. The chemical nature of the water suggests geology rich in iron sediments (Appendix 6). It has been postulated (Guan et al., 2003; Chu et al., 2003 and Bolster et al., 2001) that soils with metal hydroxide coating are able to increase deposition

rates of microbial populations and hence increase the amount of microbes attenuated. This attenuation works by electrostatic forces where the positively charged iron rich geology attracts and adsorbs the negatively charged microbe. During the a rainfall event this scenario is reversed as the ingress of fresh water into the system changes the pH momentarily and can result in the charge density of either the medium or microbe being changed and eventually reversed. This reversal of the charge on either the soil particle or microbe results in two like charged ions and subsequently results in repulsion between soil particle and microbe and hence an elution of the previously adsorbed microbe. This is noticeable in the higher coliform counts after the rainfall events in November 2007.

On an interesting note though, the borehole tested at the school principal's property adjacent to the school, showed high total coliform counts as well. The reading at the principal's home borehole was higher in general than most of the research boreholes. The water from this borehole is regularly consumed by the family and not reports of adverse effects to health has been reported by the principal (Mthembu, 2006).

It would seem that the consumers of the water has grown a tolerance to the water quality even though the South African water quality guidelines for domestic water (DWAF, 1996) is exceeded and suggests that this water is not microbiologically fit for consumption.

It would appear from these results that in an unconsolidated sandy aquifer that the horizontal distance traveled by faecal pathogens would be limited to the first few metres within the aquifer. The faecal coliforms were effectively filtered out within less than 3m around the pit latrine. The microbial contamination from surface sources poses a higher health and water quality risk and hence the value of provision of pit latrines and means of biodegradable waste disposal in the subsurface is high. Also, interestingly, over a period of time people tend to grow tolerances to certain levels of microbes and hence when setting standards and protection zones around water resources this should be taken into account. The risks posed to different communities are very specific to each community utilising the groundwater resource.

CHAPTER 6
FEASIBILITY OF IMPLEMENTING PROTECTION ZONING

1. Circumventing and minimising health, environmental and socio-economic impacts of microbiological contaminated groundwater resources

The rapid influx of people from rural areas to urban city centres has resulted in an increased demand on water resources, a notable increase in contaminant load on the land surface and sub-surface, and a decrease in effective recharge area for the aquifer. With these rapid growth rates of urban and peri-urban areas there has been insufficient planning of infrastructure required for formal sanitation and water supply.

Whilst groundwater resources, which are often the source of choice for these communities, maybe impacted on both chemically and microbiologically, resource quality that is impinged on microbiologically poses a serious threat to human health especially in the short term. Even the lowest levels of viable microbiological organisms in a water resource may cause an infectious outbreak, and remedial action should be effected immediately. Worldwide it is estimated that approximately 1.8 million deaths deaths in developing countries are as a result of microbially unsafe water supplies. Children, immuno-suppressed adults and the aged are the most susceptible to these infectious outbreaks (UNDP, 2006). These outbreaks have both short and long term implications. Repeated outbreaks and infections may result in the immune systems of individuals being compromised in the long term and thus exposing them to other opportunistic ailments. In children, a loss of educational days at school, thus impacting on the long term academic development of the individual may result. In adults who contribute to the economy, a loss in productive working days and hence an income at the family scale may result, but also a decrease in economic productivity on the regional industrial scale may be noted.

Assessments of microbiological safety of water is hence of fundamental importance but detection of viruses and bacteria cannot be always be carried out by simple and inexpensive means (Leclerc et al., 2000; Lleo et al., 2005; Grabow, 1996; Sundram et al., 2002). Bearing in mind the cost factor associated with utilization of these laboratory procedures, a more

feasible approach to the minimising outbreaks of waterborne diseases would be to limit pollution to water resources.

The concept of groundwater protection zones is one such feasible, pro-active strategy that could be applied to important South African aquifers (DWAF, 2008). The basis of protection zoning is entrenched in understanding aquifer characteristics (flow paths and velocities, fracture density and aperture size and recharge) and the risk potential (sources of contaminants and their survival and transport characteristics). Understanding these characteristics and controlling land surface activities within the recharge area of the aquifer will theoretically result in a minimisation of contaminants that reach a groundwater resource. The second zone of the zonation method tackles specifically microbiological contaminants, looking at die off times for these contaminants before reaching groundwater resources that are used for community supply. Zonation offers social, economic and environmental benefits as summarised in Table 9:

Table 9: Benefits for different water users effected from zonation (Nel et al., 2009)

Benefit	Beneficiary
Water resource protection from pollution ensuring sustainability	Water users, water service providers, water management institutions
Prevention of health effects, morbidity and deaths resulting from inappropriate water quality	Water users, health service providers, industry
Minimisation of purification costs	Water service providers, municipalities, tax payers
Sustainable and natural functioning of ecosystems	Water users, terrestrial and aquatic ecosystems, land owners

Being the custodian of South Africa's water resources, DWAF's central mission is to ensure that its water resources in terms of quality and quantity are maintained on a sustainable basis. A research project, initiated in 2005 by the national DWAF office, to address the feasibility of introducing such a policy (DWAF, 2008) investigated the concept of

implementing the concept of protection zoning in SA. Having done the groundwork in protective legislation and classification of the country's aquifers, South Africa is now in a position to move into the differentiated protection strategy of zoning. The project is currently in the feasibility stage.

However, transforming legislation and policy into implementation actions may be limited by various factors that separate the experiences in the developed world to a developing nation (Robins et al., 2007) and hence these methodologies can not be directly translated into the South African context.

2. Implications of current learning's on the fate of microbes in the subsurface

In order to fully understand how the protection zoning policy can be successfully implemented for microbiological protection of South Africa's groundwater resources cognizance must be taken of various institutional, socio, economic, legislative and technical issues. Current learning's as outlined in this thesis bring to light various technical issues around geological formations, soil cover, depth to groundwater, recharge pulses and intensity, pathogenic sources as well as their survival and transport characteristics coupled with policy and regulation and socio-economic impact.

In the South African context, where 90% of the aquifers are secondary in nature site specific characterization and protection zone delineation is necessary. Fractured rock aquifers tend to display heterogeneity in their hydraulic conductance due to their fracture density and distribution. The fractures encourage movement of groundwater in preferential pathways which in effect facilitates movement at a faster velocity thus not allowing sufficient time for contaminant attenuation and adsorption to the rock matrix.

Whilst fractured rock may encourage faster transit of pathogens into the aquifer, the effect of the soil layer and unsaturated zone hinders, slows down and eliminates pathogens by encouraging adsorption and adhesion onto soil particles. The water retention characteristics of the unsaturated zone and soil layer, determined by its clay content ensures maximum residence time within the unsaturated zone to allow for the adsorption and adhesion

processes. The unconsolidated nature of the soil particles also aids by slowing down water movement due to the pathway water molecules would have to follow around the soil particle.

The thickness of the soil mantle, unsaturated zone and hence depth to the groundwater also plays a significant role in pathogen movement. Pathogens introduced into the system, at greater proximity to the groundwater table has less retention time and hence may not be filtered out or have sufficient time to adhere to the soil particles. This increases the probability of a pathogenic microbe reaching the groundwater table and hence being introduced into the aquifer.

Pathogens in rural areas mainly arise from anthropogenic sources like pit latrines, farming practices, livestock rearing, waste disposal facilities and burial sites. Whilst the sources of pathogens can not be completely eliminated, proper planning of activities and land zoning will offer protection where it is required the most. It is impractical to locate such activities within the immediate operational area of the borehole. It must be noted that certain pathogens display a robustness in environmental survival, and coupled with geological factors as well as lack of physical borehole protection measures may increase the likelihood of microbiological contamination of the groundwater resource.

Whilst the legislation and policies currently available for protection of water resources very clearly indicate the need and level of protection required, the mechanisms for operational level implementation need to be strengthened and champion driven. Policies and strategies like that of protection zoning and the groundwater quality management strategy, if not implemented on an operational level will eventually fade away.

3. Limitations to implementation of a zoning strategy in South Africa

There is definitely a need for implementation of a formal protection programme for groundwater resources in South Africa. However, protection zoning should be seen as a part of an all encompassing groundwater resource protection programme that covers vulnerability and risk assessments, existing and future planned land uses and aquifer importance. Whilst protection zones are not a legal requirement in South Africa now, the example of the typhoid

outbreak in Delmas, Mpumalanga depicts the need for some sort land use zoning and resource protection measures. The microbial contamination in the Delmas dolomitic aquifers from pit latrines resulted in four deaths which could have been circumvented if these protection measures were put in place. The groundwater licensing and authorisation process in South Africa does enforce the need of wellhead protection within the immediate vicinity of the abstraction point, citing the standards required in the finishing off of the boreholes (sanitary seals, locking mechanism, and cement sill/shoulders). However, these are not nearly enough to effect protection of the resource in a holistic manner.

Adoption and successful implementation of a protection zoning strategy would involve the following factors (Nel et al., 2009):

- Stakeholder involvement from the onset of the process
- Defining of the aquifer characteristics and determination of vulnerability
- Identification of potential threats and determining the relative importance and priority of the aquifer
- Scientific delineation of the protection zone
- Creating and maintaining a database of the protected zones and information management
- Monitoring of protection status and effectiveness of implemented zone

Whilst it is easier to effect the scientific side of zoning, buy in from all interested and affected parties like politicians, scientists, regulators, industries and the community might be more difficult to obtain. A balance between these parties usually requires compromise from all role players to some degree. This compromise usually needs to be struck between industrialism and economic growth with environmental protection to ensure sustainability of both activities (Fricke, 1993). It becomes a difficult task to champion the perceived benefits of zonation to communities, where the zone may impact on the very activities that sustain them economically.

Another hindrance to the successful implementation of such a strategy would be the cost factor involved in setting up such a programme. In South Africa, at the local municipal level, the main thrust is water supply and sanitation provision. This is where all financial and

technical expertise is directed. In delineating the zones various simplistic, cheaper models to more expensive numerical models can be adopted. The numerical modeling requires more intense datasets, financial and human resources which are often beyond the capacity of local water service providers. In the context of a microbial protection zone, results from a numerical model would be based on log-reductions on microbial concentrations, thus giving a more realistic result than implementation of a more simplistic method of fixed radii around resources. What is required for many of the rural areas is South Africa is non-expensive yet reliable methodologies. In a comparison conducted by Strobl and Robillard, 2006, the delineation of several German agricultural boreholes using simplistic non expensive methods compared just as well with the more expensive numerical models applied by the United States of America. However these delineations were conducted on porous aquifers and may only be suitable in direct translation to South Africa's coastal unconsolidated aquifers.

Furthermore, the groundwater data required for risk assessments, vulnerability mapping and running of models to delineate zones may not readily be available in South Africa and may be a retarding factor. Costs would also have to be incurred in the collation of appropriate base data.

However, in the longer term, it can be concluded that the economic expense for the implementation of a strategy for protection and management of a groundwater resource can be justified. In towns, especially where groundwater is the sole resource, the costs incurred for the implementation and management of such a scheme may be comparable to remediative actions carried out for one pathogenic outbreak. This was clearly depicted by Nel et al., 2009, in the cost comparison of activities post pathogenic outbreak in Delmas, Mpumalanga, approximated at around R3.2 million for one outbreak compared to the hypothetical expense of R3 million for a ten year proactive implementation and management plan.

In comparison to many developed nations, where groundwater protection has been successfully implemented on a small number of large yielding aquifers, South Africa has the opposite scenario where many socially important yet low yielding aquifers require protection. This high number of sole source smaller yielding aquifers, together with limited groundwater professionals and limited budgets being allocated to groundwater management and

protection may also produce another hurdle to overcome in the implementation of a zonation programme.

Promulgation of the NWA (1998) has taken away historical 'private' status of groundwater resources. Hence many programmes and policies regarding protection of groundwater resources in South Africa are still in the infantile stages. It will take careful consideration of all factors and buy in from all role players to ensure successful implementation of a zoning policy.

CHAPTER 7
SUMMARY AND CONCLUSIONS

1. Discussion and Analysis of Case Study and Published Data

Published data was obtained from two sources, one a review type paper (Azadpour-Keeley et al., 2003 – Appendix 9) and one groundwater text book on protecting groundwater for health (WHO, 2006 – Appendix 8). Both these sources have done an extensive collation of available data on inactivation rates for various viruses and bacteria. However, many of the values quoted here are lab derived on porous medium and glass beads. Whilst the inactivation rates give us an indication of microbial survival rates in the subsurface, it must be borne in mind that microbial survival rates are organism and site specific.

Only the inactivation rates for the major viruses and bacteria will be considered excluding the data on the indicators MS2 and PRD1 phages as these are used generally to mimic actual viral and bacterial transportation in the subsurface. Inactivation rates reported outside of the normal groundwater temperature range of 10-15^{o}C were also not considered. In Appendix 9 both surface and groundwater samples are reported on. Only inactivation rates of microbes in groundwater were used. Table 10 and Figure 17 give an indication of the statistical spread of the inactivation rates for various microbes.

Table 10: Basic statistical analysis of inactivation co-efficient rates

	HIGH	LOW	MEDIAN	MEAN	NO OF DATA POINTS
Poliovirus	0.96	0.01	0.032	0.177	13
Coxsackievirus	0.49	0.012	0.031	0.103	8
Echovirus	0.038	0.019	0.032	0.029	3
Hepatitus A	0.1	0.001	0.06	0.044	4
Rotavirus	0.83	0.36		0.59	2
Fecal Streptococci	0.53	0.23	0.27	0.34	3
Fecal Coliforms	0.83	0.45		0.64	2
E. coli	0.84	0.001	0.51	0.415	6
Shigella spp.	1.7	1.4	1.6	1.56	3
Vibrio cholerae	5.3				1

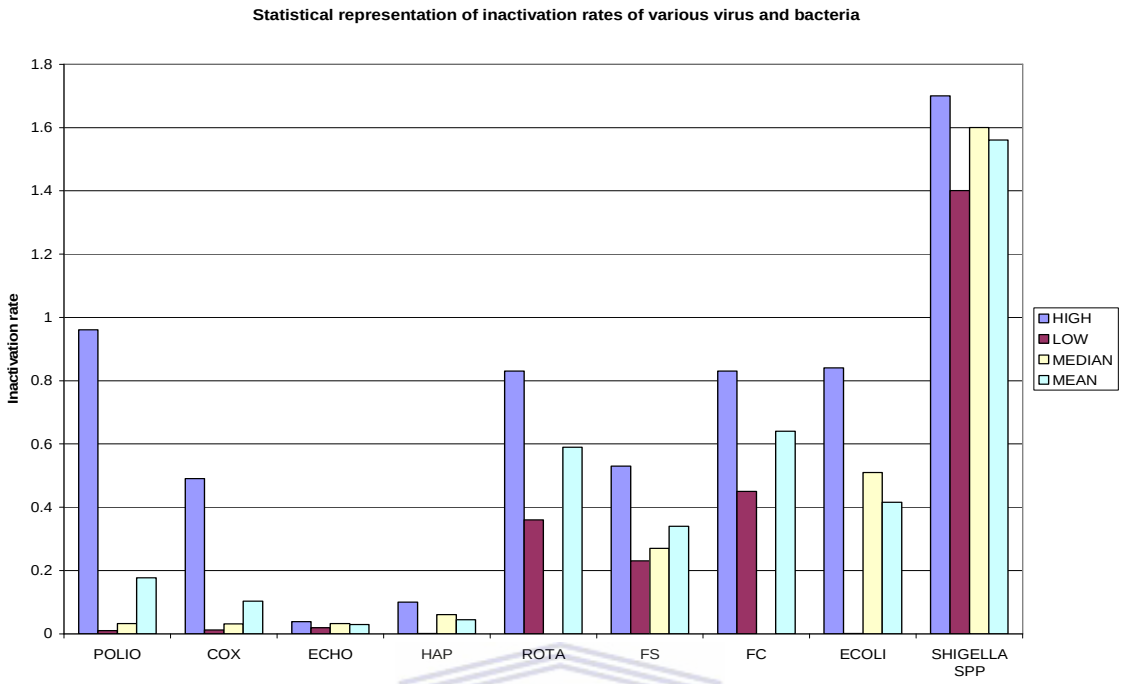

Figure 17: Graph depicting statistical analysis of inactivation rates of various microbes

From the published data it can be seen that in general bacterial species Faecal streptococci and coliforms*, E.coli* and *Shigella spp*. All have higher inactivation rates as compared to viral particles. This effectively means that bacteria will be inactivated at a higher rate within the subsurface than viral particles. Bacteria from the Shigella spp. will be inactivated at a higher rather than other bacterial species as depicted in Figure 19.

Applying this inactivation co-efficient rate analysis to the case study area in Phelendaba, the following can be noted. The permeability of the coastal Zululand aquifer is reported as 15.6 m/day. From the data obtained on site it would seem that pathogens emanating from the pit latrine are effectively filtered out within the first 3m around the borehole within the porous aquifer as there are no faecal coliform counts at the 3m borehole. If *Shigella spp*., which has the highest inactivation rate, mean inactivation rate is 1.56 $^{-}$day and the permeability as noted above is 15.6 m/day, with a high loading zone such as the school pit latrine there should be viable microbes detected at the 3m borehole. However, test site data indicates a null microbial count.

This could effectively mean that either adsorption in this geological environment, with iron rich soils is the predominant mechanism of removal or the detection methods has given a false result. From the Chapter 5, Section 5 where an indepth discussion of the microbial trends is carried out, it can be seen that metal oxide soils of the nature present at the site form favourable media for a high degree of adhesion between microbial particles and soil medium. Adsorption of the microbial particles are most probably not permanent as a higher coliform count is detected as soon as higher rainfall patterns are observed. The inflow of recharge water from the rainfall would temporarily create a change in charge on either the medium or microbe depending on the iso-electric pH of either and thus have two like charged species which would repel each other via electrostatic forces leading the desorption of the previously adsorbed microbes. This can be noted in the higher coliform counts after the November period.

2. Shortcomings of Data, Methodologies for Both Case Study and Published Data

Predicting the behaviour, transport and survival rates of pathogenic microbes in the subsurface is difficult due to the many interactive factors governing these processes (Harvey, 2005). These factors are varied and range from environmental to chemical to the microbial specific characteristics. The contribution of each of these factors to transport and survival is difficult to predict and site specific. There is a host of literature and experimental data regarding transport, attenuation and inactivation of pathogens in the subsurface however several shortcomings can be highlighted. These include issues around site specificity, enumeration and detection methods, transport in porous vs. fractured rock aquifers and lab scale issues vs. field scale. No blanket solution can be implemented for all microbes across all geologically different sites in SA.

The analysis in Chapter 7, Section 1 provides an indication to a statistical mean for inactivation of microbes in the subsurface over a range of differing geologies but mainly porous media. Whilst this gives a general indication to microbial life expectancies in the subsurface, the data can only be directly translated to the primary coastal aquifers in SA, where unconsolidated porous aquifers are present. Predicting flow paths and hence transport and inactivation of pathogens in fractured rock aquifers is much more complex than

in porous medium. Groundwater following in the preferential pathways of fractures and faults decreases the amount of time spent by microbes in the subsurface environment thus reducing the attenuation time of pathogens as well as allowing rapid transit of potential pathogens to deeper aquifers. The fractured hard rock aquifer also allows less total area surface for binding sites and hence for adsorption to occur. Site characterization to determine fracture density and degree of fracturing, groundwater flow directions and hydraulic conductivities is thus very important as a first step to determining the behaviour of microbes in the subsurface. The limiting factors of technical difficulty, time and expense to apply these experimental procedures in a field based test under natural gradients within a fractured rock geological setting maybe a reason why not much inactivation data is available for these systems.

The implementation of zonation and land use constraints may be an option in managing the impacts on groundwater quality. However, even zoning may offer limited protection and no reduction in microbial contamination if only macroscopic Darcian flow is considered. Whilst Darcian flow describes the arrival time of the average concentration for a contaminant front or the peak arrival time for a well defined pulse, field studies using tracers have shown the presence of groundwater flow paths that allow for the movement of microbiological contaminants by rapid, statistical extreme velocities. Disregarding these microscopic pathways followed by contaminants may prove detrimental especially in areas dependent on untreated groundwater as a domestic source (Taylor et al., 2004).

It is also important that column and lab scale experiments regarding transport characteristics be performed in the field as well. There are many issues that arise from lab scale experiments that need to be buffered against on a field site. These issues may give incorrect data on travel and inactivation rates of the pathogens. Issues of edge flow, repacked and disturbed cores, inadequate core diameters, simulation of waste application velocities and frequency may all give an inadequate representation of microbial transport if just extrapolated from lab tests to field. Sterile lab environments may also lead to the death of indigenous microbial species which are responsible for predation of the pathogenic species. Lab based experiments provide an excellent opportunity for stricter controls to be performed and hence provide a greater detail information on specific individualistic processes. However, in reality the rate of survival and transport of pathogens is determined by a host of

factors which work in congruence with each other and not in isolation. During field scale experiments effluent derived from septic tank effluents may contain surfactants which invariably affect pathogenic inactivation and adsorption by chemical alteration of surface binding sites on the pathogen and also suppress it survival rates. In lab based experiments the use of distilled water and other fluids differing from effluent derived from a sanitation system will give variable and false results.

Current enumeration methods widely used to detect pathogens (plate counts and membrane filtration etc.) may be under estimating actual populations as only culturable and viable pathogens are detected in the tests. Some pathogens have been noted to go into non-culturable, dormant phases to promote longevity in adverse environmental conditions. Hence these pathogens whilst present in the groundwater resource may not be detected to detection limits and methodology. The viability of pathogens in a dormant state or during release from adsorbed particles which were not detected in the enumeration method would mean that a greater set back distance of protection zone should be delineated to account for the risk posed by these pathogens (Pang et al., 2003).

It is acknowledged that the site setup and analyses carried out were elementary and could have been done on a more sophisticated and specific level, but decisions on methodology to be used were based on budgetary constraints. In terms of fine tuning the methodology and obtaining more valuable data at this site, one would have to use more specific indicators, like a bacteriophage or even conduct a tracer test using a genetically marked tracer to determine sub-surface movement. Longer sampling periods to include atleast 2years over both wet and dry seasons to give a better indication of contaminant levels would also be required. Stricter controls over animal and human access to the test site would need to be put in place to minimise introduction of source of microbes other than that from pit latrines. It would also be interesting to drill a borehole that intersects the bedrock and analyse for microbial pathogens in the deeper aquifer. It is generally assumed that the primary unconsolidated aquifer should provide a sufficient protective and attenuative barrier for the secondary basement aquifer; however evidence is now emerging suggesting the extremely rapid movement of viable microbes via preferential pathways in the fractured rock reaching depths greater than 60 mbgl (Borchardt, 2007).

The lack of proper rainfall data in the area to determine site recharge is also a shortcoming that needs to be addressed in order to have a more concrete idea on pothgen travel within the subsurface.

In order to further validify the case site data and observations, more research regarding the soil classification, isoelectric point as well as rainfall pH and microbial typing and isoelectric points are required. Indicators used should be more specific and possibly genomic typing could be carried out to determine the exact source of the coliforms.

3. Conclusion

Understanding the processes that control movements of contaminants is necessary for developing any successful strategy to protect groundwater from microbial contamination and whilst we may not yet fully understand all the mechanisms that govern subsurface transport and survival of pathogens, it is obvious from cases like the outbreak experienced in Delmas in 1993 and 2005 (Le Roux and Du Preez, 2008) where loss of life is experienced, that we require to put in some pro-active measures to ensure the preservation of potable groundwater resource quality.

Whilst protection zoning is considered an effective way to manage and restrict land uses and surface originating pollution sources, a major challenge now would be to apply this strategy to the secondary water bearing geological units of which makes up 90% of South African aquifers. The successful implementation of such a policy would require technical planning on a national, regional and local level. Aquifer classification should be carried out, in order to determine which are the most important aquifers to protect on a social, economic and productive level. Specific site characterization, determining geology type, including fracture density and distribution, type and thickness of soil cover, recharge pulses, depth to groundwater and groundwater velocity will need to be determined. This information analysed together with pathogenic type, source, load and subsurface survival characteristics will give a good indication of the size and shape of the microbiological zone to be delineated for that particular site.

The Zululand coastal aquifer can be classified as a sole source, socially important aquifer for many of the inhabitants of the former Zululand homelands, according to the aquifer classification by Parsons and Conrad, 1998. In many areas very little municipal services are available in terms of reticulated water and sanitation. The situation thus exists where there is a primary unconsolidated aquifer with shallow water levels, with many pit latrines situated with the bottom of the pits in very close proximity with the groundwater table. These are the very same aquifers through which all of their water needs are also met. The benefits that a protection measure such as zoning would offer such communities are tremendous.

From the case study data, for this particular area underlain by the unconsolidated sediments rich in iron, it would seem that a buffer zone of less than 15m would be sufficient. Whilst this is a bold conclusion to make one must also consider the current water resources and effect this has on the health of the consumers. The results also show that water consumed with a higher total coliform count at a nearby borehole has had no ill effects on the health of the consumers suggesting and intolerance build up.

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WESTERN CAPE

APPENDICES

APPENDIX 1

Groundwater regions as defined by Vegter, 2001

Ground-water Region ID	Area km^2	Groundwater Region Name	Ground-water Region ID	Area km^2	Groundwater Region Name
1	5692	Makoppa Dome	33	45349	Northeastern Upper Karoo
2	3790	Waterberg Coal Basin	34	49135	Bushmanland Pan Belt
3	13915	Beauty-Messina Granulite Gneiss	35	11329	Hantam
4	1499	Limpopo Karoo Basin	36	12283	Tanqua Karoo
5	5710	Soutpansberg Karoo Troughs	37	31876	Western Upper Karoo
6	19391	Waterberg Plateau	38	35921	Eastern Upper Karoo
7	11982	Pietersburg Plateau	39	27402	Southeastern Highland
8	6124	Soutpansberg	40	19803	Western Great Karoo
9	19790	Western Bankeveld and Bushveld	41	42370	Eastern Great Karoo
10	7862	Zeerust-Delmas Karst Belt	42	57370	Ciskeian Coastal Foreland and
11	4132	Middelburg Basin	43	30578	Transkeian Coastal Foreland and
12	15259	Eastern-Northeastern Bankeveld	44	32874	Northwestern Middleveld
13	9399	Springbok Flats	45	25630	Northeastern Middleveld
14	12473	Western Bushveld Complex	46	21948	Kwazulu-Natal Coastal Foreland
15	16797	Eastern Bushveld Complex	47	17934	Northwestern Cape Mountain Ranges
16	2449	Northern Bushveld Complex	48	6945	Southwestern Cape Mountain Ranges
17	16929	Central Highveld	49	33792	Southern Cape Mountain Ranges
18	29939	Western Highveld	50	2662	Oudtshoorn Basin
19	35475	Lowveld	51	13225	Willowmore-Grahamstown Belt
20	10136	Northern Lebombo	52	9348	Ruensveld
21	10114	Southern Lebombo	53	1342	Intermontane Tulbagh-Ashton Valley
22	50091	Eastern Kalahari	54	6197	Far Northwestern Coastal Hinterland
23	58253	Western Kalahari	55	7641	Knersvlakte
24	19957	Ghaap Plateau	56	7559	Swartland
25	17971	West Griqua Land	57	1497	Outenikwa Coastal Foreland
26	54978	Bushmanland	58	5539	Southwestern Cape Coastal Sandveld
27	32633	Namaqualand	59	192	Die Kelders
28	57805	Southeastern Highveld	60	1122	Bredasdorp Coastal Belt
29	19225	Taung-Prieska Belt	61	1798	Stilbaai Coastal Belt
30	25120	Northeastern Pan Belt	62	669	Lower Gamtoos Valley
31	41672	Central Pan Belt	63	4471	Algoa Basin
32	8014	Northeastern Highland	64	9654	North Zululand Coastal Plain

APPENDIX 2

Advantages and disadvantages of methodology for microbial source tracking (Scott et al., 2002).

Method	Advantage(s)	Disadvantage(s)
Fecal coliform/fecal streptococous ratio	Easy to perform; may be useful for recent contamination	Variable survival rates of fecal streptococci can alter ratio
Bifidobacterium sp.	Sorbitol fermenters may be human specific	Low numbers present in environment; variable survival rates; culture methods not well-defined
B. fragilis HSP40 bacteriophage	Very human specific; easy to perform	Not present in sewage in some areas
F+ RNA bacteriophage	Groups are well-correlated with source; easy to perform	Unreliable in marine and tropical waters due to variable survival rates
Human enteric virus	Human specific; Direct monitoring for pathogen circumvents need to use indicators	Low numbers in environment; labor-intensive; more sensitive methods needed
MAR	Rapid; can be used to discriminate isolates from multiple animal sources	Requires reference database; may be geographically specific; isolates that show no antibiotic resistance cannot be typed
PFGE	Extremely sensitive to minute genetic differences	May be too sensitive to broadly discriminate for source tracking
BOX-PCR	Rapid; easy to perform	Reproducibility a concern; reference database required; may be geographically specific
Ribotyping	Highly reproducible; some methods useful for classifying isolates from multiple sources	Labor-intensive; reference database required; may be geographically specific; variations in methodology exist
Bacteroides-Prevotella molecular marker	Does not require culturing of organism; PCR method is rapid, easy to perform	Little is known about survival and distribution in water systems; currently not applicable to all animals
Caffeine	Useful for assessing impact from human sewage	Minute quantities in the environment make sensitivity an issue; requires expensive analyses
Fecal sterols and/or stanols	Some sterols/stanols have greater specificity for humans and/or animals	Present naturally in sediments; requires expensive analyses; Low prevalence makes sensitivity an issue

APPENDIX 3

F - (031) 304 9546
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Yasmin Rajkumar
(031) 336 2748

DEPARTMENT OF WATER AFFAIRS AND FORESTRY : KWAZULU-NATAL
P.O. Box 1018, Durban, 4000
Southern Life House, 88 Field Street, Durban
P.O.Box 1018, **Durban**, 4000

31 October 2006

Partners in Development
PO Box 198
Mbazwana
3974

Attention: Mr. S Nash

Dear Sir

APPOINTMENT OF PARTNERS IN DEVELOPMENT FOR THE SITING AND DRILLING OF BOREHOLES IN MAPUTOLAND.

The Department of Water Affairs and Forestry signed a MoU with the University of the Western Cape in which the UWC will assist DWAF in capacity development/ building through training (academic) especially in the geohydrology/groundwater field. Such an arrangement is beginning to bear some fruits.

The Groundwater Group at UWC through Prof Yongxin Xu (UNESCO Chair of Hydrogeology) has won a tender (Project Nr 2004-256) with DWAF to develop a policy on "Groundwater Protection Zoning" in which technical and socio-economic aspects will be addressed. This 3-year project has created a prime opportunity for at least TWO students from DWAF to further their studies in the form of PhD and MSc theses. Water policy development is the key to sustaining DWAF's currently developing regulatory function.

Groundwater protection zoning is applied in many of the first world countries but has not taken off in developing countries. This protection-zoning project aims to assess the technical feasibility of protection zoning in a water supply aquifer environment in South Africa, with a proposal to test current mechanisms/ standards/ protocols for practical implementability and linkages to protection zoning. The project will entail a proposal of methodology to delineate capture zones for protection of groundwater sources. This will include the determination of travel times for biodegradable pollutants to die off, and the estimation of minimum travel distances for microbiological pollutants in South African aquifers.

To assess the travel times of E.coli bacteria from on site sanitation, a test site is to be set up for monitoring purposes. Budget for the test site has been allocated from the KZN regional Geohydrology's budget and hence it has been decided that the site will be established in the regions vulnerable Zululand coastal aquifer.

Partners in development has been identified to assist with the establishment of the site as they have worked extensively in the area and they are hereby appointed to undertake the formal siting and drilling of the five monitoring boreholes in the Maputoland area.

Kind Regards

31/10/2006

Selby Mkhize
Assistant Director Hydrology

APPENDIX 4

MWIM 181 Rev no. 06	**TOTAL AND FAECAL COLIFORM COUNT BY MEMBRANE FILTRATION METHOD**

1. PRINCIPLE:

Coliform bacteria are facultative anaerobic gram negative, oxidise negative, non-spore forming rods capable of producing colonies with a typically golden-green metallic sheen on m-Endo agar within 24 hours at 35ºC.

Faecal coliform bacteria are capable of producing colonies with typical blue colonies on m-FC agar within 24 hours at 44ºC.

Escherichia coli is a faecal coliform capable of producing indole from tryptophan within 24 hours of aerobic growth at 44º C.

2. METHOD:

11.1 Examination for total coliform bacteria

11.1.1 Immediately before use, place the sterile filtration apparatus into position on the vacuum manifold. Remove the filter flask and, using sterile forceps, place a sterile membrane filter over the porous plate, grid-side uppermost. Re-assemble the holder.

11.1.2 Thoroughly mix the water sample by inverting and righting the sample container several times.

11.1.3 Aseptically transfer the required volume of the sample into the filter funnel, and filter by applying suction to the filter flask.

11.1.4 Carry out duplicate analysis of all samples when possible.

11.1.5 After the sample has been filtered, remove the filter funnel and, using sterile forceps, aseptically transfer the membrane to a plate of m-Endo agar. Ensure that no air-bubble is trapped between the membrane and the surface of the agar.

11.1.6 Set up sterility controls.

11.1.7 Invert and incubate the dishes at 35º C ± 1ºC for 22 to 24 hours.

11.1.8 Examine the plates and count the number of coliform colonies i.e. colonies that have a pink to dark-red colour with a golden-green metallic sheen. Colonies that lack sheen are considered to be non-coliform.

11.1.9 If it is suspected that the water sample contains more than 100 colonies per 100mλ, suitable dilutions may be prepared and filtered.

11.2 Examination for faecal coliform bacteria

11.2.1 Proceed as in 11.1 but use m-FC agar, and incubate the dishes at 44ºC ± 1ºC for 18 to 24 hours.

11.2.2 Examine the plates and count the number of colonies that exhibit a blue centre with a translucent periphery. Non-faecal coliform colonies are grey to cream coloured.

APPENDIX 5

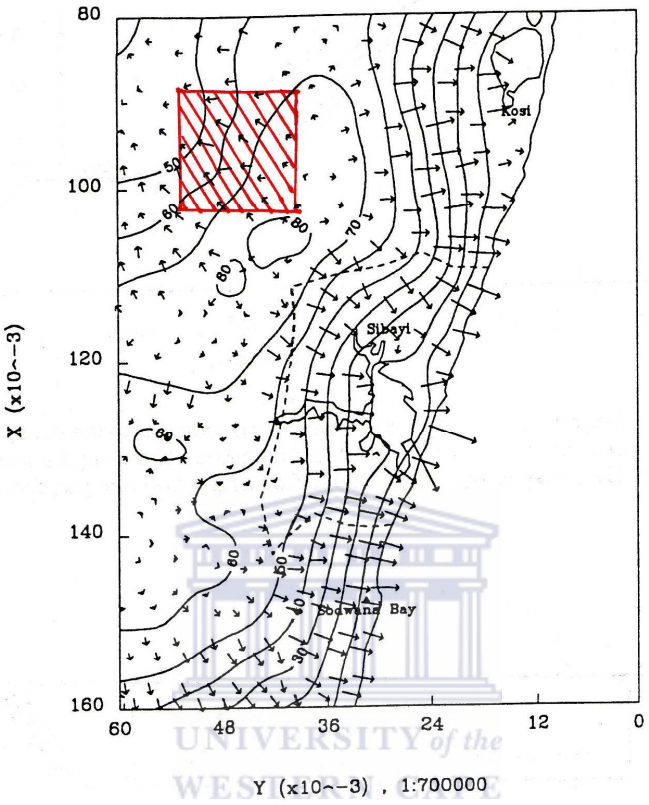

Figure 50: The direction of flow of ground water around Lake Sibayi and through the simulation area under normal conditions of 150 mm recharge per year.

***** Area highlighted in red is the Phelandaba area

APPENDIX6

SANAS ACCREDITED LABORATORY T0145

Analytical Test Report
Scientific Services

Reporting Date: 19 February 2007

MHLATHUZE WATER • AMANZI

Requested By:	Department of Water Affairs-Drbn	**Attention:**	Y. Rajkumar
Order No.:	Yasmin	**Sampled Date:**	06/02/2007
Sample Type:	Borehole Water	**Received Date:**	07/02/2007
Description:	Station 50M away		
Sample Ref. No.:	137771		
Sampling Method:	External		

Component	Result	Units	Method No.	SANS 241-2001 Specification for Drinking Water: Class 1 Aceptable Limit	Class 2 Maximum Allowable	UoM
Turbidity (NTU)	15.0	NTU	MWIM 560	1	10	±0.5
Colour (Pt/Co)	187	Hazen	MWIM 201	20	50	±3.6
Electrical conductivity at 25 °C	39.0	mS/m	MWIM 330	150	370	±3.2
pH at 25 degrees Celsius	5.71		MWIM 420	5.0 - 9.5	4.0 - 10.0	±0.06
Chloride as Cl	78	mg/L	MWIM 071	200	600	±18
Nitrate as N	<0.1	mg/L	MWIM 071	10	20	±0.4
Sulphate as SO4	7.9	mg/L	MWIM 071	400	600	±37
Fluoride as F	<0.13	mg/L	MWIM 071	1.0	1.5	±0.3
Total hardness as CaCO3	48	mg/L	MWIM 115*			
Iron as Fe	3.504	mg/L	MWIM 320	0.20	2.0	±0.09
Magnesium as Mg	4.9	mg/L	MWIM 340	70	100	±4.0
Manganese as Mn	0.056	mg/L	MWIM 320	0.10	1.0	±0.04
Potassium as K	4.8	mg/L	MWIM 340	50	100	±9.8
Sodium as Na	44	mg/L	MWIM 340	200	400	±19
Calcium as Ca	11.3	mg/L	MWIM 110	150	300	±15.9
Total dissolved solids (calculated)	222	mg/L	MWIM 115*			
Total alkalinity as CaCO3	37.2	mg/L	MWIM 040			

Environmental conditions: None

Deviation from sampling method: None

Technician | Chemist | Head: Scientific Services

Note: Methods marked with * are not by a SANAS accredited method and are not included in the SANAS Schedule of Accreditation for this laboratory. Results pertain to samples as supplied/taken.
This Analytical Test Report may only be reproduced in full unless written approval is given by the Head of Scientific Services (HSS).

UoM = Estimated uncertainty at a level of confidence of 95%

Page 1 of 1

MHLATHUZE WATER: cnr South Central Arterial & Battery Bank, Alton, Richards Bay, P.O. Box 1264, Richards Bay, 3900
Tel: +27(035) 902 1045 Fax: 27(035) 902 1107

APPENDIX 7

LEGEND

Daily rainfall (in mm) - only rainfall >= 0.1 mm is reflected on this report

---- indicates that data is not yet available

(blank) indicates that no rain fell on that day

*** indicates that data is missing or not yet available in the current month

= indicates that the total for the month is unreliable due to missing daily values

A or "B" in

but that any rainfall that did occur is included in the accumulation total at the end of the period

C next to a value indicates that the rainfall was accumulated over a number of days

South African Weather Service

Daily Rain (mm) Data for station [0412148 6] - MBAZWANA AIRFIELD -27.4770 32.5980 82 m 2007 08:00

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	10.8		***	***	***	***				7.8	30.2	
2			***	***	***	95.2					9.2	
3			***	***	***	11.2	0.2					13.4
4		24.6	***	***	***	0.4	46.4	1.6				
5			***	***	***		0.2			1.2	11.8	
6		13.6	***	***	***	11.8				2.4	5.4	41.8
7	7		***	***	***					10	0.2	12.6
8	0.2		***	***	***					0.8	26.2	4.8
9			***	***	***					0.6		
10			***	***	***					3.4		
11			***	***	***		***			0.2		
12		3.4	***	***	***							
13		0.2	***	***	***	19.2	***		2.2			
14			***	***	***		***		29.8			
15			***	***	***							0.6
16	1.6	***	***	***	***	***						0.2
17	1	***	***	***	***							20.8
18	1.6	***	***	***	***							0.2
19	0.8	***	***	***	***							
20		***	***	***	***							0.2
21		***	***	***	***						10.2	
22		***	***	***	***					0.6	25.6	
23		***	***	***						1.6	0.2	
24	9	***	***	***				0.6	1.6	1.8	3.8	
25		***	***	***	***			5.4		2.4	2.4	
26		***	***	***						0.2		18
27	2.4	***	***	***		10					0.4	0.2
28		***	***	***		0.2						
29		***	***	***					0.8			
30	18	***	***	***					3.8		9.6	
31	17.6	***	***	***		***			***		***	
Tot	70	41.8=			0.0=	148.0=	46.8=	7.6	38.2	33	135.2	112.8

Daily Rain (mm) Data for station [0412148 6] - MBAZWANA AIRFIELD -27.4770 32.5980 82 m 2008 08:00 (Extracted 2009/05/04 15:37)

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1					0.2				***		***	
2				5.8							***	
3	5.6		0.2			0.2		3.6			***	
4	1	30.8				0.4					***	9
5	2	0.2								0.6	***	1.6
6	2.6		5.4								***	0.4
7	6.6										***	
8		1									***	
9	0.2										***	
10	0.2	0.6									***	
11	0.8		0.8						0.4		***	2.4
12		0.4	20	26.8			3.8		0.2		84.4	8.2
13		***	0.2	0.2							20.6	
14											0.2	2
15			1.6									11
16			0.4					0.6	0.2	3.8		5.2
17			7.2				1.6	0.4			3	0.8
18			4.8			48.6						
19			0.2			***			9.8		4.8	
20				9.4		0.2			12.6		0.2	
21			0.2	5.6					0.8			
22			1.8	0.2		1						
23					0.8					0.6		
24	7				5.2							1.6
25	14.8				8.2							0.2
26												
27								***	8.4			8
28	4.8		5.2	14.8				***				8.2
29	0.4			0.6		0.4		***		1.4		
30		***	34.2	4				***		0.6		0.4
31		***	2	***		***		***	***	***	***	
Tot	47	33.0=	84.2	67.4	14.4	50.8=	5.4	4.6=	32.4=	7.0=	113.2=	59

APPENDIX 8

Microorganism	Temp. (°C)	Other conditions	Inactivation rate coefficient μ (1/day)	Reference
Coxsackievirus A9	10	Sterile	0.019	Matthess *et al.* (1988)
	10		0.027	
	10	Deionized	0.031	
Coxsackievirus B1	10	Sterile	0.012	Matthess *et al.* (1988)
	10		0.019	
	10	Deionized	0.040	
Coxsackievirus B3	3-15		0.49	Keswick *et al.* (1982)
Coxsackievirus B4	5		0.079	Schijven *et al.* (2003)
Coxsackievirus B5	16	1.2 mg/l O2	0.12	Jansons *et al.* (1989a)
	19.4		0.12	Jansons *et al.* (1989b)
Echovirus 1	12		0.24	Yates *et al.* (1985)
	13		0.25	
	17		0.28	
	18		0.35	
	23		0.94	
Echovirus 6	22	0.2 mg/l O2	0.25	Jansons *et al.* (1989a)
Echovirus 7	10	Sterile	0.032	Matthess *et al.* (1988)
	10		0.019	
	10	Deionized	0.038	
Echovirus 11	16	2.3 mg/l O2	0.23	Jansons *et al.* (1989a)
Echovirus 24	16	1.6 mg/l O2	0.12	Jansons *et al.* (1989a)
Hepatitis A virus	10		0.10	Nasser *et al.* (1993)
	20		0.41	
	23	Filtered bottled mineral water	0.038	Biziagos *et al.* (1988)
	25	Sterile	0.082	Sobsey *et al.* (1986)
	25		0.33	
	30		0.054	Nasser *et al.* (1993)
Poliovirus 1	3-15		0.48	Keswick *et al.* (1982)
	4		0.016	Meschke (2001)
	5		0.16	Schijven *et al.* (2003)
	10	Sterile	0.010	Matthess *et al.* (1988)
	10		0.013	
	10	Deionized	0.032	
	10		0.025	Nasser and Oman (1999)
	12		0.18	Yates *et al.* (1985)
	13		0.20	
	14	70 weeks	0.16	Meschke (2001)
	16	5.4 mg/l O2	0.21	Jansons *et al.* (1989a)
	16	0.2 mg/l O2	0.069	
	17		0.19	Yates *et al.* (1985)
	18		0.43	
	20		0.038	Nasser *et al.* (1993)
	22	0.06 mg/l O2	0.16	Jansons *et al.* (1989a)
	22		0.10	Bitton *et al.* (1983)
	23		0.17	Blanc and Nasser (1996)
	23		1.2	Yates *et al.* (1985)
	23	Filtered bottled mineral water	0.044	Biziagos *et al.* (1988)
	24		0.046	Bitton *et al.* (1983)
	25	4 weeks	0.11	Meschke (2001)
	30		0.12	Nasser *et al.* (1993)
Rotavirus	20		0.36	Pancorbo *et al.* (1987)

Microorganism	Temp. (°C)	Other conditions	Inactivation rate coefficient μ (1/day)	Reference
Simian Rotavirus	3-15		0.83	Keswick *et al.*(1982)
	23		0.28	Gerba *et al.* (Undated)
ϕX174	5		0.012	Schijven *et al.* (2002b)
F-specific RNA bacteriophages	10		0.025	Nasser and Oman (1999)
	20		0.0077	Nasser *et al.* (1993)
	30		0.031	Nasser *et al.* (1993)
MS2	2-5		0.030	Schijven *et al.* (1999)
	4		0.037	Meschke (2001)
	4		0.063	Yates *et al.* (1985)
	5		0.064	Schijven *et al.* (1999)
	5		0.082	Schijven *et al.* (2002b)
	7		0.0058-0.10	Yahya *et al.* (1993)
	12	Oxic	0.10	Schijven *et al.* (2000)
	12	Anoxic	0.024	
	12		0.16	Yates *et al.* (1985)
	12		0.065	Yates (1992, unpublished observations)
	13		0.22	Yates *et al.* (1985)
	14	70 weeks	0.45	Meschke (2001)
	17		0.17	Yates *et al.* (1985)
	18		0.19	
	23		0.36	Blanc and Nasser (1996)
	23		0.58-1.3	Yahya *et al.* (1993)
	23		0.73	Yates *et al.* (1985)
	25	4 weeks	0.41	Meschke (2001)
PRD1	5		0.0094	Schijven *et al.* (1999)
	5		0.044	Schijven *et al.* (2002b)
	7		0.010-0.10	Yahya *et al.* (1993)
	12	Oxic	0.054	Schijven *et al.* (2000)
	23		0.035	Blanc and Nasser (1996)
	23		0.12-0.30	Yahya *et al.* (1993)
Bacillus subtilis spores	14	70 weeks	0.1382	Meschke *et al.* (2001)
Cl. perfingens spores	14	70 weeks	0.0714	Meschke *et al.* (2001)
E. coli	12		0.083	Schijven *et al.* (2000)
	14	70 weeks	0.51	Meschke *et al.* (2001)
	20		0.044	Nasser and Oman (1999)
	22		0.36	Bitton *et al.* (1983)
	3-15		0.74	Keswick *et al.* (1982)
	9-13		0.84	McFeters *et al.* (1974)
E. coli O157:H7	20		0.32	Rice (1992)
Faecal coliforms	12-20		0.83	Keswick *et al.* (1982)
Faecal streptococci	22		0.066	Bitton *et al.* (1983)
	3-15		0.53	Keswick *et al.* (1982)
Klebsiella spp.	?		0.031	Dowd and Pillai (1997)
Salmonella spp.	?		0.19	Dowd and Pillai (1997)
Salmonella typhimurium	22		0.30	Bitton *et al.* (1983)
Salmonella typhimurium	9-13		0.50	McFeters *et al.* (1974)
Shigella dysentariae	9-13		1.7	McFeters *et al.* (1974)
Shigella flexeri	9-13		1.4	McFeters *et al.* (1974)
Shigella sonnei	9-13		1.6	McFeters *et al.* (1974)
Vibrio cholerae	9-13		5.3	McFeters *et al.* (1974)

APPENDIX 9

Microorganisms	*Die-off Rate (day^{-1})**	*Environmental Conditions*	*Experimental Methods*	*Reference*
Poliovirus 1	[a] 0.96	SW; pH, 8.3; T, 23-27 °C	Chamber[#]	O'Brien & Newman (1977)
	[a] 0.52	SW; pH, 8.3; T, 4-8 °C		
	0.77	SW; pH, 7.8; T, 12-20°C	Chamber	Keswick et al. (1982b)
	0.21	GW; pH, 7.8; T, 3-15 °C		
	[b] 0.01	GW; pH, 7.4; T, 10 °C	Batch test	Nasser & Oman (1999)
	[b] 0.02	GW; pH, 7.4; T, 20 °C		
	[b] 0.03	GW; pH, 7.4; T, 30 °C		
	0.013	GW saturated loamy soil; T, 10 °C	Batch test	Blanc & Nasser (1996)
	0.07	GW saturated loamy soil; T, 23 °C		
	0.016	GW saturated sandy soil; T, 10 °C		
	0.024	GW saturated sandy soil; T, 23 °C		
	[c] 0.51	GW, sandy soils; pH 8.3; T, 5 °C	Column test	Sobsey et al. (1995)
	[c] 0.66	GW, sandy soils; pH 4.3; T, 25 °C		
	[c] > 1.42	GW, clay loam; pH 8.3; T, 5 °C		
	[c] > 1.42	GW, clay loam; pH 8.3; T, 25 °C		
Poliovirus 3	1.26	SW; pH, 8.3; T, 23-27 °C	Chamber	O'Brien & Newman (1977)
	1.0	SW; pH, 7.5; T, 9-12 °C	Chamber	Keswick et al. (1982b)
Coxsackievirus A-13	[a] 3.4	SW; pH, 8.3; T, 23-27 °C	Chamber	O'Brien & Newman (1977)
Coxsackievirus B-1	0.41	SW; pH, 8.3; T, 4-8 °C		
Coxsackievirus B-3	0.19	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
Coxsackievirus A-9	[b] 2.2	Sand-silty soil; pH 7.8, T, 23 °C	Batch test	Hurst et al. (1980)
Coxsackievirus B-3	[b] 0.12	Sand-silty soil; pH 7.8, T, 23 °C		
Fecal streptococcus	[b] 0.27	GW; pH, 7.5; T, 9-12 °C	Chamber	McFeters et al. (1974)
	0.23	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
Fecal Coliforms	[b] 0.45	GW; pH, 7.5; T, 9-12 °C	Chamber	McFeters et al. (1974)
E. coli	0.32	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
E. coli	[b] 0.001	GW; pH, 7.4; T, 10 °C	Batch test	Nasser & Oman (1999)
	[b] 0.018	GW; pH, 7.4; T, 20 °C		
	[b] 0.03	GW; pH, 7.4; T, 30 °C		
Rotavirus SA-11	0.36	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
	[b] 0.20	GW; pH, 7.8; T, 23 °C	Batch test	Hurst et al. (1980)
Coliphage f2	0.39	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
F+ phage	[b] 0.01	GW; pH, 7.4; T, 10 °C	Batch test	Nasser & Oman (1999)
	[b] 0.02	GW; pH, 7.4; T, 20 °C		
	[b] 0.03	GW; pH, 7.4; T, 30 °C		

* as $-\log_{10}$ Ct/Co; GW, Ground Water; SW, Surface Water; BGS, Below ground surface

[a] One log reduction required time (LRT) was used in the reference paper for the inactivation rate.

[b] The values were estimated by curve fitting graphically.

[c] Soil columns (13.3 cm long by 2.5 cm diameter) were each dosed with 13.5 ml of virus-seeded ground water. In 53 days, a total of 16 doses were given to each column. Each dose (13.5 ml) of virus-seeded ground water was kept in a column for about 3.5 days, and then drained. Mean value of the 16 doses was presented in the reference. The values in this table are $\log_{10}$ reduction per day by dividing the mean value by 3.5 (day).

Hepatitis A virus	[b] 0.06 [b] 0.016 [b] 0.03	GW; pH, 7.4; T, 10 °C GW; pH, 7.4; T, 20 °C GW; pH, 7.4; T, 30 °C	Batch test	Nasser & Oman (1999)
	0.001 0.01	GW saturated loamy soil; T, 10 °C GW saturated loamy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	0.015 0.023	GW saturated sandy soil; T, 10 °C GW saturated sandy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	[c]0.42 [c]0.45 [c] > 0.94 [c] > 0.94	GW sandy soils; pH, 8.3; T, 5 °C GW sandy soils; pH, 8.3; T, 25 °C GW clay loam; pH, 8.3; T, 5 °C GW clay loam; pH, 8.3; T, 25 °C	Column test	Sobsey et al. (1995)